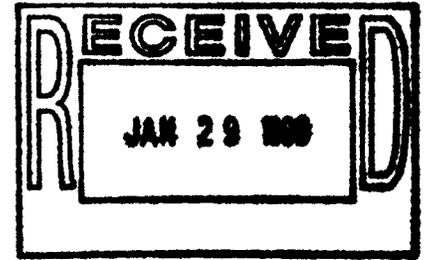


# NiPERA INC.

Nickel  
Producers  
Environmental  
Research  
Association

**Lawrence N. Curcio, Ph.D.**  
President

January 29, 1999



Dr. C.W. Jameson  
National Toxicology Program  
Report on Carcinogens  
79 Alexander Drive  
Room 3217  
P.O. Box 12233  
Research Triangle Park, NC 27709

**Re: 9th Report on Carcinogens**

Dear Dr. Jameson:

In response to the notice published in the Federal Register of December 14, 1998, 63 Fed. Reg. 68783, I am enclosing Comments of the Nickel Producers Environmental Research Association ("NiPERA") on the question of whether the broad and undifferentiated class of chemicals identified as "Nickel Compounds" should be listed as "known human carcinogens" in the 9th Report on Carcinogens ("9th RoC"). That decision, as we understand it, will be made by the Secretary of the Department of Health and Human Services ("DHHS") based in large part on a recommendation to be made by the NTP Director, Dr. Kenneth Olden, following further review and consideration by the NTP Executive Committee.

We feel strongly that listing all "Nickel Compounds" without differentiation as "known human carcinogens" would be scientifically unjustified and inconsistent with the criteria that NTP purports to apply in making these determinations. Our views on this question, along with extensive and detailed scientific reviews and analyses supporting our position, are set forth in two documents that I am enclosing with this letter. The first document is a copy of the Comments that NiPERA submitted to NTP on October 13, 1998. Those Comments present an integrated discussion of the carcinogenic potential of metallic nickel and the major classes of nickel compounds. The second document is a copy of NiPERA's Comments on NTP's Draft RoC Background Document for Nickel Compounds. Those Comments were originally submitted on November 20, 1998.

As explained at length in these two sets of Comments, certain nickel compounds (specifically, sulfidic nickel compounds and some oxidic forms of nickel) may meet the NTP's criteria for listing as "known human carcinogens." However, other nickel compounds (notably soluble compounds) do not meet those criteria. NTP and the DHHS should not gloss over these important distinctions by succumbing to the temptation to oversimplify (and, thereby, to misinterpret) an extensive and complex database.

Although both of the enclosed sets of Comments were submitted to NTP earlier, we are resubmitting them now in the hope that Dr. Olden and the NTP Executive Committee will read them attentively and give them the careful consideration we believe they deserve. As explained below, we are convinced -- and greatly disappointed -- that our Comments have not received this kind of consideration thus far in the process. In order to place the Comments in context, I want to provide some background information on NiPERA and on the process that has led to the current stage of evaluating Nickel Compounds for possible listing as "known human carcinogens" in the 9th RoC.

NiPERA is a research consortium funded by the world's primary nickel producers. Our mission is to sponsor and evaluate research on the potential human health and environmental effects associated with the extraction, manufacturing, distribution, use, and recycling/disposal of metallic nickel, nickel compounds, and nickel-containing alloys such as stainless steel. In its 20-year history, NiPERA has established itself as a center of toxicological excellence for data development and information dissemination on nickel and nickel compounds. The nickel industry, through NiPERA, has sponsored over \$25,000,000 worth of research to clarify the safety and/or hazards of nickel products and processes. The results of this research are published in peer-reviewed journals and made readily available to non-industry scientists, regulators, and the general public on an ongoing basis.

NiPERA has a vital interest in helping to ensure that regulatory and non-regulatory stakeholders possess -- and act on the basis of -- the most accurate information available regarding potential health and environmental hazards of nickel and nickel compounds. We consider NTP to be one of these stakeholders. Accordingly, through the submission of scientific/technical comments and an appearance at the Board of Scientific Counselors RoC Subcommittee meeting, we attempted to participate meaningfully in the scientific review and public participation process that was triggered by the proposal to list Nickel and Nickel Compounds as "known human carcinogens" in the 9th RoC.

Much to our dismay, we discovered that this process is seriously flawed, as is the Draft Background Document that serves as the basis for the proposed listing of all Nickel Compounds as "known human carcinogens." The points that we find most troubling include the following:

(1) While NTP invited public comment on the listing proposal, the NTP reviewers -- particularly the external peer reviewers on the Board of Scientific Counselors RoC Subcommittee -- did not have an adequate opportunity to read, to consider, or to engage in scientific discussion of the materials that were submitted by interested and knowledgeable members of the public. In particular, although NiPERA's detailed and extensive scientific Comments on the Draft RoC Background Document were submitted to NTP in a timely manner, they were not made available to members of the Subcommittee until the evening preceding the start of their meeting. That delay effectively ensured that no serious consideration or discussion of NiPERA's Comments could occur. The delay also meant that the significant errors, omissions, misinterpretations, and other shortcomings in the Draft Background Document would not be brought to the attention of Subcommittee members and, as a result, would largely escape scrutiny in the RoC listing process. When carefully reasoned and detailed scientific comments are not even read, let alone seriously considered, NTP's claim of providing for "public participation" in the RoC peer review process rings hollow. And when the peer reviewers base their recommendation on a scientifically flawed Background Document, the quality of the peer review is subject to serious question.

(2) Members of the Subcommittee were led to believe that all nickel compounds had already been listed as “reasonably anticipated to be human carcinogens” in prior editions of the RoC and that they were simply considering an “upgrading” of that classification. In fact, however, only certain specifically identified nickel compounds are identified by NTP as “reasonably anticipated to be carcinogens” in the current (8th) RoC and in previous editions of that document. For all other nickel compounds, this is the first time they are being proposed for listing as carcinogens of any sort by NTP. Subcommittee members clearly did not understand this point. (Indeed, judging from the most recent Federal Register notice, 63 Fed. Reg. 68783 (December 14, 1998), the NTP Executive Committee and the NTP Director may not understand this point either.)

(3) Scientists from NiPERA were afforded just five minutes to address complex scientific issues that can barely be identified fully, let alone discussed adequately, in such a short period of time. These NiPERA scientists devote their professional lives full-time to sponsoring and critically evaluating nickel-related health and environmental research and studies. As a result, they undoubtedly are more conversant with the epidemiological, toxicological, *in vitro*, and mechanistic literature relating to nickel and nickel compounds than any of the NTP reviewers. Yet, because NiPERA was limited to a five-minute presentation, the Subcommittee's recommendation effectively was made without the benefit of the views of NiPERA scientists on the complex issues involved and without hearing their critique of the Draft Background Document. Furthermore, even though NiPERA could have provided knowledgeable answers to many of the questions posed by the Subcommittee members, the NiPERA scientists were not allowed to answer any of these questions.

(4) The Draft RoC Background Document -- on which Subcommittee members placed primary reliance in making their recommendation -- was seriously flawed. The errors, omissions, misrepresentations, misinterpretations, and other inaccuracies in that Document are discussed at length in the November 20, 1998 Comments that accompany this letter and will not be repeated here. Suffice it to say that --

- Important points of information about certain epidemiological studies that were given most emphasis by NTP's principal reviewer were not accurately integrated into the Document. Nor was an effort made to interpret the results of these studies in light of the larger body of nickel-related epidemiological literature or to evaluate their consistency with the findings of other studies.
- Animal cancer assessments were heavily based on two studies utilizing a route of exposure (intraperitoneal) that is of questionable relevance to humans, even though there are at least eleven negative animal studies of soluble nickel chloride and sulfate salts (most of which involved inhalation or ingestion), including the NTP's own inhalation studies of rats and mice. Indeed, soluble nickel by itself was negative in one of the two intraperitoneal studies that the Background Document cites as a basis for concluding that soluble nickel is a human carcinogen. Moreover, the Background Document fails to address important aspects of the two intraperitoneal studies that call into question the significance of their positive findings.

- *In vitro* and mechanistic data (to which NTP's revised RoC criteria supposedly gave added importance<sup>1/</sup>) were misinterpreted and misrepresented.

\* \* \* \* \*

NTP's listing of a substance as a "known human carcinogen" is a portentous decision that has serious consequences both in the U.S. and abroad. A substance (or group of substances like soluble nickel compounds) should not be listed as a "known human carcinogen" as a result of a process in which public participation was more of a formality than a reality and on the basis of a Background Document that is subject to such serious criticism.

The NTP Executive Committee now must review and comment on the recommendations that have been made thus far in the process by RG1, RG2, and the Board of Scientific Counselors Subcommittee. The Director of NTP then must make a final decision on what to recommend to the Secretary of DHHS regarding the proposed listing of Nickel Compounds in the 9th RoC. Given the nearly evenly divided vote (4 yes, 3 no, 1 abstention) by the Interagency Working Group at the RG2 level and the serious procedural and substantive shortcomings that characterized the Subcommittee's consideration of this issue last December (including the flawed Background Document on which Subcommittee members relied), it is particularly important that the Executive Committee and the NTP Director conduct a careful, independent evaluation of the evidence relating to the potential carcinogenicity of the various subcategories of nickel compounds.

To facilitate such an evaluation, we are resubmitting with this letter the two sets of detailed and comprehensive Comments that were provided to NTP earlier. We believe they demonstrate that listing all nickel compounds -- without differentiation -- as "known human carcinogens" is not supported by the weight of the evidence and would be scientifically unjustified. From an epidemiological, toxicological, and mechanistic standpoint, there clearly is a difference between certain nickel compounds (such as nickel subsulfide and other forms of sulfidic nickel, for which the evidence of human carcinogenicity is strong) and other nickel compounds (notably, soluble compounds, for which the evidence of carcinogenicity is much more conflicting and uncertain).

In this connection, we wish to call your attention to a recent comprehensive Draft Toxicological Review of Soluble Nickel Salts prepared by Toxicology Excellence for Risk Assessment ("TERA"), a non-profit corporation dedicated to the best use of toxicity data for risk assessment. The TERA Review -- which was sponsored by U.S. EPA's Office of Water, the Metal Finishing Association of Southern California, and Health Canada -- was prepared by scientists from TERA with assistance from experts at Syracuse Research Corporation, Bailey Research Associates, and Mabbett & Associates. Early this month, the Draft document prepared by TERA underwent an independent peer review by a panel of expert scientists and risk assessors meeting at the University of Cincinnati College of Medicine. The peer review panel included Dr. P. Michael Bolger of the Food and Drug Administration, Dr. James J. Collins of Solutia, Inc., Dr. M. Joseph Fedoruk of the University of California Irvine, Dr. Ernest Foulkes of the University of Cincinnati, Dr. Ernest Mastromatteo of the University of Toronto, Dr. Ann G. Schwartz of Allegheny University of the Health Sciences, and Dr. Alan H. Stern of the New Jersey Department of Environmental Protection. Dr. Joyce M. Donohue of U.S. EPA, Dr. John S. Wheeler of the Agency for

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<sup>1/</sup> See NTP Revised Criteria and Process for Listing Substances in the Biennial Report on Carcinogens, 61 Fed. Reg. 50499 (September 26, 1996).

Toxic Substances and Disease Registry, and Donna J. Sivulka, a private consultant, participated as non-voting discussants.

After two days of discussions and analysis of the epidemiological, toxicological, and mechanistic database, the TERA peer review panel reached the conclusion that the carcinogenicity of soluble nickel salts via both the oral and inhalation routes "cannot be determined." For the inhalation route, this assessment was based on what was described as an "extensive but equivocal epidemiology database, together with the negative NTP bioassays in rats and mice." The peer reviewers felt that the totality of the data indicated a lack of carcinogenicity of soluble nickel by inhalation at low doses, while it remained possible that it could be carcinogenic at high doses. However, given the uncertainties and apparent conflicts in the database, this view remained speculative. The "cannot be determined" assessment for oral exposure reflected the fact that, while animal studies via the oral route are all negative, deficiencies in the studies preclude reaching a firm conclusion on the lack of oral carcinogenicity of soluble nickel. (An e-mail summary of the peer review meeting prepared by Jacqueline Patterson of TERA and distributed through TOXLIST is enclosed.)

The conclusions reached by the TERA peer reviewers are fully consistent with the analyses and conclusions presented in NiPERA's Comments to NTP. They also are consistent with last year's decision by the American Conference of Governmental Industrial Hygienists ("ACGIH") to designate soluble nickel compounds "A4" with respect to carcinogenicity -- indicating that they are "*Not Classifiable as a Human Carcinogen.*"<sup>2/</sup> At the same time, the conclusions reached by the TERA peer reviewers clearly are inconsistent with the listing of soluble nickel compounds as "known human carcinogens."

There is no question that the extensive and complex database relating to the potential carcinogenicity of soluble nickel was analyzed far more carefully and in much greater depth during the two days of discussions by the TERA peer review panel than in the rather cursory discussion of nickel compounds that occurred at last December's Board of Scientific Counselors Subcommittee meeting -- where nearly a dozen different chemicals/chemical groups and manufacturing processes were considered in just two days. We submit that the conclusions reached by the TERA peer review panel have a much sounder scientific basis and reflect a far more careful and well-informed evaluation of the overall database than the rather peremptory recommendation regarding the carcinogenic classification of all nickel compounds that was made by the Board of Scientific Counselors Subcommittee at its December 1998 meeting.

We trust that in exercising their responsibilities as part of the RoC listing process, the NTP Executive Committee and the NTP Director will not simply "rubber-stamp" the Subcommittee's flawed recommendation. Instead, we urge the NTP Director and members of the Executive Committee to read our Comments carefully and with an open mind. If they do, we believe they will reach the same conclusion as the TERA peer reviewers and ACGIH and will recognize the need to differentiate among classes of nickel compounds in deciding which of them should be recommended for listing as "known human carcinogens" in the 9th RoC.

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<sup>2/</sup>

See 1998 TLVs® and BEIs® at 51, 79.

In closing, let me say that senior scientists from NiPERA would be happy to meet with the Executive Committee and the Director to discuss the underlying data and the important issues of scientific interpretation that must be considered in deciding which nickel compounds can appropriately be listed as "known human carcinogens." As noted above, we did not have an opportunity to engage in such a discussion at the Board of Scientific Counselors Subcommittee meeting last December. We hope it is not too late to hold such a discussion with the relevant scientific decision makers at this stage of the process. Please let me know if there is a convenient time for such a meeting.

Sincerely yours,

A handwritten signature in black ink, appearing to be "Sam", with a long horizontal stroke extending to the right.

Enclosures

cc: Dr. Kenneth Olden (with enclosures)

Dr. David Satcher, Assistant Secretary for Public Health, DHHS (with enclosures)

Harriet S. Raab, Esq., General Counsel, DHHS (with enclosures)

**Comments of the Nickel Producers Environmental Research  
Association on the National Toxicology Program Carcinogen  
Classification of Nickel and Nickel Compounds**

October 13, 1998

## 1. Executive Summary

The U.S. National Toxicology Program (NTP) is reviewing the database on the potential carcinogenicity of nickel and nickel compounds. In the NTP's Eighth Report on Carcinogens, *Nickel and Certain Nickel Compounds* (*i.e.*, not including water soluble nickel compounds) were listed as substances that are "reasonably anticipated to be a carcinogen". The new proposal for the Ninth Report on Carcinogens would list *Nickel and Nickel Compounds* as substances that are "known human carcinogens." NiPERA believes that this change would be scientifically unjustified and inappropriate.

NiPERA's major objection to the NTP's proposal to list *Nickel and Nickel Compounds* as "known human carcinogens" in the Ninth Biennial Report on Carcinogens is that it fails to recognize the **critical importance of speciation in evaluating the toxicity and potential carcinogenicity of the various forms of nickel**. Each compound or species of a metal, like nickel, has its own physico-chemical properties that dictate how it behaves under a given set of conditions, including interactions with biological organisms. Thus, the fact that one form of nickel may be carcinogenic via a particular route of exposure (*e.g.*, nickel subsulfide by inhalation) does not mean that a second nickel species will be carcinogenic as well or that the first nickel species will be carcinogenic via a different route of exposure (*e.g.*, ingestion). For nickel and its compounds, this observation holds true even if the free metal ion is assumed to be the active carcinogenic agent, because the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal ion can be made bioavailable and delivered to a relevant biological site (*e.g.*, the nucleus of a lung epithelial cell).

Examination of the *in vitro*, animal, and epidemiologic data pertaining to commercially relevant nickel compounds<sup>1</sup> confirms that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity. Nickel subsulfide is likely to be carcinogenic to humans. Soluble nickel compounds, by themselves, have not been demonstrated to be carcinogenic to humans, although an enhancing (promoter) effect on other carcinogens is possible. High concentrations of oxidic nickel mixtures (*i.e.*, Ni-Cu oxides mixed with low-temperature [black] and high-temperature [green] NiO) appear to be carcinogenic in epidemiologic studies of nickel refinery workers. Exposures to nickel silicates-oxides and complex nickel oxides devoid of copper have not resulted in excess cancer risks in other human cohorts. Exposure to metallic nickel particles in the workplace does not appear to pose a respiratory carcinogenic risk for humans. Finally, nickel carbonyl is so acutely toxic that it is used in closed systems and humans are typically exposed only in accident scenarios. The high acute toxicity of nickel carbonyl has limited its examination for carcinogenic effects. The human and animal data on the potential carcinogenicity of nickel carbonyl are scant and only non-standard animals studies with exposures above the Maximum Tolerated Dose (MTD) have yielded evidence of a carcinogenic effect.

Against this background, NiPERA believes that the NTP proposal to sweep metallic nickel and all nickel compounds into the single category of "known human carcinogens" is inconsistent with both the epidemiological and toxicological data and is at odds with the best current understanding of the likely mechanism of nickel-related carcinogenicity.

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<sup>1</sup> The classes of nickel compounds discussed in this paper are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide), water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, *etc.*), and nickel carbonyl. Metallic, oxidic, and sulfidic nickel compounds and nickel carbonyl are insoluble in water.

**Table of Contents**

|   | <u>PAGE</u> |
|---|-------------|
| 1. Executive Summary .....  | 2           |
| 2. Introduction .....   | 4           |
| 3. EXPERIMENTAL DATA .....  | 5           |
| 3.1. Epidemiologic Data .....   | 5           |
| 3.1.1. Sulfidic Nickel .....  | 5           |
| 3.1.2. Oxidic Nickel .....  | 6           |
| 3.1.3. Soluble Nickel .....   | 6           |
| 3.1.4. Metallic Nickel .....  | 7           |
| 3.1.5. Nickel Carbonyl .....  | 7           |
| 3.2. ANIMAL DATA .....  | 7           |
| 3.2.1. Nickel Subulfide .....   | 7           |
| 3.2.2. Oxidic Nickel .....  | 8           |
| 3.2.3. Soluble Nickel .....   | 9           |
| 3.2.4. Metallic Nickel .....  | 9           |
| 3.2.5. Nickel Carbonyl .....  | 9           |
| 3.3. In Vitro Studies .....   | 9           |
| 4. Mechanistic Model Related to the Carcinogenicity of Nickel Compounds ..... | 10          |
| 5. Carcinogenic Assessment of Individual Nickel Compounds .....               | 10          |
| 5.1. Sulfidic Nickel .....  | 10          |
| 5.2. Oxidic Nickel .....  | 11          |
| 5.3. Soluble Nickel .....   | 11          |
| 5.4. Metallic Nickel (elemental nickel and nickel alloys) .....               | 12          |
| 5.5. Nickel Carbonyl .....  | 13          |
| 6. Conclusions .....  | 13          |
| 7. References .....   | 14          |

## 2. Introduction

The U.S. National Toxicology Program (NTP) is reviewing the database on the potential carcinogenicity of nickel and nickel compounds. In the NTP's Eighth Report on Carcinogens, *Nickel and Certain Nickel Compounds* (*i.e.*, not including water soluble nickel compounds) were listed as substances that are "reasonably anticipated to be a carcinogen". The new proposal for the Ninth Report on Carcinogens would list *Nickel and Nickel Compounds* as substances that are "known human carcinogens." NIPERA believes that this change would be scientifically unjustified and inappropriate.

NIPERA's major objection to the NTP's proposal to list *Nickel and Nickel Compounds* as "known human carcinogens" in the Ninth Biennial Report on Carcinogens is that it fails to recognize the **critical importance of speciation in evaluating the toxicity and potential carcinogenicity of the various forms of nickel**. Each compound or species of a metal, like nickel, has its own physico-chemical properties that dictate how it behaves under a given set of conditions, including interactions with biological organisms. Thus, the fact that one form of nickel may be carcinogenic via a particular route of exposure (*e.g.*, nickel subsulfide by inhalation) does not mean that a second nickel species will be carcinogenic as well or that the first nickel species will be carcinogenic via a different route of exposure (*e.g.*, ingestion). For nickel and its compounds, this observation holds true even if the free metal ion is assumed to be the active carcinogenic agent, because the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal ion can be made bioavailable and delivered to a relevant biological site (*e.g.*, the nucleus of a lung epithelial cell).

Historically, inhalation exposure to very high concentrations of certain nickel compounds in the nickel producing industry has been associated with an excess of respiratory cancer. It should be noted that only respiratory tumors have been consistently associated with these exposures and solely by the inhalation route of exposure. To understand the risks associated with exposures to nickel compounds, consideration should be given to the respiratory carcinogenic potential of the individual nickel species and the influence of particle size and mixed exposures. Examination of the *in vitro*, animal, and epidemiologic data pertaining to the four commercially relevant classes of nickel compounds<sup>2</sup> confirms that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity. Nickel subsulfide is likely to be carcinogenic to humans. Soluble nickel compounds, by themselves, have not been demonstrated to be carcinogenic to humans, although an enhancing (promoter) effect on other carcinogens is possible. High concentrations of oxidic nickel mixtures (*i.e.*, Ni-Cu oxides mixed with low-temperature [black] and high-temperature [green] NiO) appear to be carcinogenic in epidemiologic studies of nickel refinery workers. Exposures to nickel silicates-oxides and complex nickel oxides devoid of copper have not resulted in excess cancer risks in other human cohorts. Exposure to metallic nickel particles in the workplace does not appear to pose a respiratory carcinogenic risk for humans. Finally, nickel carbonyl is so acutely toxic that it is used in closed systems and humans are typically exposed only in accident scenarios. The high acute toxicity of nickel carbonyl has limited its examination for carcinogenic effects. The human and animal data on the potential carcinogenicity of nickel carbonyl are scant and only non-standard animals studies with exposures above the Maximum Tolerated Dose (MTD) have yielded evidence of a carcinogenic effect.

A brief review of the epidemiologic, animal and *in vitro* data pertinent to the understanding of the inhalation carcinogenicity of nickel and its compounds is presented in this report. Based on these data, a possible mechanistic model for the carcinogenicity of nickel compounds is discussed<sup>(1)</sup>. The carcinogenic potentials of sulfidic nickel (*e.g.*, nickel subsulfide), oxidic nickel compounds (with particular emphasis on high temperature [green] nickel oxide), soluble nickel compounds (*e.g.*, nickel sulfate hexahydrate),

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<sup>2</sup> The four classes of nickel compounds discussed in this paper are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide) and water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, etc.). Metallic, oxidic and sulfidic nickel compounds are insoluble in water.

metallic nickel, and to a lesser extent nickel carbonyl, are considered within the framework provided by this model.

Against this background, NIPERA believes that the NTP proposal to sweep metallic nickel and all nickel compounds into the single category of "*known human carcinogens*" is inconsistent with both the epidemiological and toxicological data and is at odds with the best current understanding of the likely mechanism of nickel-related carcinogenicity.

### **3. EXPERIMENTAL DATA**

#### **3.1. EPIDEMIOLOGIC DATA**

Epidemiologic data from nickel workers are difficult to interpret because of mixed exposures to not only different nickel compounds but also to other inorganic compounds (arsenic, cobalt, strong acid mists) and to organic combustion products<sup>(2)</sup>. In addition, exposure measurements are sparse, very little chemical speciation and particle size information is available, and the confounding effects of cigarette smoking on respiratory cancers have not been adequately studied. Nevertheless, with the continued acquisition of new epidemiologic data, a clearer picture is emerging with respect to the likely role that different nickel species play in human respiratory carcinogenesis. In later sections, it will be noted that this picture is largely in agreement with what is known about these compounds from animal and *in vitro* studies.

Studies of past exposures and cancer mortality reveal that only respiratory tumors have been consistently associated with inhalation exposure to certain nickel compounds. Data from ten different cohorts were presented in the report of the International Committee on Nickel Carcinogenesis in Man (ICNCM)<sup>(2)</sup>. These cohorts included approximately 80,000 workers involved in nickel operations (mostly mining, smelting, and refining, but some nickel alloy production and miscellaneous applications as well) located in the United States, Canada, England, Wales, Norway, Finland and New Caledonia.

Of the examined workers, less than 10% had clear excess respiratory cancer risks. The excess risks were confined to workers in certain types of refining operations. Only slightly elevated risks of respiratory cancer were seen in some (but not all) smelting and mining workers; these appeared to be attributable to other causes<sup>(3,4)</sup>. There was no evidence of risk for workers in the manufacturing of barrier material for gaseous diffusion (uranium enrichment process), nor in workers involved in alloy production. An additional 50,000 workers in nickel-using industries and applications (stainless steel and nickel alloy production, welding, and plating) have given no evidence of excess respiratory cancer risks from exposures to metallic and/or complex nickel oxides largely free of copper<sup>(5-9)</sup>.

Thus, of the large number of nickel-exposed workers comprising a variety of occupations, only a small proportion have shown excess respiratory cancer risks. Nickel-related cancer risks appear to have been confined to certain types of refining operations, most of which are no longer in existence today. No nickel-related excess respiratory cancer risks have been found in any nickel-using industry workers.

##### **3.1.1. Sulfidic Nickel**

The ICNCM report<sup>(2)</sup> concluded that much of the excess respiratory cancer risk in workers involved in certain types of nickel refining operations appeared to be associated with exposure to a mixture of sulfidic and oxidic nickel compounds at high concentrations ( $\geq 10$  mg Ni/m<sup>3</sup>). In the case of sulfidic nickel, both lung and nasal cancers were associated with exposure to this nickel compound in Canadian sinter plant workers. In refinery workers in Clydach, Wales excess lung cancers were associated with high cumulative exposures to sulfidic nickel and low-level exposures to other nickel compounds. It should be noted that the risks of developing respiratory cancers in this cohort dramatically dropped after 1930 despite the continued presence of some high levels of sulfidic nickel into the late 1930s, suggesting that other factors (*e.g.*, possible presence of arsenic) could have contributed to the cancer risks seen in these workers. However, clear evidence of respiratory carcinogenicity in animals administered nickel subsulfide (see

below) indicates that the association of exposures to sulfidic nickel and lung and nasal cancer in humans is likely to be causal.

### **3.1.2. Oxidic Nickel**

With respect to oxidic nickel, excess lung and nasal cancers reported in refinery workers in Clydach and in Kristiansand, Norway who were exposed to high concentrations of oxidic nickel (mainly as nickel copper oxides, but with the possible presence of both high-temperature and low-temperature NiO as well), strongly suggests that these forms of oxidic nickel are likely human respiratory carcinogens<sup>3</sup>. Conversely, in nickel-using industry workers exposed to metallic nickel and/or complex nickel oxides free of copper, with no exposure to sulfidic nickel, there have been no nickel-related excess risks of respiratory cancer. Likewise, nickel production workers involved in the mining and smelting of lateritic ores have shown no nickel-related excess respiratory cancer risks. The oxidic nickel to which these workers were exposed would have mainly been nickel silicates-oxides and complex nickel oxides devoid of copper. It should be mentioned that oxidic nickel exposures in the latter groups were considerably lower than those experienced by workers in certain types of nickel refining operations. It is uncertain, therefore, whether the lack of increased respiratory cancer risk in these workers was due to the low concentrations of oxidic nickel to which they were exposed and/or to the physicochemical properties (including particle size) of the particular oxidic nickel compounds present.

### **3.1.3. Soluble Nickel**

The role of soluble nickel in respiratory carcinogenesis is less evident than that of sulfidic and certain oxidic nickel compounds. Comparisons of electrolysis workers at Port Colborne, Canada and Kristiansand, Norway reveal that only Kristiansand workers had excess lung cancers. Because of differences in processes, the Kristiansand workers were thought to be exposed to slightly higher levels of soluble nickel and also to handle approximately seven times more insoluble nickel (per unit of soluble nickel) than those at Port Colborne. In addition, basic nickel carbonate (water insoluble) was included in the soluble compounds category at Kristiansand, whereas it was classified as insoluble at Port Colborne. While the amounts involved were not large, they would have exaggerated the differences in exposure to soluble compounds between the two operations. In another cohort of hydro-metallurgical workers at Clydach that had high cumulative exposure to soluble forms of nickel but low exposures to oxidic and sulfidic forms of nickel, there was no evidence of increased risks of respiratory cancer. From these studies, the ICNCM Report concluded that, while there was evidence that soluble nickel exposure ( $\geq 1 \text{ mg Ni/m}^3$ ) could increase the risk of respiratory cancers, the effect might be one of enhancing risks associated with co-exposure to less soluble forms of nickel or other non-nickel compounds.

Recent studies have provided supportive evidence for the possible role of soluble nickel as a promoter of carcinogenicity. In particular, in a recent study of the Kristiansand cohort that has updated cancer morbidity, newly available information on the smoking characteristics of the workers has been included<sup>(10)</sup>. A synergistic lung cancer response between smoking and exposure to a mixture of soluble and insoluble nickel compounds was observed. In the small number of nickel-exposed workers who did not smoke, there was no evidence that nickel exposure increased the risk for lung cancer. A similar lack of excess respiratory cancers was noted in a 1996 cancer mortality study in a relatively small population of nickel platers exposed solely to nickel chloride and sulfate mists<sup>(9)</sup>. The results from these two studies are consistent with those of the ICNCM Report.

In a 1998 study of Finnish refinery workers exposed predominantly to soluble nickel three nasal cancer cases were identified and a 2-fold increase in lung cancer risk was found in nickel workers with more than

<sup>3</sup> It should be noted that these workers were also exposed to various levels of metallic, sulfidic and/or soluble nickel compounds, since no workplace in the producing industry had "pure" exposure to any individual nickel compound.

20 years employment<sup>(11)</sup>. Unfortunately, smoking data are unavailable for these workers. As indicated in the above study on Norwegian electrolysis workers, such data would be helpful in interpreting the significance of the lung cancers seen in these workers. In the case of the observed nasal cancers, even though the Finnish workers were predominantly exposed to soluble nickel during their employment at the refinery, their previous job experiences, as well as concomitant exposures to insoluble nickel compounds and acid mists, make the establishment of a causal association with soluble nickel compounds difficult.

Taken together, the epidemiologic results from all the above studies are most consistent with soluble nickel compounds enhancing, rather than initiating, cancer. The animal data on soluble nickel compounds strongly support this interpretation (see next section).

#### **3.1.4. Metallic Nickel**

The ICNCM Report found no evidence that exposure to metallic nickel in industrial plants increased respiratory cancer risk. The lack of excess respiratory cancer risks in workers at a gaseous diffusion barrier manufacturing plant was particularly notable as these workers were exposed solely to metallic nickel. Likewise, in a recent update of a study on 715 hydrometallurgical workers in Canada, no excess lung or nasal cancers was reported<sup>(12)</sup>. Although the size of the cohort was small, exposures in this plant were solely to nickel concentrates and metallic nickel. In a recent study of nickel alloy workers, Redmond and coworkers updated the cancer mortality data from more than 30,000 people employed in 13 nickel alloy plants in the U.S.A. Exposures were primarily to metallic nickel and complex nickel oxides devoid of copper. No excess mortality rates were observed for respiratory cancers in these workers when compared to local population rates<sup>(5,6)</sup>. Examination of the available data shows that, even in the past, exposures to metallic nickel have generally been low ( $\leq 1 \text{ mg Ni/m}^3$ ) compared to exposures to other nickel compounds found in certain types of nickel refining operations. The overwhelming lack of epidemiologic carcinogenic evidence for metallic nickel could be due to the combination of low-dose exposures, the particle size of the metallic nickel found in the workplace, and the limited bioavailability of the nickel ion from nickel metal itself. It is clear then, that under past and current industrial practices, exposure to metallic nickel does not pose a respiratory carcinogenic risk for humans.

#### **3.1.5. Nickel Carbonyl**

The severe acute toxicity effects of nickel carbonyl have been recognized for decades. It is because of this acute toxicity that short-term exposure limits are usually set. The only human study investigating the possible health effects of nickel carbonyl involved the examination of causes of death in 69 men who worked at Clydach, Wales from 1933 to 1966<sup>(13)</sup>. Their SMR for lung cancer was 152 and was not considered to be statistically significant. The presence of other confounding exposures at Clydach was not considered in this study.

### **3.2. ANIMAL DATA**

Animal data are often useful in helping to elucidate mechanisms of carcinogenesis. As noted above, this is particularly true in the case of nickel and its compounds where the animal data are in good agreement with the human lung carcinogenicity data. The ICNCM Report, recognizing the limitations of human studies involving mixed exposures, pointed out the importance of the results of animal carcinogenesis studies (using inhalation as the route of exposure) to help understand the human health risks associated with individual nickel compounds. It should be noted that under the conditions used in the studies, none of the rodent species showed evidence of nasal tumors after inhalation exposure to any one of the nickel compounds tested. The animal data are reviewed herein.

#### **3.2.1. Nickel Subsulfide**

In a 1974 study, inhalation of nickel subsulfide ( $\text{Ni}_3\text{S}_2$ ) resulted in the induction of lung tumors in rats<sup>(14)</sup>. The U.S. National Toxicology Program (NTP) recently completed two-year inhalation cancer bioassays in rats and mice with three nickel compounds, including nickel subsulfide<sup>(15,16)</sup>. In the nickel subsulfide study, rats were exposed to 0, 0.1 or 0.7 mg  $\text{Ni}/\text{m}^3$ ; mice were exposed to 0, 0.4, or 0.8 mg  $\text{Ni}/\text{m}^3$ . After two years exposure, there was clear evidence of carcinogenic activity in male and female rats, with a dose-dependent increase in lung tumor response. No evidence of carcinogenic activity was detected in male or female mice. No nasal tumors were detected in rats or mice, but various non-malignant lung effects were seen.

### 3.2.2. Oxidic Nickel

In the case of oxidic nickel, few properly designed chronic inhalation studies had been performed prior to the NTP studies<sup>(15,17)</sup>. The first inhalation studies that were carried out on hamsters and rats with different nickel oxides were either negative or inconclusive due to high mortality at toxic concentrations<sup>(18-23)</sup>.

In the recently completed NTP study<sup>(15,17)</sup>, rats were exposed to high temperature, green NiO (calcined at 1,350 °C) at concentrations of 0, 0.5, 1.0, or 2.0 mg  $\text{Ni}/\text{m}^3$ . After two years, no increased incidence of tumors was observed at the lowest exposure level in rats. At the intermediate and high concentrations, 12 out of 106 rats and 9 out of 106 rats, respectively, presented with either adenomas or carcinomas. These numbers were not statistically different from those seen both in the control and low dose groups, but were statistically significant compared to historical controls (cancer incidence in ~200 control rats per sex used in previous NTP studies). Therefore, the NTP concluded that there was some evidence of carcinogenic activity in rats. NTP also found equivocal evidence of carcinogenicity in female mice based on excess tumors found in animals exposed to 1 but not 2 or 4 mg  $\text{Ni}/\text{m}^3$ . Other findings in rodents included inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes. No nasal tumors were observed in rats or mice.

Two clearance studies of high temperature, green NiO particles from the respiratory tract of rats and mice showed impaired clearance of NiO after two to six months exposure to the same concentrations used in the NTP studies<sup>(24-26)</sup>. These results indicate that impairment of lung clearance was likely present in the NTP rats at the concentrations at which tumors were found.

It has been shown in rats that prolonged exposures to high concentrations of particles of low toxicity can result in lung tumors independent of the composition of the particles<sup>(27,28)</sup>. The mechanism for tumor induction involves an impairment of lung clearance that leads to chronic inflammation. Chronic inflammation can result in enhanced cell proliferation, and, indirectly, in increases in mutations through the action of oxygen radicals produced by the activated inflammatory cells<sup>(29-32)</sup>. The tumors found in rats exposed to high concentrations of high temperature green nickel oxide in the NTP studies could have been the result of the indirect particle effect described above, rather than resulting from the direct genotoxic effects of  $\text{Ni}^{2+}$ . It is uncertain at present, whether the induction of tumors secondary to a particle effect observed in rats could occur in humans, since no excess tumor incidence has been observed in workers exposed to very high concentrations of low solubility and low toxicity dusts<sup>(33)</sup>.

In evaluating the carcinogenicity of oxidic nickel compounds, it is important to consider the concentration and physicochemical characteristics of the particles (including particle size). The physicochemical characteristics of oxidic nickel produced by different processes as well as the presence of other metals (*e.g.*, Ni-Cu oxides) may result in different biological activities. For example, a nickel oxide produced at lower calcining temperatures than the green NiO may have increased solubility resulting, perhaps, in enhanced toxicity as well as clearance. Depending on the balance of these effects, the ultimate result may be an increase or decrease in the respiratory carcinogenic potential of the various oxidic nickel compounds relative to high temperature, green nickel oxide.

### 3.2.3. Soluble Nickel

No inhalation studies with soluble nickel compounds had been conducted prior to the NTP studies. Soluble nickel compounds gave consistent negative results by oral<sup>(34-37)</sup> and intramuscular<sup>(38-41)</sup> routes of exposure; only intraperitoneal injection studies gave positive results with nickel acetate<sup>(42-45)</sup>. Injection studies are not appropriate to evaluate hazards for predicting human risk from inhalation exposures. This is due to the fact that injection bypasses natural protective mechanisms and causes unrealistically high spikes of exposure to occur in various organ systems.

In the recently conducted NTP inhalation study<sup>(16,46)</sup>, rats were exposed to NiSO<sub>4</sub>·6H<sub>2</sub>O at concentrations up to 0.11 mg Ni/m<sup>3</sup>; mice were exposed to up to 0.22 mg Ni/m<sup>3</sup>. These concentrations were chosen based on the toxicity observed in the 13-week studies and corresponded to the maximum tolerated doses (MTD). After two years of continuous exposure, there was no evidence of lung or nasal carcinogenic activity in mice or rats. Various combinations of non-carcinogenic lung effects were seen in both sexes in rats and mice. Overall, the non-carcinogenic effects were similar to those seen with the other two nickel compounds.

### 3.2.4. Metallic Nickel

A limited number of animal inhalation studies with elemental nickel powder have not indicated carcinogenicity in rats or hamsters<sup>(47)</sup>. In one intratracheal instillation study, 9 mg Ni/rat of nickel powder (unspecified particle size) produced malignant lung tumors in rats<sup>(48)</sup>. However, the relevance of such a route of administration for humans is highly questionable, given that the lung burden by intratracheal instillation is massive, potentially overloading lung clearance mechanisms and affecting the animal's ability to eliminate the material. Intratracheal instillation of 10 mg nickel powder did not induce tumors in hamsters<sup>(49)</sup>.

### 3.2.5. Nickel Carbonyl

Published studies on the carcinogenicity of nickel carbonyl were all performed prior to present day standardized testing protocols and because of the extreme acute toxicity of this material, more recent studies have not been conducted.

Sunderman and co-workers<sup>(50)</sup> exposed 64 male rats to 30 mg/m<sup>3</sup> and 32 male rats to 60 mg/m<sup>3</sup> of nickel carbonyl vapor three times a week for one year. Only 20 test animals survived the study. In a second study 80 rats were exposed to a single dose of 250 mg/m<sup>3</sup> and observed for effects for two years. Of the 176 animals exposed to nickel carbonyl in these two experiments, only 9 survived for two years, and of these, 4 had tumors.

In later studies, Sunderman and Donnelly<sup>(51)</sup> exposed 285 male rats to 600 mg/m<sup>3</sup> of nickel carbonyl for 30 minutes. Only 71 rats survived for longer than 3 weeks, with roughly equivalent numbers of tumors found in both the exposed and the control animals. The experiments of Sunderman and coworkers are the only animal studies linking nickel carbonyl to respiratory cancer. Thus the high rate of early mortality, the fact that in some studies the controls also developed tumors, as well as the possible secondary effects of acute nickel carbonyl poisoning preclude definitive evaluation of carcinogenicity.

## 3.3. IN VITRO STUDIES

*In vitro*, the Ni<sup>2+</sup> ion does not behave like a typical mutagen; it does not show high affinity for DNA and lacks mutagenicity in most bacterial and mammalian assays<sup>(52-59)</sup>. Only chromosomal aberration assays (indicative of chromosomal damage) have been positive with nickel compounds *in vitro*<sup>(60-67)</sup> and *in vivo*<sup>(68-73)</sup>.

However, *in vitro* cell transformation assays have been positive with soluble and insoluble nickel compounds<sup>(54,55,74-77)</sup>. It was shown that endocytosis by target cells was likely to play an important role in the *in vivo* transforming potential of nickel compounds<sup>(77)</sup>. Endocytized particles release Ni<sup>2+</sup> ions and are transported to the nuclear membrane where the endocytic vesicles deliver Ni<sup>2+</sup> ions in close proximity to the chromosomes<sup>(78)</sup>. Some of the characteristics of insoluble nickel compounds that increase their ability to be endocytized include: crystalline nature, negative surface charge, 2-4 µm range particle size, and low solubility in biological fluids<sup>(79-81)</sup>. Even though water soluble nickel compounds are not endocytized, they are positive in *in vitro* transformation assays due to the persistent high concentration of Ni<sup>2+</sup> ions that can be achieved in the cell culture medium. The high nickel gradient in the medium allows Ni<sup>2+</sup> ions to concentrate at the nuclear target sites. However, *in vivo*, Ni<sup>2+</sup> ions from soluble compounds are unlikely to be bioavailable due to their rapid clearance from the lung and excretion in urine (t<sub>1/2</sub> in rats of ~ 2-3 days<sup>(24)</sup>).

The Ni<sup>2+</sup> ion present at nuclear sites has been shown *in vitro* to bind to proteins within heterochromatin regions of DNA<sup>(82-84)</sup>. This binding may enhance DNA condensation and methylation in nearby regions<sup>(85-87)</sup> and may result in nickel-mediated induction of oxidative DNA damage<sup>(44,82, 88-92)</sup>. These actions could have similar effects on senescence or tumor suppressor genes; the former, by diminishing gene expression, the latter by resulting in deletion of these genes<sup>(86,87,93-96)</sup>.

#### **4. Mechanistic Model Related to the Carcinogenicity of Nickel Compounds**

There are two components that may contribute to the development of lung tumors by certain nickel compounds<sup>(1)</sup>: (1) heritable changes in gene expression and (2) cell proliferation. Heritable changes in gene expression can be the result of: (i) genetic changes such as mutations (changes in DNA sequence) or chromosomal aberrations (changes at the chromosome level), and (ii) epigenetic changes that affect gene expression without altering DNA sequences. Nickel compounds have not been shown to directly induce mutations, but some nickel compounds are able to cause heritable chromosomal aberrations and epigenetic changes through methylation. These direct effects could be specific for Ni<sup>2+</sup> ions and dependent on its bioavailability (the delivery of the nickel ion to sites within the nuclei of the target cells). In addition, some nickel compounds may have indirect effects as a consequence of an inflammatory response. The indirect effects could be attributed to DNA damage caused by oxygen radicals and are not specific to nickel.

Cell proliferation is required to convert DNA lesions into mutations and is also involved in clonal expansion of the initiated cell population, a factor that increases the probability of occurrence of a second mutating event. Only sustained increases in cell proliferation, as seen in chronic exposures, are likely to be significant in carcinogenesis<sup>(97)</sup>. Expression of pro-inflammatory cytokines was found to be increased in lungs of rats and mice after subchronic exposure to nickel subsulfide<sup>(26)</sup>. Cell proliferative responses in alveolar epithelial cells from rats and mice exposed to high temperature green NiO, paralleled the inflammatory responses<sup>(26)</sup>. Collectively, these studies suggest that some nickel compounds can stimulate cell proliferation *in vivo*. Again, this effect may not be specific for nickel compounds, and it could be similar for other substances that can induce proliferative responses. Both components: heritable changes in gene expression and cell proliferation, are needed for tumor development.

### **5. Carcinogenic Assessment of Individual Nickel Compounds**

#### **5.1. SULFIDIC NICKEL**

The human data provide evidence of an association of excess respiratory cancer risk with inhalation of aerosols containing high concentrations of sulfidic nickel (>10 mg Ni/m<sup>3</sup>). Positive animal carcinogenicity results from inhalation exposure to nickel subsulfide have been found in rats (with evidence of dose-response).

Nickel subsulfide particles need to be oxidized to release  $\text{Ni}^{2+}$  ions. Nickel subsulfide is quite insoluble in water, but shows enhanced release of  $\text{Ni}^{2+}$  ions in biological fluids. *In vivo*, since nickel subsulfide is likely to be readily endocytized by the target cells, this compound is likely to affect both components of the carcinogenic process (induction of heritable changes and increases in cell proliferation). Because of its enhanced "solubility" in biological fluids, efficient delivery of  $\text{Ni}^{2+}$  ions to the target site within the cell nucleus is likely. The release of  $\text{Ni}^{2+}$  ions on the alveolar surface can result in cell toxicity and directly induce inflammation and proliferation of initiated cells.

With regard to carcinogenicity assessment, an NTP category of "*known human carcinogen*" seems appropriate. Because nickel subsulfide may efficiently affect both components of the carcinogenic process, this compound appears to present the highest respiratory carcinogenic potential relative to other nickel compounds.

## 5.2. OXIDIC NICKEL

Historical human data indicate that inhalation exposure to high concentrations ( $>10 \text{ mg Ni/m}^3$ ) of oxidic nickel (consisting of Ni-Cu oxides mixed with low-temperature (black) and high-temperature (green) NiO), was associated with respiratory carcinogenicity. Conversely, exposures to approximately  $\leq 1 \text{ mg Ni/m}^3$  of silicate oxides and complex Ni oxides (largely free of Cu) did not result in any nickel-related respiratory cancer risk.

The animal data suggest that high-temperature green NiO is a weakly positive carcinogen in rats by inhalation, with negative or equivocal evidence of carcinogenicity in mice. It is possible that the lung tumors seen in rats exposed to green NiO may have been generated by an inflammatory/proliferative response that results from the impaired function and chronic activation of macrophages, rather than by a direct heritable effect of  $\text{Ni}^{2+}$  ions. At present, it is not known if this high-concentration, low-solubility particle effect can occur in humans. Compared to nickel subsulfide, high temperature, green NiO appears to pose a lower risk for respiratory carcinogenicity. The relative carcinogenic potential of other nickel oxides (NiO) and complex oxides will likely depend on their concentration, solubility and ease of phagocytosis/endocytosis.

With regard to carcinogenicity assessment, it seems appropriate to draw a distinction between two groups of nickel oxides. An NTP category of "*known human carcinogen*" seems appropriate for Ni-Cu oxides as well as high and low temperature NiO found in nickel refineries; while silicate oxides and complex nickel oxides (devoid of copper) found in nickel using industries can best be classified as "*reasonably anticipated to be a carcinogen*."

## 5.3. SOLUBLE NICKEL

The human evidence does not establish that soluble Ni compounds by themselves act as complete respiratory carcinogens. In the ICNCM Report <sup>(2)</sup>, there were no cohorts where exposure was solely to soluble nickel compounds. While the Report concluded that exposures to soluble nickel compounds (predominantly in excess of  $1 \text{ mg Ni/m}^3$ ) were associated with excess respiratory cancers, the authors suggested the possibility that the role of soluble nickel may be one of enhancement, since the evidence for soluble nickel compounds being carcinogenic was inconsistent across cohorts. The recent negative rodent NTP inhalation studies of nickel sulfate hexahydrate appear to confirm that soluble nickel compounds, by themselves, are not likely to cause respiratory tumors.

The relevance of the animal data for human extrapolation has been questioned on the grounds that the highest concentration to which rats were exposed was  $0.1 \text{ mg Ni/m}^3$  while workers in some of the cohorts studied by the ICNCM experienced soluble nickel exposures  $\geq 1 \text{ mg Ni/m}^3$ . It should be noted that the aerosol used in the NTP studies (mist) had an average size of  $2\text{-}3 \mu\text{m}$  while the particle size of the aerosols in the workplace has a much larger distribution with aerosols of  $2\text{-}3 \mu\text{m}$  comprising less than 5%

of the total. Preliminary results from an animal to human extrapolation study based on deposition/clearance models for rat and human lungs, indicate that after accounting for particle size distribution, the exposures experienced by the rats in the NTP studies appear equivalent (in terms of nickel lung burden) to those experienced by workers in the epidemiologic nickel refinery studies <sup>(98)</sup>.

In the lung, soluble nickel compounds are not endocytized; rather, they dissociate to release nickel ions and are rapidly cleared from the lungs.  $\text{Ni}^{2+}$  ions may cross the cell membrane using the  $\text{Mg}^{2+}$  ion transport system, as has been seen in microorganisms <sup>(99)</sup>. Because  $\text{Mg}^{2+}$  ions are present in cells at mM levels, high concentrations of  $\text{Ni}^{2+}$  ions are needed to compete with  $\text{Mg}^{2+}$  ions for their uptake <sup>(100)</sup>. It is possible to speculate that if the dose were sufficiently high (as happens in *in vitro* assays) enough  $\text{Ni}^{2+}$  ions could reach the nucleus to have an effect. This is unlikely *in vivo* since the toxic effects of soluble nickel compounds <sup>(46)</sup> would be evident long before a sufficiently high concentration of  $\text{Ni}^{2+}$  ions in the nucleus could be achieved.

The solubility of nickel sulfate hexahydrate in biological fluids results in release of  $\text{Ni}^{2+}$  ions at the bronchioalveolar surface, causing cell toxicity and some inflammation. Proliferation rates are enhanced, but given that the background (spontaneous) number of initiated cells is presumed to be very low, and many of these cells could be killed by the toxic effects of  $\text{Ni}^{2+}$  ions, no tumors are expected to develop. Because soluble nickel compounds may stimulate cell proliferation (the second component of the cancer process), they may act as enhancers of other compounds that are able to induce heritable changes. Furthermore, the presence of soluble nickel compounds could adversely affect the macrophage-mediated clearance of more insoluble nickel compounds <sup>(79)</sup>.

With regard to carcinogenicity assessment, because soluble nickel compounds (such as hydrated nickel sulfate and nickel chloride) do not appear to be carcinogenic by themselves, they should not be listed as either "*known*" or "*reasonably anticipated to be a carcinogen*." Because soluble compounds may affect only one of the components of the carcinogenic process (cell proliferation), they present negligible risk of carcinogenicity acting alone.

#### **5.4. METALLIC NICKEL (ELEMENTAL NICKEL AND NICKEL ALLOYS)**

Epidemiologic studies have not shown an association between the relatively low ( $\leq 1 \text{ mg Ni/m}^3$ ) metallic nickel exposure found in industrial settings and respiratory carcinogenesis. Animal evidence regarding the potential carcinogenicity of metallic nickel by a relevant route of exposure is limited but suggests the absence of respiratory carcinogenic risk.

For metallic nickel, as for nickel subsulfide, the release of  $\text{Ni}^{2+}$  ion is not based on solubility. Rather, deposited or endocytized particles need to be oxidized to release  $\text{Ni}^{2+}$  ions. The particle size and the presence of oxidants in the lung surface and inside the cells could influence the kinetics of this reaction. Small size particles are expected to have a higher release of  $\text{Ni}^{2+}$  ion resulting in greater toxicity but faster lung and renal clearance than larger particles.

In addition, the presence of other metals in Ni-containing alloys may increase or decrease the rates of oxidation and release of  $\text{Ni}^{2+}$  ions. Therefore, more research is needed to determine the relative rates of nickel corrosion from elemental nickel and individual alloys under biologically relevant conditions.

With regard to carcinogenicity assessment, no NTP classification is justified. Past and current exposures to metallic nickel particles in occupational settings do not appear to pose a respiratory cancer risk for humans. Thus, rather than elevating it to the "*known*" category, NTP should remove metallic nickel from the list of substances that are "*reasonably anticipated to be a carcinogen*."

### 5.5. NICKEL CARBONYL

Exposure to nickel carbonyl can result in severe acute respiratory damage. The extreme acute toxicity of this compound has resulted in its use in closed circuit applications that limit human contact with nickel carbonyl to accidental exposures. Therefore, there is a paucity of either human or animal data on the potential effects of chronic exposure to nickel carbonyl. Review of the limited information that is available demonstrates an absence of human evidence for the carcinogenicity of nickel carbonyl. The equivocal evidence of a carcinogenic effect of nickel carbonyl comes from animal studies where exposures clearly exceeded the Maximum Tolerated Dose (MTD). Therefore, NTP should not categorize nickel carbonyl as either a "known human carcinogen" or a compound that is "reasonably anticipated to be a carcinogen."

## 6. Conclusions

Examination of the *in vitro*, animal and epidemiologic data indicates that **speciation is of paramount importance for assessing the respiratory carcinogenicity of individual nickel species**. The concentration as well as the ability of nickel compounds to be phagocytized/endocytized and their *in vivo* solubility may be the most important factors in determining the bioavailability of Ni<sup>2+</sup> ions at target sites in the nucleus of respiratory tract cells, and hence, the respiratory carcinogenic potential of these compounds.

The nickel species discussed in this paper have very different biological behaviors. With regard to carcinogenicity assessment:

- Sulfidic nickel (including nickel subsulfide) could appropriately be included by NTP in the "known human carcinogen" category. Because nickel subsulfide may efficiently affect both components of the carcinogenic process, this compound appears to present the highest respiratory carcinogenic potential relative to other nickel compounds.
- A distinction should be drawn between two groups of nickel oxides. An NTP category of "known human carcinogen" seems appropriate for the mixtures of Ni-Cu oxides, high-temperature NiO, and low-temperature NiO found in nickel refineries; while silicate oxides and complex nickel oxides (devoid of copper) found in nickel using industries can best be classified as "reasonably anticipated to be a carcinogen."

When low-temperature (black) or high-temperature (green) NiO are not mixed with Ni-Cu oxides (as they were in the nickel refineries) their carcinogenic potential is less clear. High-temperature green NiO is a weakly positive carcinogen in rats by inhalation. It is possible that the lung tumors seen in rats exposed to this compound were generated as a consequence of a particle effect, rather than by a direct heritable effect of Ni<sup>2+</sup> ions. At present, it is not known if this high-concentration, low-solubility particle effect can occur in humans. The carcinogenic potential of other individual nickel oxides may depend on their concentration, manufacturing history, and solubility.

- Soluble nickel compounds (such as hydrated nickel sulfate and nickel chloride) should not be listed as either "known" or "reasonably anticipated to be a carcinogen." Because soluble nickel compounds may affect only one component of the carcinogenic process (cell proliferation), they present a negligible risk of carcinogenicity acting alone.
- For metallic nickel, no NTP classification is justified. Past and current exposures to metallic nickel particles in occupational settings have shown no respiratory cancer risk for humans.
- An NTP category of "known human carcinogen" for nickel carbonyl is totally unjustified. This is based on the absence of human evidence for the carcinogenicity of nickel carbonyl and the limited

animal carcinogenicity studies. These studies caused high mortality in all the exposure groups (clearly exceeding the Maximum Tolerated Dose) resulting in an equivocal carcinogenic effect.

For the reasons set forth above, *Nickel and Nickel Compounds* should not be listed as a "known human carcinogen" in the NTP Ninth Biennial Report on Carcinogens. Instead, NTP should make species-specific carcinogen determinations for the various forms of nickel, as suggested above.

## 7. References

1. Oller, A. R., Costa, M., and Oberdörster, G. (1997). Carcinogenicity assessment of selected nickel compounds. *Toxicol. Appl. Pharmacol.*, 143, 152-166.
2. ICNCM Report. (1990). Report of the International Committee on Nickel Carcinogenesis in Man. *Scand. J. Work Environ. Health* 16(1), 1-82.
3. Verma, D. K., Julian, J. A., Roberts, R. S., Muir, D. C. F., Jadon, N., and Shaw, D. S. (1991). Polycyclic aromatic hydrocarbons (PAHs): A possible cause of lung cancer mortality among nickel/copper smelter and refinery workers. *Am. Ind. Hyg. Assoc. J.*, 7, 277-294.
4. Muller, J., Wheeler, W. C., Gentleman, J. F., Suranyi, G., and Kusiak, R. A. (1983). Study of the mortality of Ontario miner, 1955-1977: Part 1. Toronto, Canada: Atomic energy Control Board of Canada, Ontario Workmen's Compensation Board, Ontario Ministry of Labour.
5. Redmond, C. K., Arena, V. C., Costantino, J. P., Trauth, J. M., Bass, G., and LeGasse, A. A. (1994). High nickel alloys workers study update. University of Pittsburgh. Final report to NIPERA.
6. Redmond, C. K., Sussman, N. B., Arena, V. C., and Costantino, J. P. (1996). Supplemental analysis of high nickel alloys workers. University of Pittsburgh. Final report to NIPERA.
7. Simonato, L., Fletcher, A. C., Andersen, A., Andersen, K., Becker, N., Chang-Claude, J., Ferro, G., Gérin, M., Gray, C. N., Hansen, K. S., Kalliomaäki, P.-L., Kurppa, K., Långard, S., Meriö, F., Moulin, J. J., Newhouse, M. L., Peto, J., Pukkala, E., Sjögren, B., Wild, P., Winkelmann, R., and Saracci, R. (1991). A historical prospective study of European stainless steel, mild steel, and shipyard welders. *Br. J. Ind. Med.*, 48, 145-154.
8. Moulin, J. J., Mantout, B., Portefaix, P., Wild, P., Fournier-Betz, M., Mur, J. M., and Smagghe, G. (1992). Etude épidémiologique de mortalité dans deux aciéries d'acier inoxydable. [Historical prospective mortality study in two stainless steel factories]. *Arch. Mal. Prof. Secur. Soc.*, 53, 157-166.
9. Pang, D., Burges, D. C., and Sorahan, T. (1996). Mortality study of nickel platers with special reference to cancers of the stomach and lung, 1945-93. *Occup. Environ. Med.*, 53, 714-717.
10. Andersen, A., Engeland, A., Berge, S.R., and Norseth, T. (1996). Exposure to nickel compounds and smoking in relation to incidence of lung and nasal cancer among nickel refinery workers. *Occup. Environ. Med.*, 53, 708-713.
11. Anttila, A., Pukkala, E., Aitio, A., Rantanen, T., and Karjalainen, S. (1998). Update of cancer incidence among workers at a copper/nickel smelter and nickel refinery. *Int. Arch. Occup. Environ. Health*, 71, 245-250.
12. Egedahl, R. D., Carpenter, M., and Homik, R. (1993). An update of an epidemiological study at a hydrometallurgical nickel refinery in Fort Saskatchewan, Alberta. *Health Reports*, 5, 291-302.
13. Morgan, L. G. (1992), *Problems in the toxicology, diagnosis, and treatment of nickel carbonyl poisoning*. In: Neiboer, E.; Nriagu, J. O., eds. Nickel and human health: Current perspectives. New York, NY: John Wiley & Sons, Inc.; pp. 261-271.
14. Ottolenghi, A. D., Hasegan, J.K., Payne, W. W., Falk, H. J., and MacFarland, H. N. (1974). Inhalation studies of nickel sulfide in pulmonary carcinogenesis in rats. *J. Natl. Cancer Inst.*, 54, 1165-1172.
15. Dunnick, J. K., Elwell, M. R., Radovsky, A. E., Benson, J. M., Hahn, F. F., Nikula, K. J., Barr, E. B., and Hobbs, C. H. (1995). Comparative carcinogenic effects of nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate on chronic exposures in the lung. *Cancer Res.*, 55, 5251-5256.
16. NTP (National Toxicology Program) Draft Technical Report (1994a). Toxicology and carcinogenesis studies of nickel subsulfide in F344/N rats and B6C3F<sub>1</sub> mice. NTP TR 453, NIH publication No. 94-3369.
17. NTP (National Toxicology Program) Draft Technical Report (1994b). Toxicology and carcinogenesis studies of nickel oxide in F344/N rats and B6C3F<sub>1</sub> mice. NTP TR 451, NIH publication No. 94-3363.
18. Tanaka, I., Horie, A., Haratake, J., Kodama, Y., and Tsuchiya, K. (1988). Lung burden of green nickel oxide aerosol and histopathological findings in rats after continuous inhalation. *Biol. Trace Elem. Res.*, 16, 19-26.
19. Takenaka, S., Hochreiner, D., and Oldiges, H. (1985). Alveolar proteinosis induced in rats by long-term inhalation of nickel oxide. In *Progress in Nickel Toxicology* (S.S. Brown and F. W. Sunderman Jr. Eds.), pp. 89-92. Blackwell Scientific Publications, Oxford.
20. Horie, A., Haratake, J., Tanaka, I., Kodama, Y., and Tsuchiya, K. (1985). Electron microscopical findings with special reference to cancer in rats caused by inhalation of nickel oxide. *Biol. Trace Elem. Res.*, 7, 223-239.
21. Glaser, U., Hochrainer, D., Oldiges, H., and Takenaka, S. (1986). Long-term inhalation studies with NiO and As<sub>2</sub>O<sub>3</sub> aerosols in Wistar rats. In *Health hazards and biological effects of welding fumes and gases: Proceedings of the international conference, February 1985*; (R. M. Stern, A. Berlin, A. C. Fletcher, J. Jarvisalo, Eds.) pp. 325-328. The Netherlands: Excerpta medica (International Congress series No. 676). Amsterdam, Copenhagen, Denmark.
22. Wehner, A. P., Busch, R. H., Olson, R. J., and Craig, D. K. (1975). Chronic inhalation of nickel oxide and cigarette smoke by hamsters. *Am. Indust. Hyg. Assoc. J.*, 36, 801-810.

23. Wehner, A. P., Dagle, G. E., and Busch, R. H. (1984). Pathogenicity of inhaled nickel compounds in hamsters. In *Nickel in the human environment: Proceedings of a joint symposium: March 1983* (Sunderman, F. W. Jr., Ed.), pp. 143-151. International Agency for Research on Cancer, (IARC scientific publications no. 53). Lyon, France.
24. Benson, J. M., Chang, I.-Y., Cheng, Y. S., Hahn, F. F., Kennedy, C. H., Barr, E. B., Maples, K. R., and Snipes, M. B. (1995). Particle clearance and histopathology in lungs of F344/N rats and B6C3F<sub>1</sub> mice inhaling nickel oxide or nickel sulfate. *Fundamental and Applied Toxicology*, 28, 232-244.
25. Benson, J. M., Barr, E. B., Bechtold, W. E., Cheng, Y. S., Dunnick, J. K., Eastin, W. E., Hobbs, C.H., Kennedy, C. H., and Maples, K. R. (1994). Fate of inhaled nickel oxide and nickel subsulfide in F344/N rats. *Inhalation Toxicology* 6, 167-183.
26. Oberdörster, G., Baggs, R. B., and Finkelstein, J. (1995). Pulmonary retention and effects of inhaled NiO and Ni<sub>3</sub>S<sub>2</sub> in rats and mice: indicators of maximum tolerated dose? *Annals of Clinical and Laboratory Sciences*, 25, pp. 441, abstract 101.
27. Mauderly, J. L. (1994). Non-cancer pulmonary effects in chronic inhalation exposure of animals to solid particles. In *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract* (U. Mohr, D. L. Dungworth, J. L. Mauderly, and G. Oberdörster, Eds.), pp.43-55. ILSI Monographs ILSI Press, Washington, DC.
28. Mauderly, J. L., Burton Snipes, M., Barr, E. D., Belinsky, S. A., Bond, J. A., Brooks, A. L., Chang, I-Y, Cheng, Y. S., Gillet, N.A., Griffith, W. C., Henderson, R. F., Mitchell, C. E., Nikula, K. J., and Thomassen, D. G. (1994). Pulmonary toxicity of inhaled diesel exhaust and carbon black in chronically exposed rats. Part I: neoplastic and nonneoplastic lung lesions. *Health Effects Institute (HEI)*. HEI Research report No. 68. Montpelier, VT: Capital City Press.
29. Cerutti, P.A. (1985). Pro-oxidant states and tumor promotion *Science*, 227, 375-381.
30. Driscoll, K. E., Carter, J. M., Howard, B. W., and Hassenbein, D. G. (1994). Mutagenesis in rat lung epithelial cells after in vivo silica exposure or ex vivo exposure to inflammatory cells. *A. J. Respir. Crit. Care Med.*, 149, A553.
31. Driscoll, K. E., Carter, J. M., Howard, B. W., and Hassenbein, D. G., Pepelko, W., Baggs, R. B., Oberdörster G. (1996). Pulmonary inflammatory, chemokines and mutagenic responses in rats after subchronic inhalation of carbon black. *Tox. Appl. Pharmacol.*, 136, 372-380.
32. Oberdörster, G. (1995). Lung particle overload: implications for occupational exposures to particles. *Reg. Toxicol. and Pharmacol.*, 21, 123-135.
33. Snipes, M. B. (1996) Current information on lung overload in nonrodent mammals: contrast with rats. *Inh. Toxicol.*, 8(suppl), 91-109.
34. Schroeder, H. A., Balassa, J. J., and Vinton, W. H. (1964). Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumors and tissue levels. *J. Nutr.*, 83, 239-250.
35. Schroeder, H.A., Mitchener, M., and Nason, A.P. (1974). Life-term effects of nickel in rats: survival, tumors, interactions with trace elements and tissue levels. *J. Nutr.*, 104, 239-243.
36. Schroeder, H. A. and Mitchener, M. (1975). Life-term effects of mercury, methyl mercury, and nine other trace metals on mice. *J. Nutr.*, 105, 452-458.
37. 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.
38. Gilman, J. P. W. (1962). Metal Carcinogenesis. II. A study of the carcinogenic activity of cobalt, copper, iron and nickel compounds. *Cancer Res.*, 22, 158-162.
39. Payne, W. W. (1964). Carcinogenicity of nickel compounds on experimental animals. *Proc. Am. Assoc. Cancer Res.*, 5, 50.
40. Kasprzak, K. S., Gabryel, P., and Jarczewska, K. (1983). Carcinogenicity of nickel (II) hydroxides and nickel(II) sulfate in Wistar rats and its relation to the in vitro dissolution rates. *Carcinogenesis*, 4, 275-279.
41. Kasprzak, K. S. (1994). Lack of carcinogenic activity of promptly soluble (hydrated) and sparingly soluble (anhydrous) commercial preparations of nickel (II) sulfate in the skeletal muscle of male F334/NCR rats. *Toxicologist*, 14, 239.
42. Stoner, G. D., Shimkin, M. D., Troxell, M. C., Thompson, T. L., and Terry, L. S. (1976). Test for carcinogenicity of metallic compounds by the pulmonary tumor response in Strain A mice. *Cancer Res.*, 36, 1744-1747.
43. Poirier, L. A., Theiss, J. C., Arnold, L. J., and Shimkin, M. B. (1984). Inhibition by magnesium and calcium acetates, of lead subacetate- and nickel acetate-induced lung tumors in strain A mice. *Cancer Res.*, 44, 1520-1522.
44. Kasprzak, K. S., Diwan, B. A., Konishi, N., Misra, M., and Rice, J. M. (1990). Initiation by nickel acetate and promotion by sodium barbital of renal cortical epithelial tumors in male F344 rats. *Carcinogenesis*, 11(4), 647-652.
45. Pott, F., Rippe, R. M., Roller, M., Csicsaky, M., Rosenbruch, M., and Huth, F. (1992). Carcinogenicity of nickel compounds and nickel alloys in rats by intraperitoneal injection. In *Nickel in human health: current perspectives* (E. Nieboer, and J. O. Nriagu, Eds.) pp. 491-502. John Wiley and Sons, Inc., New York, NY.
46. NTP (National Toxicology Program) Draft Technical Report (1994c). Toxicology and carcinogenesis studies of nickel sulfate hexahydrate in F344/N rats and B6C3F<sub>1</sub> mice. NTP TR 454, NIH publication No. 94-3370.
47. Hueper, W. C. and Payne, W. W. (1962). Experimental studies in metal carcinogenesis. *Arch. Environ. Health*, 5, 445-462.
48. Pott, F., Ziem, U., Reiffer, F. J., Huth, F. Ernst, H., and Mohr, U. (1987). Carcinogenicity studies on fibers, metal compounds, and some other dusts in rats. *Exp. Pathol.* 32, 129-152.
49. Muhle, H., Bellman, B., Takenaka, S., Fuhst, R., Mohr, U., and Pott, F. Chronic effects of intratracheally instilled nickel-containing particles in hamsters. In: *Nickel and Human Health: Current Perspectives*. Nieboer, E.; Nriagu, N. O., eds. New York, NY: John Wiley & Sons, Inc. p. 467-479 (1992).
50. Sunderman, F.W. ; Donnelly, A.J.; West, B.; Kincaid, J.F. (1959) Nickel poisoning: IX. Carcinogenesis in rats exposed to nickel carbonyl. *AMA Arch. Ind. Health*, 20, 36-41.
51. Sunderman, F.W. and Donnelly, A.J. (1965) Studies of nickel carcinogenesis metastasizing pulmonary tumors in rats induced by the inhalation of nickel carbonyl. *Am. J. Pathol.*, 46, 1027-1041.
52. Biggart, N. W. and Costa, M. (1986). Assessment of the uptake and mutagenicity of nickel chloride in Salmonella tester strains. *Mutat. Res.*, 175, 209-215.

53. Miura, T., Patierno, S. R., Sakuramoto, T., and Landolph, J. R. (1989). Morphological and neoplastic transformation of C3H/10T1/2 Cl 8 mouse embryo cells by insoluble carcinogenic nickel compounds. *Environ. Mol. Mutagen.*, 14, 65-78.
54. Little, J. B., Frenial, J.-M., and Coppey, J. (1988). Studies of mutagenesis and neoplastic transformation by bivalent metal ions and ionizing radiation. *Terato., Carcino., Mutagen.*, 8, 287-292.
55. Gurley, L. R., Valdez, J. G., Miglio, J. J., Cox, S. H., and Tobey, R. A. (1986). Biological availability of nickel arsenides: Cellular response to soluble Ni<sub>5</sub>As<sub>2</sub>. *J. Toxicol. Environ. Hlth.*, 17, 101-117.
56. Arroujial, F. Z., Hildebrand, H. F., Vopfi, H., and Marzin, D. (1990). Genotoxic activity of nickel subsulfide α-Ni<sub>3</sub>S<sub>2</sub>. *Mutagenesis*, 5(6), 583-589.
57. Kargacin, B., Klein, C. B., and Costa, M. (1993). Mutagenic responses of nickel oxides and nickel sulfides in Chinese hamster V79 cell lines at the xanthine-guanine phosphoribosyl transferase locus. *Mutat. Res.*, 300, 63-72.
58. Biedermann, K. A. and Landolph, J. R. (1987). Induction of anchorage independence in human diploid foreskin fibroblasts by carcinogenic metal salts. *Canc. Res.*, 47, 3815-3823.
59. Skopek, (1995). Mutagenic potential of nickel compounds in human lymphoblastoid cells in vitro. Final report to NIPERA.
60. Sen, P. and Costa, M. (1985). Induction of chromosomal damage in Chinese hamster ovary cells by soluble and particulate nickel compounds: Preferential fragmentation of the heterochromatic long arm of the X-chromosome by carcinogenic crystalline NiS particles. *Canc. Res.*, 45, 2320-2325.
61. Sen, P., Conway, K., and Costa, M. (1987). Comparison of the localization of chromosome damage induced by calcium chromate and nickel compounds. *Canc. Res.*, 47, 2142-2147.
62. Lin, X., Sugiyama, M., and Costa, M. (1991). Differences in the effect of vitamin E on nickel sulfide or nickel chloride-induced chromosomal aberrations in mammalian cells. *Mutat. Res.*, 260, 159-164.
63. Howard, W., Leonard, B., Moody, W., and Kochhar, T. S. (1991). Induction of chromosome changes by metal compounds in cultured CHO cells. *Tox. Lett.*, 56, 179-186.
64. Conway, K., Wang, X-W, Xu, L.-S., and Costa, M. (1987). Effect of magnesium on nickel-induced genotoxicity and cell transformation. *Carcinogenesis*, 8(8), 1115-1121.
65. Christie, N. T., Sen, P., and Costa, M. (1988). Chromosomal alterations in cell lines derived from mouse rhabdomyosarcomas induced by crystalline nickel sulfide. *Biol. Metals*, 1, 43-50.
66. Larramendy, M. L., Popescu, N. C., and DiPaolo, J. A. (1981). Induction by inorganic metal salts of sister chromatid exchanges and chromosome aberrations in human and Syrian hamster cell strains. *Environ. Mutagen.*, 3, 597-606.
67. Montaldi, A., Zentilin, L., Zordan, M., Bianchi, V., and Levis, A. G. (1987). Chromosomal effects of heavy metals (Cd, Cr, Hg, Ni and Pb) on cultured mammalian cells in the presence of nitrotri-acetic acid (NTA). *Tox. Environ. Chem.*, 14, 183-200.
68. Sharma, G. P., Sobti, R. C., Chaudhry, A., Ahluwalia, K. K., and Gill, R. K. (1987). Effect of some nickel compounds on the chromosome of mice and mosquitoes. *La Kromosomo II*, 45, 1423-1432.
69. Mohanty, P. K. (1987). Cytotoxic effect of nickel chloride on the somatic chromosomes of swiss albino mice *mus musculus*. *Current Sci.*, 56(22), 1154-1157.
70. Chorvatovicova, D. (1983). The effect of nickel chloride on the level of chromosome aberrations in Chinese hamsters *Cricetulus griseus*. *Biologia (Bratislava)*, 38(11), 1107-1112.
71. Chorvatovicova, D. (1987). Synergic effects on chromosome aberration frequency of chromium and nickel ions *in vivo*. *Biologia*, 42(11), 1047-1052.
72. Zhong, B.-Z., Li, Z.-Q., Ma, G.-Y., and Wang, B.-S. (1989). Study of the mutagenicity and carcinogenicity of produced nickel dust. *Environ. Mol. Mutagen.*, 14 (Suppl. 15), Abstract no. 669.
73. Zhong, Z., Troll, W., Koenig, K. L., and Frenkel, K. (1990). Carcinogenic sulfide salts of nickel and cadmium induce H<sub>2</sub>O<sub>2</sub> formation by human polymorphonuclear leukocytes. *Canc. Res.*, 50, 7564-7570.
74. Costa, M., Abbracchio, M. P., and Simmons-Hansen, J. (1981). Factors influencing the phagocytosis, neoplastic transformation, and cytotoxicity of particulate nickel compounds in tissue culture systems. *Toxicol. Appl. Pharmacol.*, 60, 313-323.
75. Patierno, S. R., Dirscherl, L. A., and Xu, J. (1993). Transformation of rat tracheal epithelial cells to immortal growth variants by particulate and soluble nickel compounds. *Mutat. Res.*, 300, 179-193
76. DiPaolo, J. A. and Casto, B. L. C. (1979). Quantitative studies of in vitro morphological transformation of Syrian hamster cells by inorganic metal salts. *Cancer Res.*, 39, 1008-1313.
77. Costa, M. and Mollenhauer, H. H. (1980). Phagocytosis of nickel subsulfide particles during the early stages of neoplastic transformation in tissue culture. *Canc. Res.*, 40, 2688-2694.
78. Abbracchio, M. P., Simmons-Hansen, J., and Costa M. (1982a). Cytoplasmic dissolution of phagocytized crystalline nickel sulfide particles: a prerequisite for nuclear uptake of nickel. *J. Toxicol. and Environ. Health*, 9, 663-676.
79. Heck, J. D. and Costa, M. (1983). Influence of surface charge and dissolution on the selective phagocytosis of potentially carcinogenic particulate metal compounds. *Canc. Res.*, 43, 5652-5656.
80. Costa, M. and Heck, J. D. (1984). Perspective on the mechanism of nickel carcinogenesis. In *Advances in Inorganic Biochemistry*, (G. L. Eichhorn and L. Marzilli, Eds.), Vol. 6, Chapter 8, pp. 285-309. Springer-Verlag, New York.
81. Sunderman, F. W., Jr., Hopfer, S. M., Knight, J. A., McCully, K. S., Cecutti, A. G., Thornhill, P. G., Conway, K., Miller, C., Patierno, S. R., and Costa, M. (1987). Physicochemical characteristics and biological effects of nickel oxides. *Carcinogenesis*, 8(2), 305-313.
82. Huang, X., Kitahara, J., Zhitkovich, A., Dowjat, K and Costa, M. (1995). Heterochromatic proteins specifically enhance nickel-induced 8-oxo-dG formation. *Carcinogenesis*, 16, 1753-1759.
83. Patierno, S. R., Sugiyama, M., Basilion, J. P., and Costa, M. (1985). Preferential DNA-protein cross-linking by NiCl<sub>2</sub> in magnesium-insoluble regions of fractionated Chinese hamster ovary cell chromatin. *Can. Res.*, 45, 5787-5794.
84. Sen, P. and Costa, M. (1986). Pathway of nickel uptake influences its interaction with heterochromatic DNA. *Toxicol. Appl. Pharmacol.*, 84, 278-285.

85. Klein, C. B., Conway, K., Wang, X. W., Bhamra, R. K., Lin, X., Cohen, M. D., Annab, L., Barrett, J. C., and Costa, M. (1991). Senescence of nickel-transformed cells by an X chromosome: possible epigenetic control. *Science*, 251, 796-799.
86. Costa, M. (1991). Molecular mechanisms of nickel carcinogenesis. *Ann. Rev. Pharmacol. Toxicol.*, 31, 321-337.
87. Lee, Y.-W., Klein, C. B., Kargacin, B., Salnikow, K., Kitahara, J., Dowjat, K., Zhitkovich, A., Christie, N. T., and Costa, M. (1995). Carcinogenic nickel silences gene expression by chromatin condensation and DNA methylation: a new model for epigenetic carcinogens. *Mol. Cell. Biol.*, 15(5), 2547-2557.
88. Kasprzak, K. S., Diwan, B. A., Rice, J. M., Misra, M., Riggs, C. W., Olinski, R., and Dizdaroglu, M. (1992). Nickel(II)-mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis. *Chem. Res. Toxicol.*, 5, 809-815.
89. Datta, A. K., Misra, M., North, S. L., and Kasprzak, K. S. (1992). Enhancement by nickel(II) and L-histidine of 2'-deoxyguanosine oxidation with hydrogen peroxide. *Carcinogenesis*, 13(2), 283-287.
90. Kawanishi, S., Inoue, S., and Yamamoto, K. (1989). Site-specific DNA damage induced by nickel(II) ion in the presence of hydrogen peroxide. *Carcinogenesis*, 10(12), 2231-2235.
91. Nackerdien, Z., Kasprzak, K. S., Rao, G., Halliwell, B., and Dizdaroglu, M. (1991). Nickel(II)- and Cobalt(II)-dependent damage by hydrogen peroxide to the DNA bases in isolated human chromatin. *Canc. Res.*, 51, 5837-5842.
92. Misra, M., Olinski, R., Dizdaroglu, M., and Kasprzak, K. S. (1993). Enhancement by L-histidine of Nickel(II)-induced DNA-protein cross-linking and oxidative DNA base damage in the rat kidney. *Chem. Res. Toxicol.*, 6, 33-37.
93. Trott, D. A., Cuthbert, A. P., Overell, R. W., Russo, I., and Newbold, R. F. (1995). Mechanisms involved in the immortalization of mammalian cells by ionizing radiation and chemical carcinogens. *Carcinogenesis*, 16, 193-204.
94. Zhang, Q. and Barrett, J. C. (1988). Dose-response studies of nickel-induced morphological transformation of Syrian hamster embryo fibroblasts. *Toxic. In Vitro*, 2(4), 303-307.
95. Wang, X. W., Lin, X., Klein, C. B., Bhamra, R. K., Lee, Y. W., and Costa, M. (1992). A conserved region in human and Chinese hamster X chromosomes can induce cellular senescence of nickel-transformed Chinese hamster cell lines. *Carcinogenesis*, 13, 555-561.
96. Conway, K. and Costa, M. (1989b). Nonrandom chromosomal alterations in nickel-transformed Chinese hamster embryo cells. *Canc. Res.*, 49, 6032-6038.
97. Swenberg, J. A. (1995). Bioassay design and MTD setting: old methods and new approaches. *Reg. Toxicol. and Pharm.*, 21, 44-51.
98. Yu, C. P., Hsieh, T. H., and Oberdörster, G. (1998). Dosimetry of inhaled nickel compounds. Abstract and presentation made at the American Association for Aerosol Research Annual Meeting held June 22-26, 1998 in Cincinnati, Ohio.
99. Hausinger, R. P. (1992). Biological utilization of nickel. In *Nickel in human health: current perspectives* (E. Nieboer, and J. O. Nriagu, Eds.), pp. 21-36. John Wiley and Sons, Inc. New York, NY.
100. Abbraccio, M. P., Evans, R. M., Heck, J. D., Cantoni, O., and Costa M. (1982b). The regulation of ionic uptake and cytotoxicity by specific amino acids and serum components. *Biol. Trace Element Res.*, 4, 289.

**Comments of the Nickel Producers Environmental Research  
Association on the National Toxicology Program  
Draft RoC Background Document for Nickel Compounds**

November 20, 1998

## 1. Executive Summary

The U.S. National Toxicology Program (NTP) is reviewing the database on the potential carcinogenicity of nickel and nickel compounds. In the NTP's Eighth Report on Carcinogens, *Nickel and Certain Nickel Compounds* (*i.e.*, not including water soluble nickel compounds) were listed as substances that are "reasonably anticipated to be a carcinogen". The new proposal for the Ninth Report on Carcinogens would list *Nickel and Nickel Compounds* as substances that are "known human carcinogens." NIPERA believes that this change would be scientifically unjustified and inappropriate. NIPERA's major objection to the NTP's proposal to list *Nickel and Nickel Compounds* as "known human carcinogens" in the Ninth Biennial Report on Carcinogens is that it fails to recognize the **differences in the carcinogenic potential of the various forms of nickel.**

The NTP proposal to list *Nickel and Nickel Compounds* as substances that are "known human carcinogens" is presumed to be based on the information contained in the Draft RoC Background document. This draft document concludes that "nickel ion is a human carcinogen and all compounds that contain nickel ions should be considered human carcinogens". Based on these conclusions, the NTP proposal would appear to be fully justified. However, a closer look at the Draft RoC Background document reveals that its conclusions are based on a less than objective selection, presentation, and interpretation of the data for nickel and its compounds. The consistently low quality of the data presentation and interpretation is particularly alarming in view of the excellent analyses presented by the NTP in its discussions of the toxicology of nickel compounds included in the NTP Technical reports for nickel subsulfide, high temperature [green] nickel oxide, and nickel sulfate hexahydrate (NTP, 1996a-c).

One of the main problems found in this document relates to the selection, presentation, and interpretation of the epidemiological, animal and *in vitro* data pertaining to soluble nickel compounds.

- In some cases, isolated studies are discussed without making any attempts to integrate the data from one study with the rest of the available data. In many cases, the reporting is superficial and incomplete, in other cases, the reporting is just wrong.
- A discussion of the epidemiologic studies in which exposures to soluble nickel compounds occur in refinery workers should take into account the fact that all cohorts had mixed exposures to more insoluble nickel compounds and to other confounders (*e.g.*, acid mists, arsenic, chromium, cigarette smoking). In addition, studies of platers (almost exclusively exposed to soluble nickel compounds) should be included in the discussions. Finally, an effort should be made to look at the consistency of the data from all studies in assessing the human carcinogenic potential of soluble nickel compounds.
- Two rat studies by intraperitoneal route of exposure are featured prominently in the report while a dozen other negative studies (including relevant routes of exposures such as the inhalation NTP 1996a bioassay) are ignored.
- The significance of these two intraperitoneal studies for the carcinogenic assessment of soluble nickel compounds can be seriously questioned given the very unique conditions under which rats developed renal tumors (only males with concurrent exposure to sodium barbital) and the high toxicity experienced by the pups with pituitary tumors in the transplacental carcinogenicity study.

A second significant problem is the lack of understanding of the mechanistic data pertaining to the carcinogenicity of certain nickel compounds.

- It is not just the presence of nickel in any compound that will determine the positive respiratory carcinogenic potential of the compound but rather the availability at nuclear sites within the target epithelial cells of the lung or nose of the nickel ion released from this compound.
- A nickel-containing compound that is highly insoluble may not cause tumors because even if particles are endocytized by the epithelial cells, not enough nickel ions will reach the nucleus.

- On the other extreme, a nickel compound that is completely soluble will be cleared from the nose and lung very quickly (no accumulation), will not be able to enter the cell by endocytosis, and will not be available at nuclear sites of target cells (see NTP 1996 bioassay).
- Only those nickel compounds (*e.g.*, nickel subsulfide) that are readily endocytized by epithelial cells, have intermediate clearance rates, have increased solubility under acidic endocytic pH, will result in sufficient amount of nickel ions at nuclear sites to induce tumors (see NTP 1996b bioassay). The overall human, animal and mechanistic data are consistent with this interpretation but are not consistent with all nickel compounds being human carcinogens.

The NTP Draft RoC Background Document concludes that *nickel and all nickel compounds should be human carcinogens* based on biased and inaccurate data discussions. **NIPERA strongly urges NTP to undertake a revision of the NTP Draft RoC Background Document, including a careful re-analysis of the available data and elimination of obvious errors, before this document is used as a background document to evaluate the carcinogenic potential of nickel compounds.** The NTP technical reports with nickel compounds (NTP, 1996) should be used as a model since they present a more thoughtful data integration and critical examination of the many animal and human studies. In addition, the NTP Technical reports display a significant understanding of the mechanistic data, an area that was quite misunderstood in the NTP Draft RoC Background Document.

A more thorough examination of the *in vitro*, animal, and epidemiologic data pertaining to commercially relevant nickel compounds<sup>1</sup> will reveal that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity (see the October 13, 1998 NIPERA comments on the NTP proposal for Classification of Nickel and Nickel Compounds).

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<sup>1</sup> The classes of nickel compounds discussed in this paper are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide), water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, *etc.*), and nickel carbonyl. Metallic, oxidic, and sulfidic nickel compounds and nickel carbonyl are insoluble in water.

## Table of Contents

|   | <u>PAGE</u> |
|---|-------------|
| 1. Executive Summary .....  | 2           |
| 2. Comments Arranged by Page and Document Section .....   | 5           |
| 2.1. Carcinogenicity .....  | 5           |
| 2.2. Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis .....  | 6           |
| 2.3. Identification and Chemical-Physical Properties of Nickel Compounds .....  | 6           |
| 2.4. Human Exposure.....  | 7           |
| 2.5. Studies Post IARC (1990) .....   | 9           |
| 2.6. Other Occupational Exposure Studies .....  | 12          |
| 2.7. Experimental Carcinogenesis.....   | 12          |
| 2.8. Genotoxicity.....  | 13          |
| 2.9. Other Relevant Data.....   | 15          |
| 2.10. Mechanisms of Carcinogenesis .....  | 15          |
| 3. Conclusion.....  | 17          |
| 4. References Cited in these Comments that are not Included in the NTP Draft RoC Background Document .....  | 19          |
| <br>Appendix A: Review of the Manuscript by Diwan et al. Titled, <i>Transplacental carcinogenic effects of nickel(II) acetate in the renal cortex, renal pelvis and adenohipophysis in F344/NCR rats.</i> ..... | <br>A-1     |
| Appendix B: U.S. and Foreign Mining, Milling, and Smelting Operations .....   | B-1         |
| Appendix C: Review of the Manuscript by Andersen et al. Titled, <i>Exposure to Nickel Compounds and Smoking in Relation to Incidence of Lung Cancer Among Nickel Refinery Workers.</i> .....                    | C-1         |
| Appendix D: Detailed Comments Regarding the Finnish Refinery Studies .....  | D-1         |

## 2. Comments Arranged by Page and Document Section

### 2.1. CARCINOGENICITY

#### FIRST PAGE (NOT NUMBERED), PARAGRAPH 1:

The NTP Draft RoC Background Document concludes that all nickel compounds should be classified as "...known to be human carcinogens based on increased risk of cancer in workers and evidence of malignant tumor formation by multiple routes of exposure, at various sites, in multiple species of experimental animals." This statement is flawed in that consistent human and animal data showing increased carcinogenicity (at several sites and by several routes of exposure) are available for only one nickel compound: sulfidic nickel including nickel subsulfide. For all other nickel compounds (nickel carbonyl, oxidic nickel and soluble nickel compounds) the statement is not true and does not even agree with the data reviewed in this report.

The NTP Draft RoC Background Document states that because all "...nickel compounds act by the generation of nickel ions at critical sites in target cells all these compounds can be evaluated as a single group." This statement is false and it appears to be based on the limited consideration of a subset of animal studies with disregard for the results demonstrated in other studies (including the NTP 1996a-c studies). The consideration of all nickel compounds as a single group for carcinogenic evaluation demonstrates a lack of understanding of the mechanistic and toxicokinetic data related to nickel compounds.

It is not just the presence of nickel in any compound that will determine the positive respiratory carcinogenic potential of the compound but rather the availability at nuclear sites within the target epithelial cells of the lung or nose of the nickel ion released from this compound. A nickel-containing compound that is highly insoluble may not cause tumors because even if particles are endocytized by the epithelial cells, not enough nickel ions will reach the nucleus. At high concentration, a highly insoluble nickel compound may cause tumors only secondary to a particle effect. On the other extreme, a nickel compound that is completely soluble will be cleared from the nose and lung very quickly (no accumulation), will not be able to enter the cell by endocytosis, and will not be available at nuclear sites of target cells due to rapid binding to cytoplasmic proteins (see NTP 1996 bioassay). Only those nickel compounds (*e.g.*, nickel subsulfide) that are readily endocytized by epithelial cells, have intermediate clearance rates, have increased solubility under acidic endocytic pH, will result in sufficient amount of nickel ions at nuclear sites to induce tumors (see NTP 1996b bioassay). The overall human, animal and mechanistic data are consistent with this interpretation but are not consistent with all nickel compounds being human carcinogens.

#### FIRST PAGE (NOT NUMBERED), PARAGRAPH 2:

NTP should remember that IARC's pronouncements in 1990 were not based on the datasets available today, therefore pronouncements that may have been justified at the time need to be re-evaluated considering the current body of data. More recent assessments by ACGIH for example have taken speciation of nickel compounds into account for carcinogenic classification.

Of all the categories of nickel compounds, the need for speciation is most compelling for soluble nickel compounds. There are a large number of negative animal carcinogenicity studies by relevant routes of exposure, starting with the well-conducted NTP inhalation bioassays in mice and rats (NTP 1996c) and continuing with five negative oral studies in mice, rats, and dogs (Schroeder *et al.*, 1964; Schroeder *et al.*, 1974; Schroeder and Mitchner, 1975; Ambrose *et al.*, 1976; Kurokawa *et al.*, 1985). Even a non-relevant route of exposure like intramuscular injection gave negative results (Gilman, 1962; Payne, 1964; Kasprzak *et al.*, 1983; Kasprzak, 1994; in rats). In an intraperitoneal study, the administration of a soluble nickel compound by itself was also negative (Kasprzak *et al.*, 1990). In that study, administration of the non-genotoxic carcinogen sodium barbital resulted in kidney tumors in male rats (only). When the soluble nickel compound was administered with sodium barbital, a higher number of kidney tumors (in male rats

only) were induced (Kasprzak *et al.*, 1990; Diwan *et al.*, 1992). This phenomenon was later explained by the enhanced susceptibility of male kidneys to the sodium barbital effects (possibly involving the  $\alpha$ -2 microglobulin mechanism). EPA and other regulatory agencies agree that these type of tumors should not be considered in carcinogenicity assessment.

Therefore, out of 14 animal studies, there is only one positive study, by one route of exposure, in one animal species with a soluble nickel compound. This study is a transplacental rat carcinogenicity study in which dams were injected intraperitoneally with a soluble nickel compound and the surviving pups were examined for tumors. A significant fraction of both male and female pups developed pituitary tumors (Diwan *et al.*, 1992). In the context of a dozen negative studies, the relevance of one transplacental study for the carcinogenic assessment of soluble nickel compounds should be seriously questioned. This is particularly true based on fact that the study used an irrelevant route of exposure for risk assessment, the study caused high toxicity resulting in 88% mortality, and the fact that this tumor type has never been observed in any other animal study (even those that used a clearly carcinogenic nickel compound such as nickel subsulfide) or in human studies (+50,000 workers). The transplacental study of Diwan *et al.* is discussed further in comments provided under Section 4 and in Appendix A.

The overwhelmingly negative animal data, together with the epidemiological data that suggests an enhancing rather than a direct carcinogen role for soluble nickel compounds, does not justify the classification of soluble nickel compounds as *Known to be Human Carcinogens*.

**FIRST PAGE (NOT NUMBERED), PARAGRAPH 2:**

With regard to the human epidemiologic data pertaining to soluble nickel compounds, the document wrongly states that exposure to soluble nickel alone in a refinery resulted in excess lung and nasal cancers. Such a refinery cohort does not exist and as mentioned in Andersen *et al.* (1996), workers always had mixed exposures to soluble and insoluble nickel compounds, arsenic, acid mists, *etc.* There are however, smaller nickel plater cohorts that have exposures almost exclusively to soluble nickel compounds and show no excess risk of respiratory tumors (Burgess *et al.*, 1980; Pang *et al.*, 1996). Unfortunately, these references were totally left out of the NTP Draft RoC Background Document. [See further comments on this issue under Section 3.2.4.]

**2.2. OTHER INFORMATION RELATING TO CARCINOGENESIS OR POSSIBLE MECHANISMS OF CARCINOGENESIS**

**SECOND PAGE (NOT NUMBERED), PARAGRAPH 2:**

At the end of this section, the intraperitoneal transplacental study by Diwan and coworkers is featured again as evidence of the carcinogenicity of soluble nickel compounds while the recent negative inhalation studies by the NTP, which used a relevant route of exposure and two animal species, are not even mentioned. Furthermore, the document gives great relevance to the kidney tumors seen only in males, and only after sodium barbital exposure. At the same time, the NTP Draft RoC Background Document ignores all the information pertaining to the association between the nongenotoxic carcinogen, sodium barbital, and the kidney tumors in seen only male animals (possibly involving the  $\alpha$ -2 microglobulin mechanism). The document also fails to mention that soluble compounds alone did not cause tumors while sodium barbital alone did.

**2.3. IDENTIFICATION AND CHEMICAL-PHYSICAL PROPERTIES OF NICKEL COMPOUNDS**

**PAGE 1-1, SECTION 1.0, PARAGRAPH 1:**

In line 1 the word "thousands" should be changed to "many" since the number of nickel compounds is on the hundreds rather than thousands range. The list of compounds with potential for occupational exposure (Table 1-1) actually has less than 80 compounds.

**PAGE 1-1, SECTION 1.0, PARAGRAPH 1:**

In line 5 "*melting*" should be added along with "*fabrication and joining*" since the risk of exposure to nickel is important during this stage of processing.

**PAGE 1-1, PARAGRAPH 5:**

The NTP Draft RoC Background Document states categorically: "*It is expected that ionic nickel may arise from any nickel compound at physiological pH.*" This is incorrect given that one nickel compound listed in Table 1-1 of the NTP Draft RoC Background Document itself is stated to "*dissolve in hot sulfuric or nitric acid only.*" Clearly no physiological pH on this planet will dissolve this compound. In fact, since the solubilization or corrosion of nickel ions from a nickel compound particle is important in eliciting a biological response, it should be noted that solubilization/corrosion of a nickel compound is directly relevant to the route of exposure and the appropriate subcellular organelle. Sweeping generalizations of this type should be avoided.

**TABLE 1-1:**

The information in Table 1-1 (pages 1-2 to 1-10) is reproduced from various sources and assumed to have been accurately copied. However, the formula for Raney Nickel, which is the last entry in the Table, should be "nickel", it is just porous nickel.

**2.4. HUMAN EXPOSURE****PAGE 2-1, SECTION 2.1, PARAGRAPH 1:**

The corresponding sentences in this paragraph should be corrected as follows:

*"In the United States, consumption reached about 200,000 tons per year (U.S. Bureau of Mines, 1991; cited by NTP, 1996), but has recently fallen to the 180,000 ton level. The use of primary nickel can be divided into six sectors: stainless steel, alloy steel, nickel alloys, electroplating, foundry and other. In 1996, approximately 73% of primary nickel was used for the production of stainless and alloy steels, 14% went into nonferrous and superalloys, 9% for electroplating, 3 % into foundry products and the balance of 2% was used in other applications such as, chemicals, catalysts, batteries, pigments and ceramics (Kuck, 1997a; NiDI, 1997)."*

**PAGE 2-1, SECTION 2.2, PARAGRAPHS 2 AND 3:**

For clarification the corresponding sentences in these paragraphs should be corrected as follows:

*"Metallic nickel is produced from sulfide ores and oxide (laterite) ores. The oxide ores are found in tropical regions and areas that were once considered tropical such as parts of the Pacific Northwest. Neither type of ore currently processed averages more than 3% nickel (Warner, 1984; cited by IARC, 1990). Nickel and co-products are recovered from sulfide ores by a combination of flotation, roasting, smelting, electrolysis or decomposition processes. (IARC, 1990). Nickel is recovered from oxide ores by either hydrometallurgical or pyrometallurgical techniques (IARC, 1990). Other ways of obtaining nickel units are through the recycling process, consumer scrap, and as a by-product from the refining of other metals such as copper and platinum (Sibley, 1985; cited by IARC, 1990).*

*Nickel products are broadly classified by the amount of nickel they contain. Class I products are defined as containing > 99.8% nickel, whereas Class II products vary in their nickel content (NiDI, 1997). Class I nickel products are refined using a variety of processes which decrease impurities such as antimony, cobalt, arsenic, zinc, copper, iron and lead. Cobalt closely resembles the physical and chemical properties of nickel and is often difficult to remove completely from the mined ores, therefore many Class I products may contain minor amounts of residual cobalt. Nickel products designated as Class II material such as nickel oxide, metallized oxide and ferronickel are produced directly by hydrometallurgical or pyrometallurgical techniques and are*

*sufficiently pure to be used without further refining in applications like stainless steel production (Ullman, 1985)."*

**PAGE 2-2, TABLE 2-1:**

The section on the uses of "Nickel" should be replaced with the following:

*"Wrought and cast stainless steels, alloy steels, cupronickels, superalloys, electroplating, magnets, coinage, catalysts, batteries, electrical contacts, and electrodes, pigments."*

**PAGE 2-5, SECTION 2.2.1, PARAGRAPH 4:**

For clarification, the second line in this paragraph should read as follows:

*"...demand for primary nickel increased significantly in 1995 when it rose by 15%..."*

and the following sentence should be added after (Kuck, 1997b).

*"Current consumption is near the 1 M ton mark."*

**PAGE 2-5, SECTION 2.3.1, PARAGRAPH 5:**

In the second sentence "*mechanically*" should be replaced by "*by flotation*" while in the third sentence "*is*" should be replaced by "*maybe*."

**PAGE 2-5, SECTION 2.3.1, PARAGRAPH 5:**

Delete the last line "*Lateritic ores may be...*" It is repeated on the top of page 2-6.

**PAGE 2-6, SECTION 2.3.1, PARAGRAPH 3:**

For clarification starting at the first line, the paragraph should be modified to read, "*...a nickel-copper matte. The nickel is leached from the matte and recovered by electrolysis of the solution. The atmospheric.....nickel and cobalt in the feed. A series...*"

**PAGE 2-6, SECTION 2.3.2, PARAGRAPH 4:**

In the third line "*most*" should be replaced by "*over half*."

**PAGE 2-8, SECTION 2.3.3, PARAGRAPH 2:**

Delete first sentence starting with "*Table 2-2 is....*" and add the following sentence at the end of the paragraph:

*"Table 2-2 gives a summary of the current producers of refined nickel and indicates the type of material processed, the process technology used, and the nickel products produced."*

**PAGE 2-9, TABLE 2.2:**

This Table is not complete. A substitute Table is enclosed in Appendix B which lists all current producers, including a brief description of the type of material they process, the main process technology and the products made. No reference is made to the specific types of nickel-bearing materials involved in processing since the chemistry is complex and would require a very detailed analysis. It would be virtually impossible to summarize all the materials used in a table. Facilities no longer in production have not been included.

**PAGE 2-10, SECTION 2.4.1:**

The average levels of nickel found in the ambient air ought to be reported. As noted in the document, they are very low (much lower than most of the occupational values reported in Table 2-3). In as much as inhalation is the main exposure route of concern regarding the health effects of nickel, the reader should understand that health risks due to the inhalation of ambient nickel will likely be negligible given the minute amounts of nickel present in the air (see later comments regarding such risks).

**PAGES 2-10, SECTION 2.4.2:**

It is not clear why mining, milling, smelting, and refining should be considered among the most relevant industrial sectors with respect to this document. The NTP guidelines require that this document be focused on the United States. There are no nickel mining, milling, smelting or refining operations in the U.S. Even in the past, the presence of nickel production operations in the US has been very limited. US operations where nickel is potentially present are confined to using-industries where exposures will mainly be to oxidic, metallic, and soluble nickel. Furthermore, the oxidic nickel exposures in using industries tend to be different from the oxidic nickel exposures that were associated with the nasal and lung cancers seen in the past in producing industries. No respiratory cancers have been associated with exposures to nickel-using industries. This is an important point that ought to be elaborated on in later sections of the NTP Draft RoC Background Document.

**PAGE 2-12, SECTION 2.4.2, PARAGRAPH 1:**

"Table 2-1" should be cahnged to "Table 2-4."

**PAGE 2-14, SECTION 2.5:**

The NIOSH REL is dated (1977) and was proposed long before scientists knew much about the health effects and cancer mechanisms of nickel and individual nickel compounds. NIOSH has not been active in researching the health effects of nickel, nor in up-dating its recommendations. Either the REL should not be reported or should only be mentioned with appropriate qualifying statements regarding its obsolescence.

**PAGE 3-1, SECTION 3.1, PARAGRAPH 2:**

The second and third sentences of this paragraph have been taken out of context from an occupational criteria document that was prepared by a group of independent scientists for the Directorate General V of the CEC.<sup>2</sup> These sentences are not generic to all oxidic and sulfidic cancer incidences and refer only to a group of refinery workers employed at Falconbridge's Kristiansand, Norway operation. In addition, it appears that the reference to Ni-Cu oxides and impure NiO has been confused. Exposure levels at a nickel refinery in Clydach, Wales were estimated at 1-10 mg Ni/m<sup>3</sup> (mainly as Ni-Cu oxides) prior to 1936 and 1-5 mg Ni/m<sup>3</sup> (mainly as impure NiO) in subsequent years. Exposures at Kristiansand were mainly to Ni-Cu oxides.

**PAGE 3-1, SECTION 3.1, PARAGRAPH 3:**

The end of paragraph three would be a useful place to integrate the data presented under Section 2.4.1. The conclusions of Steenland *et al.* (1996, cited in the text, but missing from the references) are much the same as the ICNCM which noted that "*the risk to the general population from exposure to the extremely small concentrations [of nickel] (less than 1 µg Ni/m<sup>3</sup>) to which it is exposed in the ambient air is minute, if indeed, there is any risk at all.*"

**2.5. STUDIES POST IARC (1990)****PAGE 3-2, SECTION 3.2.1:**

The Moulin *et al.* (1990) study of stainless steel and ferrochromium production workers is essentially a negative study for nickel. While elevated odds ratios were seen for nickel and/or chromium workers in a nested case control study (OR=3.4 and OR=2.75), they were not statistically elevated. In contrast, the ORs for workers definitely or possibly exposed to PAHs were 4.51 and 14.86, respectively. These ORs were statistically raised. These results agreed with the significantly high SMRs observed in the case of people hired during the early years of the plant when PAH pollution was likely to be at its highest in the

<sup>2</sup> This document has elsewhere been cited as "NIPERA, 1996". However, it should be noted that this document, which was a Criteria Document on Nickel and its Compounds, was authored by a group of independent scientists for DGV (Drs. Agius, Crawford, Goyer, Hewitt, Mark, Rappaport, Skopek, Templeton, Vincent, and Zatka). NIPERA served purely in a coordinating and editing capacity.

ferrochromium workshops. The authors of the study concluded that their findings clearly suggested that the excess of deaths from lung cancer seen in the cohort (no nasal cancers were observed) was attributable to former PAH exposures in the ferrochromium production workshops rather than to exposures in the stainless steel manufacturing areas. Similar attributions of cancer risk to PAH exposures have been seen among nickel/copper smelter and refinery workers in Sudbury (Verma *et al.*, 1992). In a second study by Moulin *et al.* (1992), in which data from a second factory was added to the original study, the overall SMR for lung cancer, again, was not significantly raised at 130 (95% CI 94-175).

**PAGE 3-3, SECTION 3.2.3:**

It would be worth noting in the discussion of the Simonato *et al.* (1991) study, that the complex nickel oxides found in welding stainless steel do not contain copper. This is important because one of the predominant theories for the existence of lung and nasal cancer in nickel refinery workers in the past was due to their exposure to nickel-copper oxides, per se, rather than other complex nickel oxides. Lack of evidence of excess cancer deaths in workers in nickel-using industries (*e.g.* stainless steel and high nickel alloy workers) and producing industries where exposures were predominantly to silicate oxides or complex nickel oxides free of copper (New Caledonia, Oregon) lends credence to this theory. Only in workers who were exposed to high concentrations of nickel-copper oxides have excess respiratory cancers been seen.

**PAGE 3-4, SECTION 3.2.4:**

The two studies regarding the Finnish nickel refinery workers (Karjalainen *et al.*, 1992; Anttila *et al.*, 1998) and the up-date of the Norwegian refinery workers (Andersen *et al.*, 1996) require additional analysis from that which has been presented in this document. The interpretation and discussion of the epidemiologic findings, particularly with respect to soluble nickel exposures, are oversimplified. In particular, the papers inadequately discuss several factors that argue against the authors' conclusions that soluble nickel is mainly responsible for the elevated respiratory cancer risks in these cohorts. As indicated by the discussion that follows, the author's conclusions are largely speculative, as alternative hypotheses for the observed respiratory cancer risks are equally plausible.

**The Norwegian Study**

It is true that excess lung and nasal cancer risks were observed among Kristiansand workers in the electrolysis department exposed mainly to soluble nickel (ICNCM, 1990; Andersen *et al.*, 1996). However, it should be noted that insoluble nickel was also present. In fact, it was the presence of greater amounts of insoluble nickel at Kristiansand that was believed to account for the differences seen in cancer risks between electrolysis workers at Kristiansand and Port Colborne, Sudbury (ICNCM, 1990). Both groups of workers were exposed to approximately similar concentrations of soluble nickel (they were slightly higher at Kristiansand), but insoluble nickel concentrations at Kristiansand were seven times those at Port Colborne. Only the Kristiansand workers developed excess lung cancers.<sup>3</sup> The conclusions reached by Andersen *et al.* in their 1996 follow-up of the cohort, therefore, are not materially different from the ICNCM conclusions. This would be expected in as much as most of the cohort was hired prior to 1960 and a considerable amount of the follow-up in the latter study (at least 24 years) had already occurred at the time the cohort was studied by the ICNCM.

What is unfortunate in the up-dated study is that, while Andersen *et al.* noted the association of lung cancer with soluble nickel exposures, they failed to thoroughly explore the likely role of soluble nickel acting indirectly as a promoter of lung cancer in cigarette smokers. Indeed, the most important new information to be derived from the Andersen follow-up is the prominent role that cigarette smoking played in the lung cancers seen at Kristiansand. A distinctly synergistic

<sup>3</sup> With respect to nasal cancers, Andersen *et al.* concluded that the evidence for linking nasal cancer to oxidic nickel exposures was much stronger than it was for soluble nickel. Further, no new nasal cancers have occurred in Kristiansand workers first employed since 1956, strongly suggesting that the nasal cancer cases seen in this study were linked to the early mixed exposures of insoluble and soluble nickel at relatively high concentrations.

lung cancer response between smoking and exposure to the mixture of soluble and insoluble nickel compounds that the workers were exposed to was observed. In the small number of nickel-exposed workers who did not smoke, there was no evidence that nickel exposures increased the risk for lung cancer (see Appendix C for further comments on this study). A similar lack of excess respiratory cancers was noted in a 1996 cancer mortality study in a relatively small population of nickel platers exposed solely to nickel chloride and sulfate mists (Pang *et al.*, 1996). This study should be included in the NTP Draft RoC Background Document.

The results from the above studies are in good agreement with the original theory advanced by the ICNCM that the role of soluble nickel was likely the enhancement of the carcinogenicity of other agents present, including insoluble nickel compounds and, as strongly suggested by the recent Andersen study, cigarette smoking.

### **The Finnish Studies**

In the Finnish refinery studies, three nasal cancer cases were identified and a 2-fold increase in lung cancer risk was found in nickel workers with more than 20 years employment. While these cancers have been attributed to soluble nickel exposures at fairly low levels, this claim is not well-supported.

First, it is questionable whether the "low-levels" of soluble nickel reported in these studies are pertinent to the analyses of the respiratory cancers seen in these workers. The use of 1979-1980 exposure measurements (reported to be below 0.5 mg Ni/m<sup>3</sup>) as the basis for the analyses of cancers that were likely induced in the 1960s (particularly the nasal cancers) is questionable. Data available from the company suggest that earlier exposures--not only to soluble nickel, but also insoluble nickel and acid mists containing sulfuric acid--may have been higher. Technological changes purposely implemented to lower exposures prior to 1980 bear this out (see appendix).

Second, in the case of the lung cancers, smoking data are unavailable for these workers. As indicated in the above study on Norwegian electrolysis workers, such data would be helpful in interpreting the significance of the lung cancers seen in the Finnish workers. A smoking prevalence in the Finnish workers similar to that observed in the Norwegian workers could readily explain the increased lung cancer rates seen in this study. This needs to be examined further.

Third, in the case of the nasal cancers, even though the Finnish workers may have been predominantly exposed to soluble nickel during their employment at the refinery, their previous job experiences as well as concomitant exposures to insoluble nickel compounds and acid mists make the establishment of a causal association with soluble nickel difficult. The very large nasal cancer risk in the Finnish workers is inconsistent with that found in other nickel refinery workers with a comparable (or higher) degree of soluble nickel exposure. It is notable that in up-dates of other cohorts, nickel-related nasal cancers have not been observed in workers first employed since around the mid-1950s. Adequate follow-up time exists for many of these workers. While it might be argued that the ability to detect such rare cancers in occupational workers is limited, if soluble nickel is really as potent a nasal cancer inducer as some would have the regulatory community believe, it is curious that only in the Finnish cohort have nasal cancers been detected in workers first employed since the mid-century. As these findings are inconsistent with all other studies on nickel workers, careful scrutiny must be given to these nasal cancers.

In short, there are many problems surrounding the Finnish studies and further information critical to their interpretation is required. A thorough discussion of these problems (and additional information) is provided in Appendix D.

## 2.6. OTHER OCCUPATIONAL EXPOSURE STUDIES

### PAGES 3-6 THROUGH 3-7, SECTION 3.3:

First, data from the Wortley *et al.* (1992) and Horn-Ross *et al.* (1997) have been mixed-up in the NTP Draft RoC Background Document. A job matrix was used in the Wortley study, not the Horn-Ross study.

More importantly, it is highly questionable whether the studies by Wortley *et al.*, (1992) and Horn-Ross *et al.*, (1997) show any association of occupational exposure to nickel and cancer. In the case of Wortley *et al.*, the authors, themselves, note that potential exposure to chromium or nickel was not associated with significantly increased risk, nor could the exposures to the two metals be separated. Further, the fact that elevated laryngeal cancers have not been seen in other, much larger cohorts where workers have been involved in grinding operations (Arena *et al.*, 1998), suggests that the results seen in the Wortley *et al.* study may be due to chance or limitations in the design of the study (misclassification in job titles, multiple exposures, multiple statistical comparisons, *etc.*).

The Horn-Ross study is even more questionable as a useful source of information in that a self-reporting questionnaire was used to determine whether workers were "exposed" to nickel. Scanty information is provided on the questionnaire, and it is indeterminate whether the questionnaire was properly validated. The reader is only told that phrasing of questions was drawn from validated instruments "whenever possible." More importantly, although salivary cancer may be rare, elevated rates of it have never been reported in any other individual nickel cohorts studied, nor in the pooled analysis of cancer data in the ICNCM study. This lack of substantiating evidence from other nickel studies--some of which are very large (30,000-50,000+ workers)--renders the salivary cancers "found" in the Horn-Ross study particularly suspect. Either this study should not be reported, or its deficiencies and inconsistencies should be clearly noted.

## 2.7. EXPERIMENTAL CARCINOGENESIS

### PAGE 4-1, SECTION 4.2.1, PARAGRAPH 3:

Evaluation of the adrenal tumorigenicity data from the NTP rat studies of inhalation exposure to nickel subsulfide and nickel sulfate hexahydrate demonstrated that at equivalent exposures of nickel in the nickel subsulfide-treated and nickel sulfate hexahydrate-treated animals, there was a completely different response with regard to the occurrence of pheochromocytomas. Inhalation of 0.1 mg Ni/m<sup>3</sup> of nickel subsulfide caused an increase in this spontaneously occurring tumor while inhalation of 0.1 mg Ni/m<sup>3</sup> of nickel sulfate hexahydrate did not. This is a particularly important observation given the fact that the water soluble nickel sulfate hexahydrate would have caused higher blood Ni<sup>2+</sup> levels than the poorly soluble nickel subsulfide. Higher blood Ni<sup>2+</sup> would have resulted in higher Ni<sup>2+</sup> levels in the adrenal medulla. The lack of adrenal tumors in the nickel sulfate hexahydrate treated animals **clearly suggests that the nickel ion is not responsible for the induction of these tumors.** Given that pheochromocytomas are spontaneously occurring endocrine tumors in the Fisher 344 rat, it is likely that the increase in these tumors over control levels seen in the nickel subsulfide and nickel oxide studies are related to secondary effects on endocrine homeostasis at the toxic doses of these compounds that were used in the studies.

It should also be noted that a similar response was observed in animals that inhaled talc (NTP, 1993). This response may be a particle effect-related response although it was not observed in animals that inhaled antimony trioxide or titanium dioxide. Ultimately, the significance of these tumors is unclear, but the NTP's own data show that they cannot have occurred as a direct effect of the nickel ion in the adrenal medulla!

### PAGES 4-2 TO 4-4, SECTION 4.2.1-3:

It is disturbing that the discussion on animal carcinogenicity post IARC, would include just a cursory presentation of the data derived from the well-conducted animal bioassay in rats and mice by relevant

route of exposure (NTP, 1996), while focussing the discussion on the results of studies conducted by irrelevant routes of exposure, such as intraperitoneal, intrarenal or intramuscular, at one particular laboratory. Other post 1990 studies were ignored (*e.g.*, Muhle *et al.*, 1992).

**PAGE 4-4, SECTION 4-2-3:**

Even though the document focuses almost exclusively on the studies coming from one research group, the presentation and interpretation of the results from these studies is misleading and inaccurate. In the discussion of the Kazprzak *et al.* (1990) study, it should be noted that after 96 weeks the group of animals exposed solely to nickel acetate by intraperitoneal injection did not get excess tumors compared to saline controls (only one adenoma among 23 rats was found). Therefore, nickel acetate was not shown to be carcinogenic to rats by i.p. injection. A group of animals exposed solely to sodium barbital did get excess tumors (6 of 24 animals had adenomas, some had more than one), indicating complete carcinogenic activity for this compound in the rat kidneys. This important control is not included in Table 4-1. In the presence of nickel acetate and sodium barbital more tumors were observed (13 of 24 animals had adenomas and 4 of them had carcinomas). These results are consistent with a possible "enhancing" role for soluble nickel in the kidney rather than an initiator/complete carcinogen role. These results are also in agreement with the results from the Kurokawa *et al.* (1985) study.

In the Diwan *et al.* (1992) study, again intraperitoneal injection of nickel acetate by itself fails to induce kidney tumors in the offspring of treated female rats. These results confirm the lack of kidney carcinogenicity seen with nickel acetate alone by Kazprzak *et al.* (1990). Surprisingly, this study shows three-times as many pituitary tumors in offspring of nickel acetate treated rats (42%) than in offspring of sodium acetate ones (13%). It should be noted that the historical data for the Fischer 344 rat indicate an average of 23 percent and 45 percent pituitary adenoma incidence for males and females, respectively (Haseaman *et al.*, 1990). The observed increases in pituitary tumors in offspring of animals treated with nickel acetate may be explained by a disruption of the endocrine system due to the toxic effects of the Ni<sup>2+</sup> ion (quite evident in this study with 88% pup mortality) rather than to a carcinogenic effect. It has been shown that in the rat, pituitary tumors can occur as a consequence of hormonal disruption (Mennel, 1978). The lack of synergistic effects between sodium barbital and nickel acetate, as well as the lack of pituitary tumors in other studies (with soluble and insoluble nickel compounds) such as: transplacental study (Sunderman *et al.*, 1981), intraperitoneal study (Kasprzak *et al.*, 1990), oral studies (Ambrose *et al.*, 1976; Schoeder and Mitchener 1975), and inhalation NTP studies (NTP 1996) are consistent with this explanation. In addition no pituitary tumors have been detected in human epidemiologic studies.

The whole animal section should be rewritten with careful consideration of interpretation and conclusions derived from all the animal studies available in the nickel literature.

## **2.8. GENOTOXICITY**

The section on genotoxicity and mechanism of carcinogenesis shows a very poor understanding of the significance and limitations of *in vitro* assays. In some cases, it appears that only the title of the articles cited were reviewed. Some examples of mistakes or omissions are listed below:

**PAGE 5-3, PARAGRAPH 3:**

It is stated that nickel sulfate induced transformation to anchorage-"dependent" instead of "independent" growth of primary human foreskin fibroblasts. This is not just a typo since it is repeated again on page 5-4 for nickel acetate.

**PAGE 5-3, PARAGRAPH 3:**

Regarding the work by Tveito *et al.* (1989), it is reported that "human fetal kidney cortex explants did not become tumorigenic after 70-100 days of exposure to nickel sulfate." This statement is correct. However, some of the other important findings in this work were not reported while the same type of

findings in other studies were. For example, the fact that exposure to nickel sulfate resulted in immortalization and growth in soft agar (anchorage independence) was not reported.

**PAGE 5-5, PARAGRAPH 4:**

It is reported that "... $Ni^{2+}$  was effective in causing 8-OH-dG formation and double strand breaks in calf thymus DNA." What was not reported is that this effect was only seen when significant concentrations of *t*-butyl hydroperoxide and glutathione were also added to the reaction. Addition of 1 mM nickel chloride by itself did not cause any induction of oxidative damage.

A review of the genotoxicity data indicates that, in general, nickel compounds are not very genotoxic in standard *in vitro* assays. Nickel compounds have not been shown to induce gene locus mutations, but DNA strand breaks, chromosomal aberrations, and cell transformation have been consistently observed. It should be noted that although there are differences in the concentrations needed to see these effects, both soluble and insoluble nickel compounds can induce them. In general, much higher concentrations of soluble nickel compounds than of more insoluble nickel compounds are needed to see an effect. These results can be reconciled with the negative animal respiratory carcinogenicity data for soluble nickel compounds that have been discussed above. As mentioned before, ***it is the availability of nickel ions at nuclear sites within target cells that is important for carcinogenesis.*** *In vivo*, the clearance of soluble nickel compounds is so fast, nickel ion their transport into the cells is so inefficient (*i.e.*, nickel competes with mM levels of magnesium for transport via magnesium channels), and the affinity of nickel ions for proteins in the cytoplasm is so strong, that no accumulation of nickel ions at nuclear sites is expected to occur. When animals or humans are exposed to soluble nickel compounds by inhalation, the toxic response to soluble nickel compounds is evident before high enough concentrations of nickel ions can accumulate in the nucleus and cause heritable changes (see data from NTP 1996c report). *In vitro*, however, there is no clearance, and if high enough concentrations are added to the culture, the nuclear effects of nickel ions will also be observed with soluble nickel compounds.

A consideration of the interrelationship among clearance, toxicity, and availability of nickel ions at target sites needs to be taken into account when extrapolating *in vitro* results to the *in vivo* situation.

**PAGE 5-5, PARAGRAPH 5:**

Again, the data from another Kasprzak study (Kasprzak *et al.*, 1997) is incorrectly described. Male rats were injected with 90  $\mu$ moles (or 23 mg) nickel acetate tetrahydrate per kg body weight. The formation of oxidative damage in DNA isolated from liver or kidney tissue was examined as a function of time. Several oxidative lesions were found to be increased 1 day after injection. The increases in both tissues were very small (*e.g.*, 8-OH-dG went from 12.18 to 17.69 mol 8-OH-dG/ $10^5$  mol of dG in kidney and from 10.20 to 14.95 mol 8-OH-dG/ $10^5$  mol of dG, in liver). Some lesions persisted more in kidney ( $\sim$ 15 mol 8-OH-dG/ $10^5$  mol of dG in treated versus 12.5 in controls) after 14 days, than in liver ( $\sim$ 11 mol 8-OH-dG/ $10^5$  mol of dG versus 10 in controls). The NTP Draft RoC Background Document considers that these results are an indication of the "*tissue-specific [kidney] response to Ni(II)-mediated oxidative DNA damage*", and that they are "*consistent with the kidney as primary target for Ni(II) carcinogenicity from soluble salts.*" These conclusions have to be seriously questioned based on the following facts:

- It cannot be concluded that nickel-induced oxidative damage is tissue specific to the kidney when only kidney and liver were looked at and when the increases at these two tissues were similar at 5.5 and 4.8 8-OH-dG/ $10^5$  dG, respectively.
- Kidney is not the target organ for the carcinogenicity of certain nickel compounds. Animals and humans exposed by inhalation to certain nickel compounds experienced lung and/or nasal sinus tumors only. Animal exposed to nickel acetate by intraperitoneal injection did not get kidney tumors either. In addition, the NTP studies of nickel sulfate hexahydrate also showed no effects on the kidneys of exposed rats. This is particularly important since the nickel sulfate hexahydrate study would have had higher peak blood levels of nickel ion at equivalent doses than either the nickel oxide or nickel subsulfide studies. This is due to the dissolution (solubilization) rates of the different nickel

compounds in the respiratory tract (a fact which should have been made clear in Page 1-1, Paragraph 5 – see the corresponding comments for that section).

- Only male rats exposed to sodium barbital, with or without prior intraperitoneal injection of sodium acetate, got kidney tumors. Of the animal studies conducted with nickel subsulfide, only rats injected intrarenally got kidney tumors.

A reference is made in the NTP Draft RoC Background Document to another study by the Kasprzak group (Higinbotham *et al.*, 1992). In this study 12 renal tumors induced by intrarenal injection with nickel subsulfide were analyzed for mutations at the K-ras oncogene. Only one tumor showed a mutation in codon 12 of the K-ras gene. This mutation was a G to T transversion. According to the NTP Draft RoC Background Document this mutation is the result of a 8-OH-dG lesion induced by nickel. This conclusion seems premature at best, given that it is not known with certainty whether the observed oncogene mutations seen in some tumors (ras, p53) are directly related to the treatment that induced that tumors or simply the result of selection by the treatment of preexisting mutations. Analysis of mutations at the p53 gene of the nickel subsulfide-induced tumors mentioned above failed to reveal any changes (Weghorst *et al.*, 1994). A further study of the human kidney cells immortalized *in vitro* by exposure to nickel sulfate (Tveito *et al.*, 1989) revealed clones with T to C transition mutations (rather than G to T transversions) in the p53 gene (Maehle *et al.*, 1992). What does it all mean? At present, the significance of these findings remains to be determined.

## 2.9. OTHER RELEVANT DATA

### PAGE 6-2, SECTION 6.2, PARAGRAPH 3:

The information in the last paragraph of this section comes from a study by Sunderman and co-workers (1989). This study is cited but not referenced. The importance of fasting is critical to the results and understanding of this study. Intestinal absorption of nickel will largely depend upon the presence of food already in the stomach and the type of food ingested (Solomons *et al.*, 1982; Foulkes and McMullen, 1986). It should be clarified in the text that the maximum absorption of nickel from an oral dose of nickel sulfate (25%) was only observed in volunteers that fasted overnight before drinking water. Under more common intake conditions about 5% of nickel will be absorbed orally.

### PAGE 6-2, SECTION 6.3, PARAGRAPH 4:

The NTP Draft RoC Background Document states that "*In blood and urine, soluble nickel compounds and nickel metal powder are more easily measured than less soluble nickel compounds (Sunderman et al., 1986).*" This statement is incorrect. The only thing that can be found and measured in blood and urine is the Ni<sup>2+</sup> ion. What the Sunderman paper concluded was that monitoring Ni<sup>2+</sup> levels in blood or urine could be useful as an indication of exposure to soluble nickel compounds or very finely divided nickel metal powder. However, these parameters would not be useful to evaluate inhalation exposure to less soluble nickel compounds due to the slow lung clearance of these compounds.

## 2.10. MECHANISMS OF CARCINOGENESIS

### PAGE 7-1, SECTION 7.0, PARAGRAPHS 1-2:

The first two paragraphs in this section indicate that the fact that high concentrations of soluble nickel compounds can, in some studies, induce DNA damage *in vitro* and *in vivo* (after injection), is a demonstration that the ionic nickel may be the carcinogenic agent. A more appropriate conclusion would be that because soluble and insoluble nickel compounds can produce some level of DNA damage *in vitro* and *in vivo*, the nickel ions present at cellular nuclear sites appear to have the potential to cause adverse genotoxic effects. Whether this genotoxic potential will translate or not into carcinogenic potential will depend on many other factors (such as route of exposure, particle size, solubility of the compound, clearance, *etc.*) that will ultimately determine the availability of nickel ions at nuclear sites within target

cells. Animal and human data needs to be used to ultimately determine the carcinogenic potential of the individual nickel compounds.

**PAGE 7-1, PARAGRAPH 3:**

The animal data for soluble compounds are indeed reviewed on paragraph 3. However, only the Kasprzak studies by intraperitoneal route are mentioned excluding the other dozen negative studies, by relevant routes of exposure and in multiple animal species. The significance of the intraperitoneal studies is further obfuscated by failing to mention the very unique conditions under which rats developed renal tumors (males only, in the presence sodium barbital exposure only) and the high toxicity experienced by the pups with pituitary tumors in the transplacental carcinogenicity study.

It is surprising that again, the NTP inhalation studies (1996) with nickel subsulfide, high temperature (green) nickel oxide and nickel sulfate hexahydrate in two animal species were not mentioned at all.

**PAGE 7-1, PARAGRAPH 4:**

The next few paragraphs in the NTP Draft RoC Background Document address a possible mechanism for nickel-induced carcinogenesis. It is apparent that the authors of this section of the NTP Draft RoC Background Document do not quite understand some of the issues pertaining to respiratory tract physiology. Two different issues are of concern for inhalation exposure to nickel compounds: toxicity and carcinogenicity. With regard to toxicity, damage to lung cells appears to occur by the action of nickel ions at the cell surface or in the cytoplasm, due to the great affinity of nickel ions for proteins. In this regard, soluble nickel compounds are toxic to the lungs of animals at lower concentrations than insoluble nickel compounds are. Toxicity for particulate nickel compounds appears to be related to their solubility in biological fluids<sup>4</sup>.

With regard to carcinogenicity, the target cells for tumors in the lung are epithelial cells. The lungs contain a mucociliary escalator that moves particles up towards the throat for elimination by oral route. They also contain alveolar macrophages. It is the function of the macrophages to phagocytize foreign particles and bacteria that get deposited deep into the lung. Macrophages possess a very specialized way of engulfing particles and disposing of them by dissolving them under acidic pH in the phagosomes or carrying them via the lymphatic system for elimination. They also have a great capacity to generate oxidative damage. If the above mentioned mechanisms fail to completely eliminate all the particles (high exposure), lung epithelial cells themselves may come in contact with particles. All cells, including lung epithelial cells, have the ability to endocytize particles to varying degrees. If the epithelial cells endocytize nickel-containing particles (insoluble), nickel ions may be released inside the acidic endocytic vesicles. Furthermore, these vesicles appear to fuse with the nuclear membrane allowing a high pulsatile delivery of nickel ions to nuclear chromatin. Once in the nucleus, nickel ions can replace magnesium binding to histones in the chromatin. It is not clear what the exact changes caused by nickel ions are that can result in tumor induction (*e.g.*, changes in chromatin condensation, oxidative damage, *etc.*). In this case, the bioavailability of nickel ions at nuclear sites within the epithelial cells will be greater for particulate compounds of intermediate solubility (*e.g.*, nickel subsulfide). In contrast, soluble nickel compounds are rapidly eliminated by dissolution into the blood and excretion through the kidney. Soluble nickel compounds cannot be endocytized by epithelial cells (*i.e.*, they quickly dissociate to free ions hence, there are no particles to endocytize). Therefore, the only way in which soluble nickel compounds could cause a high accumulation of nickel ions in the nucleus of epithelial cells is by being present in the lung at such high concentrations that even with rapid clearance they can compete with magnesium (mM) levels for uptake into the cytoplasm. They also have to be able to concentrate in the cytoplasm at high enough levels to reach the nucleus in spite of their high binding affinity for cytoplasmic proteins. *In vivo*, this will not be achieved due to the high toxicity of nickel ions that will be manifested before such high inter- and intracellular concentrations of nickel ions can be achieved.

<sup>4</sup> For low-solubility particles, toxicity and carcinogenicity may occur at high concentrations through a secondary mechanism related to impaired clearance.

The NTP Draft RoC Background Document continues to confuse the concepts of toxicity and carcinogenicity as shown in the following examples:

**PAGE 7-2, PARAGRAPH 1:**

It is mentioned here that *"Tumor induction was thought to be related to ...or by the ability of the cell to incorporate the compound (e.g., phagocytosis). However, Kasprzak and Ward (1991) found that stimulated phagocytes, rather than enhancing carcinogenic response, actually strongly inhibited muscle tumor development in rats injected with nickel subsulfide."* It is expected that stimulated phagocytosis by macrophages will have the opposite effect on tumor induction (by decreasing particle availability to target cells) than stimulated endocytosis by target cells would (by increasing nickel ion availability at nuclear sites). By contrast, the language in the NTP Draft RoC Background Document suggests that all processes of phagocytosis by macrophages or endocytosis by target cells are irrelevant for tumor induction.

**PAGE 7-2, PARAGRAPH 2:**

It is mentioned here that *"...particles dissolved in the acidic pH of cytoplasm."* This is incorrect, cytoplasm is not acidic, phagosomes or endocytic vesicles are.

The last two sentences of this paragraph were obviously intended by the NTP Draft RoC Background Document authors to support each other. Unfortunately, although each statement is correct by itself, they do not relate to each other in the context of this document. The authors have confused phagocytosis by macrophages, a protective mechanism that reduces the toxicity/carcinogenic potential of insoluble nickel compound particles, and endocytosis by respiratory epithelial cells which precipitates tumor formation. Enhancing macrophage phagocytosis will enhance clearance of particles reducing the chance that epithelial cells will endocytize particles and become transformed.

**PAGE 7-3, PARAGRAPH 2:**

The first sentence in this paragraph is wrong. Soluble nickel salts are not less toxic than insoluble nickel compounds in animal models. The NTP Draft RoC Background Document appears to be misquoting the following statement included in Costa (1991) and cited in Costa 1995: *"Water soluble nickel salts are generally less carcinogenic in experimental animals because they do not get taken up to a degree similar to that for the particulate nickel compounds that yield high concentrations of nickel inside cells."*

**PAGE 7-3, PARAGRAPH 2:**

In this paragraph, the Kasprzak intraperitoneal studies are cited again as compelling evidence for the carcinogenicity of soluble nickel compounds, the NTP inhalation studies are left out again, and this time the whole discussion is wrapped up with the categorical statement that *"...macrophage solubilization is not required for carcinogenesis to occur with nickel."* This is the last of the many examples in this NTP Draft RoC Background Document showing a lack of understanding of the mechanistic data (see comments for Page 7-2, Paragraphs 1 and 2).

**PAGE 7-4, PARAGRAPH 2:**

Surprisingly, the last sentence in the document makes a reasonable assessment even if it is ignored for the purposes of nickel compounds carcinogenic classification:

*"Overall, it appears that the ionic form of nickel is the ultimate carcinogenic species, and biokinetic factors may dictate the carcinogenic potential of the various soluble and insoluble nickel compounds."*

### **3. Conclusion**

NIPERA's major objection to the NTP's proposal to list *Nickel and Nickel Compounds* as "known human carcinogens" in the Ninth Biennial Report on Carcinogens is that it fails to recognize differences in the carcinogenic potential of the various forms of nickel. Each compound or species of a metal, like nickel, has its own physico-chemical properties that dictate how it behaves under a given set of conditions,

including interactions with biological organisms. Thus, the fact that one form of nickel may be carcinogenic via a particular route of exposure (*e.g.*, nickel subsulfide by inhalation) does not mean that a second nickel species will be carcinogenic as well or that the first nickel species will be carcinogenic via a different route of exposure (*e.g.*, ingestion). For nickel and its compounds, this observation holds true even if the free metal ion is assumed to be the active carcinogenic agent, because the different physico-chemical properties of various forms of the metal will largely determine the extent to which the free metal ion can be made bioavailable and delivered to a relevant biological site (*e.g.*, the nucleus of a lung epithelial cell).

Examination of the *in vitro*, animal, and epidemiologic data pertaining to commercially relevant nickel compounds<sup>5</sup> confirms that these compounds have very different biological behaviors, particularly with regard to respiratory carcinogenicity. Nickel subsulfide is likely to be carcinogenic to humans. Soluble nickel compounds, by themselves, have not been demonstrated to be carcinogenic to humans, although an enhancing (promoter) effect on other carcinogens is possible. High concentrations of oxidic nickel mixtures (*i.e.*, Ni-Cu oxides mixed with low-temperature [black] and high-temperature [green] NiO) appear to be carcinogenic in epidemiologic studies of nickel refinery workers. Exposures to nickel silicates-oxides and complex nickel oxides devoid of copper have not resulted in excess cancer risks in other human cohorts. Exposure to metallic nickel particles in the workplace does not appear to pose a respiratory carcinogenic risk for humans. Finally, nickel carbonyl is so acutely toxic that it is used in closed systems and humans are typically exposed only in accident scenarios. The high acute toxicity of nickel carbonyl has limited its examination for carcinogenic effects. The human and animal data on the potential carcinogenicity of nickel carbonyl are scant and only non-standard animals studies with exposures above the Maximum Tolerated Dose (MTD) have yielded evidence of a carcinogenic effect.

Against this background, NIPERA believes that the NTP proposal to sweep metallic nickel and all nickel compounds into the single category of "*known human carcinogens*" is inconsistent with both the epidemiological and toxicological data and is at odds with the best current understanding of the likely mechanism of nickel-related carcinogenicity.

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<sup>5</sup> The classes of commercially relevant nickel compounds are: metallic nickel, oxidic nickel (including nickel oxides, hydroxides, silicates, carbonates, and complex nickel oxides), sulfidic nickel (including nickel sulfide and subsulfide), water soluble nickel compounds (including hydrated forms of nickel acetate, sulfate, chloride, *etc.*), and nickel carbonyl. Metallic, oxidic, and sulfidic nickel compounds and nickel carbonyl are insoluble in water.

#### **4. References Cited in these Comments that are not Included in the NTP Draft RoC Background Document**

- 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.
- Arena, V. C.; Sussman, N. B.; Redmond, C. K.; Costantino, J. P. and Trauth, J. M. (1998). Using alternative comparison populations to assess occupation-related mortality risk. *Journal of Occupational and Environmental Medicine*, 40, 907-916.
- Burges, D. C. L. (1980). Mortality study of nickel platers. In: Brown, S. S. and Sunderman, F. W., Jr., eds. Nickel toxicology: Proceedings of the 2nd international conference, September, Swansea, Wales. London, United Kingdom: Academic Press, pp. 15-18.
- Foulkes, E. C. and McMullen, D. M. (1986). On the mechanism of nickel absorption in the rat jejunum. *Toxicology*, 38, 5-42.
- Gilman, J. P. W. (1962). Metal Carcinogenesis. II. A study of the carcinogenic activity of cobalt, copper, iron and nickel compounds. *Cancer Res.*, 22, 158-162.
- Haseman, J. K.; Eustis, S. L.; and Arnold, J. (1990). Tumor Incidences in Fischer 344 Rats: NTP Historical Data. In: *Pathology of the Fischer Rat: Reference and Atlas*, edited by Boorman, G.A.; Eustis, S.L.; Elwell, M.R.; Montgomery, Jr., C.A.; and MacKenzie, W.F., pp. 555-564, Academic Press, San Diego, California.
- IARC (1992). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Occupational Exposures to Mists and Vapors from Strong Inorganic Acids and Other Industrial Chemicals; v. 54. Geneva, Switzerland: World Health Organization.
- Kasprzak, K. S., Gabryel, P., and Jarczewska, K. (1983). Carcinogenicity of nickel (II) hydroxides and nickel(II) sulfate in Wistar rats and its relation to the *in vitro* dissolution rates. *Carcinogenesis* 4, 275-279.
- Kurokawa, Y.; Matsushima, M.; Imazawa, T.; Takamura, N.; Takahashi, M. (1985). Promoting effect of metal compounds on rat renal tumorigenesis. *J. Am. Coll. Toxicol.* 4, 321-330.
- Maehle, L.; Metcalf, R. A.; Ryberg, D.; Bennett, W. P.; Harris, C. C.; and Haugen, A. (1992). Altered p53 gene structure and expression in human epithelial cells after exposure to nickel. *Cancer Res.*, 52, 218-221
- Mennel, H.D. (1978). Transplantation of tumors of the nervous system induced by resorptive carcinogens. *Neurosurg. Rev.*, 1, 123.
- Moulin, J. J., Mantout, B., Portefaix, P., Wild, P., Fournier-Betz, M., Mur, J. M., and Smagghe, G. (1992). Etude épidémiologique de mortalité dans deux aciéries d'acier inoxydable. [Historical prospective mortality study in two stainless steel factories]. *Arch. Mal. Prof. Med. Trav. Secur. Soc.*, 53, 157-166.
- Muhle, H., Bellman, B., Takenaka, S., Fuhst, R., Mohr, U., and Pott, F. (1992). Chronic effects of intratracheally instilled nickel-containing particles in hamsters. In: Nickel and Human Health: Current Perspectives. Nieboer, E.; Nriagu, N. O., eds. New York, NY: John Wiley & Sons, Inc. p. 467-479.
- NTP (National Toxicology Program) Technical Report. (1993). Toxicology and carcinogenesis studies of talc (CAS No. 14807-96-6) in F344/N rats and B6C3F<sub>1</sub> mice (inhalation studies). NIH publication No. 93-3152.
- Pang, D., Burges, D. C., and Sorahan, T. (1996). Mortality study of nickel platers with special reference to cancers of the stomach and lung, 1945-93. *Occup. Environ. Med.*, 53, 714-717.
- Payne, W. W. (1964). Carcinogenicity of nickel compounds on experimental animals. *Proc. Am. Assoc. Cancer Res.* 5, 50.
- Schroeder, H. A., Balassa, J. J., and Vinton, W. H. (1964). Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumors and tissue levels. *J. Nutr.*, 83, 239-250.
- Schroeder, H.A., Mitchener, M., and Nason, A.P. (1974). Life-term effects of nickel in rats: survival, tumors, interactions with trace elements and tissue levels. *J. Nutr.*, 104, 239-243.
- Schroeder, H. A., and Mitchener, M. (1975). Life-term effects of mercury, methyl mercury, and nine other trace metals on mice. *J. Nutr.*, 105, 452-458.

Solomons, N. W., Viteri, F., Shuler, T. R., and Nielsen, F. H. (1982). Bioavailability of nickel in man: Effects of foods and chemically-defined dietary constituents on the absorption of inorganic nickel. *J. Nutr.*, 112, 39-50.

Steenland, K.; Schnorr, T.; Beaumont, J.; Halperin, W.; and Bloom, T. (1996) Incidence of laryngeal cancer and exposure to acid mists. *Br. J. Ind. Med.*, 45, 766-776.

Sunderman, F. W.; McCully, K. S.; and Rinehimer, L. A. (1981). Negative test for transplacental carcinogenicity of nickel subsulfide in Fischer rats. *Res. Commun. Chem. Pathol. Pharmacol.* 31, 545-554.

Sunderman, F. W., Jr., Hopfer, S. M., Swenney, K. R., Marcus, A. H., Most, B. M., and Creason, J. (1989). Nickel absorption and kinetics in human volunteers. *Proc. Soc. Exp. Biol. Med.*, 191, 5-11.

Verma, D. K.; Julian, J. A.; Roberts, R. S.; Muir, D. C. F.; Jadon, N.; and Shaw, D. S. (1992) Polycyclic aromatic hydrocarbons (PAHs): A possible cause of lung cancer mortality among nickel/copper smelter and refinery workers. *Am. Ind. Hyg. Assoc. J.*, 53, 317-324.

Weghorst, C. M.; Dragnev, K. H.; Buzard, G. S.; Thorne, K. L.; Vandeborne, G. F.; Vincent, K. A.; and Rice, J. M. (1994). Low incidence of point mutations detected in the p53 tumor suppressor gene from chemically induced rat renal mesenchymal tumors. *Cancer Res.*, 54, 215-219.

**APPENDIX A**

**REVIEW OF THE STUDY BY DIWAN *ET AL.* TITLED, *TRANSPLACENTAL CARCINOGENIC EFFECTS OF NICKEL(II) ACETATE IN THE RENAL CORTEX, RENAL PELVIS AND ADENOHYPOPHYSIS IN F344/NCR RATS.***

An examination of the potential for nickel to induce transplacental carcinogenesis was conducted by Diwan and co-workers (1992) at the U.S. National Cancer Institute. In that study, the soluble compound, nickel acetate (NiAct), was administered to pregnant Fischer 344 rats by intraperitoneal injection during the last third of their gestation period. Three treatment groups were utilized; the first exposed to 90  $\mu\text{mol/kg}$  of nickel acetate dissolved in distilled water on gestation day 17 (the day mating was confirmed was designated gestation day 1); the second exposed to 45  $\mu\text{mol/kg}$  of nickel acetate on each of days 16 and 18 of gestation; and the third also exposed to 45  $\mu\text{mol/kg}$  of nickel acetate, but on each of gestation days 12, 14, 16, and 18. Control animals were exposed to 180  $\mu\text{mol/kg}$  of sodium acetate on gestation day 18. The animals were allowed to deliver and nurse their young. After weaning, the pups were randomly divided into two subgroups (A and B) within each prenatal exposure group. The A subgroups were maintained on-study with no further treatment while the B subgroups were administered the tumor promoter, sodium barbital (NaBB), in their drinking water at a 4 percent concentration from the fourth week postpartum until the study end at 85 weeks postpartum. All the pups on this study were necropsied for histopathological assessment upon their death or at the study termination.

The authors reported that the four day exposure regimen used for the animals in group three resulted in 100 percent mortality. In examining the offspring in groups one and two they stated that nickel acetate was a complete carcinogen for the induction of pituitary tumors seen in the A subgroups which were not exposed to the tumor promoter NaBB. In addition, sodium barbital (NaBB)-promoted renal tumors were observed in both male adult rats administered nickel acetate (an earlier study by Kasprzak *et al.* employing a single i.p. injection of 90  $\mu\text{mol NiAct/kg bw}$ ) and the male offspring of dams administered NiAct (either single i.p. injection, 90  $\mu\text{mol NiAct/kg}$  on day 17 of gestation or two i.p. injections, 45  $\mu\text{mol NiAct/kg}$  each, on days 16 and 18 of gestation). Animals administered NiAct but not NaBB did not develop renal tumors. The authors concluded that nickel acetate initiated the formation of renal cortical and pelvis (medullary) tumors in the two surviving B subgroups exposed to the tumor promoter NaBB during their lifetimes.

#### **Study Critique**

A number of considerations in the design, conduct and interpretation of this study cast doubt on the validity of the conclusion reached by Diwan and co-workers. These considerations are discussed as follows:

#### **Sodium Barbital Carcinogenicity**

Subsequent work by these authors (Kurata *et al.*, 1993) has demonstrated that sodium barbital by itself is a nongenotoxic nephrotoxicant carcinogen and an inducer of neoplastic tubular lesions in the Fisher 344/NCr rat. The fact that NaBB by itself acted as a complete kidney carcinogen (initiation and promotion) while NiAct by itself did not, can certainly not be used as evidence for the carcinogenicity of soluble nickel compounds.

Diwan *et al.* (1992) concluded that NiAct was behaving as a tumor initiator in their study. An alternative explanation for the increased presence of tumors in NaBB-exposed animals whose mothers received NiAct is possible and more in agreement with the rest of the animal and human data on soluble nickel compounds. The presence of Ni ions during kidney development in the fetus could have caused toxicity and increased cell proliferation leading to a greater fixation of spontaneous lesions. The increased number of lesions at birth would not, by themselves, result in tumors unless they were promoted further in males by NaBB. This explanation is consistent with a promoter rather than an initiating role for soluble nickel compounds.

#### **Design Considerations**

Review of this study revealed a number of design flaws. Specifically, the study was designed with a small number of  $F_1$  animals (approximately 60/group; 30/subgroup A and B) which were assessed for transplacental carcinogenicity. The authors do not say how many dams were actually treated in this study, but the Fischer 344 rat has a litter size of approximately nine pups indicating that either there was a large incidence of postnatal mortality (*i.e.* offspring dead at birth) or the number of litters that were treated with nickel acetate was approximately 8/group. The data from this study were erroneously analyzed on the basis of the pup as the unit of statistical significance. The authors stated that they had an  $n$  of approximately

30/subgroup. The smallest "unit" that can be individually treated in a study such as this is the litter; therefore, the actual  $n$  for this study is the number of maternal animals treated in each group (*i.e.* approximately 8/group). This design flaw is further exacerbated by an inappropriate selection of statistical methods for the analysis of some of the data generated in this study. Specifically, t-test's were used to analyze data from multiple groups. This approach increases the possibility of obtaining a false positive result.

### **Tumorigenicity Issues**

The results of this study indicated an increase in renal and pituitary tumors in the offspring exposed to nickel while in utero. Renal tumors in rats have been associated with a gender dependent susceptibility pattern which has been observed with a variety of renal carcinogens, including unleaded gasoline (Montgomery and Seely, 1990). This syndrome, known as  $\alpha 2\mu$ -globulin ( $\alpha 2\mu$ -g) nephropathy, has been associated with dose-related increases in renal adenomas and adenocarcinomas in male Fischer 344 rats (the rats used in this study). The EPA, after considerable study and panel review, issued a science policy that states: "Male rat renal tubule tumors arising as a result of a process involving  $\alpha 2\mu$ -g accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk" (U.S. EPA, 1991).

In this study, renal tumors were seen only in male offspring of exposed maternal animals. Diwan and co-workers stated that they did not think this syndrome was operating in their study because no lesions or hyaline droplets compatible with  $\alpha 2\mu$ -g nephropathy were found in the kidneys in male offspring of either the subgroup A (NiAct alone) or subgroup B (NiAct + NaBB) animals "in spite of careful scrutiny of histologic sections." However, Montgomery and Seely (1990) state that "...although the lesions associated with short-term exposure to chemicals causing increased  $\alpha 2\mu$ -g in the kidney may be marked and characteristic of hyaline droplet nephropathy, in some studies there are minimal changes on routine hematoxylin and eosin stained sections." They note that other staining methods must be used to detect the  $\alpha 2\mu$ -g lesions. Diwan and co-workers used only a routine hematoxylin and eosin staining technique. Without a definitive assessment of the potential role of  $\alpha 2\mu$ -g in this study, no conclusions based on the renal tumor incidence in the study should be made.

In evaluating the pituitary tumor data in this study, Diwan and co-workers concluded that nickel acetate is a complete carcinogen since it did not require the presence of a promoter (NaBB) to cause a significant increase in tumorigenicity. The tumorigenicity data for the pituitary were analyzed by the authors based on the total tumor incidence. No differentiation between adenomas and carcinomas was made in the analysis although, the incidence of each type of tumor was reported. Adenoma incidence ranged from 7 to 29 percent in the control groups and from 19 to 29 percent in the treated groups. Historical data for the Fischer 344 rat indicate an average of 23 percent and 45 percent adenoma incidence for males and females, respectively (Haseman *et al.*, 1990). Therefore, the data from this study do not support the assertion of an effect on pituitary adenoma incidence. The incidence of pituitary carcinoma in this study ranged from 7 to 31 percent in the treated subgroups, and was nonexistent in the control subgroups.

To ascertain the significance of the pituitary tumor findings in this study it should be considered that pituitary tumors can occur as a consequence of hormonal disruption in the rat (Mennel, 1978). This mechanism has not been shown to have a corollary in humans and therefore, may not be relevant for risk assessment purposes. It is possible therefore, that toxicity of nickel could disrupt endocrine homeostasis producing the indirect effect of inducing hormonal disruption in the rat which could lead to pituitary tumors. The toxic effects of the  $Ni^{2+}$  ion were quite evident in this study and resulted in 88% pup mortality. The lack of synergistic effects between sodium barbital and nickel acetate, as well as the lack of pituitary tumors in other studies (with soluble and insoluble nickel compounds) such as: a transplacental study by Sunderman *et al.* (1981), an intraperitoneal study by Kasprzak *et al.* (1990), oral studies by Ambrose *et al.* (1976) and by Schoeder and Mitchener (1975), and the inhalation studies by the NTP (1996) are consistent with this explanation. In addition no pituitary tumors have been detected in human epidemiologic studies.

### **Relevance of Route of Exposure**

Many of these studies utilize a route of exposure chosen to maximize effect rather than reproduce human exposure patterns. The relevance of the route of exposure is exemplified by Diwan and co-worker's statement that "*the realistic routes of exposure to [nickel] are air, drinking water, and food*" (Diwan *et al.*,

1992). In view of this fact, one of Diwan's co-authors conducted a study to test the transplacental carcinogenic potential of NiAct via the route of oral exposure. This study showed no increased incidence of tumors in the kidney of exposed rats (Kasprzak, 1995).

Further evidence of the inadequacy of the routes of exposure used in the studies by Diwan and co-workers can be found in the Environmental Protection Agency's 1991 revised drinking water document. The Agency states that "[M]any nickel compounds cause tumors via intraperitoneal (i.p.) or intrarenal injection. In general, these studies have found tumors only at the site of injection, although a few distant site responses were also seen. However, injection studies are not particularly relevant to human exposure." The Agency further notes that "[A]s expected from the low gastrointestinal absorption, the toxicity of nickel in animal studies is much lower by the oral route than by parenteral routes. In the excretion study by Ho and Furst (1973) no overt toxic effects were observed in anesthetized rats given an oral dose of  $\leq 64$  mg Ni/kg body weight in the form of nickel chloride. The same dose given i.p. resulted in the death of 60 % of the animals." The LD<sub>50</sub> for NiAct given i.p. is 8 mg Ni/kg body weight whereas the LD<sub>50</sub> for NiAct given orally is 116-120 mg Ni/kg body weight (Haro *et al.*, 1968).

### Inconsistencies With Other Studies

Neither the NTP inhalation study with rats and mice (NTP 1996) or several oral studies in various animal species (Schroeder *et al.*, 1974, 1964; Schroeder and Mitchener, 1975; Ambrose *et al.*, 1976) provide evidence of carcinogenicity for soluble nickel compounds. In the recent NTP inhalation study, about 100 rats and 100 mice were exposed for two years to near MTD concentrations of nickel sulfate hexahydrate and not tumors were observed at any site. The Ambrose study (1976) is particularly important because pathology was done on both adult rats in the 2-year feeding study and the F<sub>3b</sub> offspring (10 males and females each) of the reproductive study (a fact often overlooked when this study is evaluated.) Renal tissues were examined. No nickel-related tumors were found in the adult rats and no nickel-related kidney lesions were found in the offspring. This study, therefore, suggests that nickel sulfate (NiSO<sub>4</sub>), a more soluble compound than NiAct, did not behave as a complete carcinogen (in the case of the 2-year study) and did not result in kidney lesions in the offspring exposed *in utero*. The relevance of this work to the Diwan study (1992) concerns the pituitary tumors observed in that study. The renal tumors seen in that study only developed when promoted, but the pituitary tumors ostensibly were the result of a complete carcinogenic effect of NiAct which was not replicated in the Ambrose study (1976).

### Formation of DNA Adducts

In search of the mechanism of the carcinogenesis evidenced in NiAct + NaBB treated rats in Diwan's study (1992), co-workers Kasprzak and Mishra have published a series of studies detailing the formation of DNA adducts formed in the kidneys of male and female adult rats as well as their offspring. The authors theorized that 8-hydroxy deoxyguanosine (8-OH-dG) DNA adducts might be the initial damage that lead to the renal carcinogenicity. This purine adduct was found to be elevated in the kidneys of adult male rats administered NiAct (Kasprzak *et al.*, 1992). Likewise, adult male rats administered NiAct + NaBB developed tumors (Kasprzak *et al.*, 1990). The offspring (males and females combined) of dams administered NiAct had elevated 8-OH-dG levels and, likewise, the male offspring administered NiAct + NaBB developed kidney tumors (Diwan *et al.*, 1992). As further "evidence" of the 8-OH-dG/tumor induction theory, the authors noted that 8-OH-dG was not elevated in the liver and, likewise, no liver tumors developed.

The authors of these papers failed to consider the *in vitro* literature in formulating their 8-OH-dG theory. Since 8-OH-dG adducts affect single DNA base pairs the heritable mutagenic outcome of the adduct would be a point mutation. The *in vitro* literature has demonstrated conclusively that nickel does not cause point mutations. Therefore, 8-OH-dG adducts cannot be the mechanism by which renal tumors are caused in the Diwan/Kasprzak/Mishra series of studies. In fact, the concentration mechanisms of renal excretion indicate that high levels of nickel ion are probably produced within the nephrons of the kidney. It is not surprising therefore, that 8-OH-dG adducts are seen in renal DNA, but *in vitro* research has also demonstrated that there are ample repair mechanisms for such damage in the kidney. In addition, it has been demonstrated that oxygen radicals are needed to induce point mutations with nickel. Since the kidney has extremely high concentrations of the oxygen radical quenching proteins glutathione and metallothionein, it is unlikely that the 8-OH-dG adducts seen in these studies have any bearing on the induction of the renal tumors seen in rats.

In the Kasprzak 1992 paper on DNA base damage, the authors mentioned that marked sex differences in the susceptibility of rats to renal carcinogenesis have been seen and they, therefore, stated that the possible significance of 8-OH-dG formation in renal DNA "must be viewed with caution." Since it is clear that mechanism of tumor induction is extremely unlikely to involve 8-OH-dG adducts, the role of  $\alpha_2\mu$ -globulin in inducing these tumors is still the only theory that accounts for the male specific pattern of tumorigenicity seen in the Diwan/Kasprzak/Mishra studies.

### References

- Ambrose, A.M.; Larson, P.S.; Borzelleca, J.F.; and Hennigar, G.R., Jr. Long term toxicologic assessment of nickel in rats and dogs. *J. Food Sci. Technol.* 13: 181-187 (1976).
- Creason, J.P.; Svendsgaard, D.; Bumgarner, J.; Pinkerton, C. and Hinners, T. Maternal-fetal tissue levels of 16 trace elements in 8 selected continental United States communities. In: *Trace Substances in Environmental Health--X*, edited by Hemphill, D.D., pp. 53-62 (1976).
- Diwan, B.A., Kasprzak, K.S., and Rice, J.M. Transplacental carcinogenic effects of nickel(II) acetate in the renal cortex, renal pelvis and adenohypophysis in F344/NCr rats. *Carcinogenesis* 13(8): 1351-1357 (1992).
- EPA. *Alpha 2 $\mu$ -Globulin: Association With Chemically Induced Renal Toxicity and Neoplasia in the Rat.* EPA/625/3-91/019F, U.S. Environmental Protection Agency, Washington, D.C. (1991).
- Haro, R.T.; Furst, A.; and Falk, H.L. Studies on the acute toxicity of nickelocene. *Proc. West. Pharmacol. Soc.* 11:39-42 (1968).
- Haseman, J.K.; Eustis, S.L.; and Arnold, J. Tumor Incidences in Fischer 344 Rats: NTP Historical Data. In: *Pathology of the Fischer Rat: Reference and Atlas*, edited by Boorman, G.A.; Eustis, S.L.; Elwell, M.R.; Montgomery, Jr., C.A.; and MacKenzie, W.F., pp. 555-564, Academic Press, San Diego, California (1990).
- Ho, W. and Furst, A. Nickel excretion by rats following a single treatment. *Proc. West. Pharmacol. Soc.* 16: 245-248 (1973).
- Kasprzak, K. S. personal communication with NiPERA. (1995).
- Kasprzak, K. S.; Diwan, B. A.; Konishi, N.; Misra, M.; Rice, J. M. Initiation by nickel acetate and promotion by sodium barbital of renal cortical epithelial tumors in male F344 rats. *Carcinogenesis* 11: 647-652 (1990).
- Kasprzak, K. S.; Diwan, B. A.; Rice, J. M.; Misra, M.; Riggs, C. W.; Olinski, R.; Dizdaroglu, M. Nickel(II)mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis. *Chem. Res. Toxicol.* 5: 809-815 (1992).
- Kuehn, K. and Sunderman, F.W. Dissolution half-times of nickel compounds in water, rat serum, and renal cytosol. *J. Inorg. Biochem.* 17: 29-39 (1982).
- Kurata Y.; Diwan, B. A.; Uno, H.; Rice, J. M.; and Ward, J. M. pathology of preneoplastic and neoplastic renal tubular lesions induced in F-344 rats by sodium barbital, a nongenotoxic renal carcinogen and nephrotoxicant. *Toxicol. Pathol.*, 21(1):35-45 (1993).
- Mennel, H.D. Transplantation of tumors of the nervous system induced by resorptive carcinogens. *Neurosurg. Rev.*, 1: 123 (1978).
- Metzler, M. Mechanisms of Carcinogenesis Induced by Diethylstilbestrol. In: *Comparative Perinatal Carcinogenesis*, edited by Schuller, H.M., pp. 137-150, CRC Press, Boca Raton, Florida (1984).
- Misra, M.; Olinski, R.; Dizdaroglu, M.; Kasprzak, K.S. Enhancement by L-histidine of nickel(II)-induced DNA-protein cross-linking and oxidative DNA base damage in the rat kidney. *Chem. Res. Toxicol.* 6: 33-37 (1993).

Montgomery, Jr., C.A. and Seely, J.C. Kidney. In: *Pathology of the Fischer Rat: Reference and Atlas*, edited by Boorman, G.A.; Eustis, S.L.; Elwell, M.R.; Montgomery, Jr., C.A.; and MacKenzie, W.F., pp. 127-154, Academic Press, San Diego, California (1990).

NTP (National Toxicology Program) Draft Technical Report (1994c). Toxicology and carcinogenesis studies of nickel sulfate hexahydrate in F344/N rats and B6C3F<sub>1</sub> mice. NTP TR 454, NIH publication No. 94-3370.

Olsen, L. and Jonsen, J. Whole body autoradiography of <sup>63</sup>Ni in mice throughout gestation. *Toxicology*, 12:165-172 (1979).

Rice, J.M. Transplacental carcinogenesis. In: *Developmental Toxicology*, edited by Kimmel, C.A. and Buelke-Sam, J., pp. 191-212, Raven Press, New York (1981).

Schroeder, H.A.; Balassa, J.J.; and Vinton, W.H. Chromium, lead, cadmium, nickel and titanium in mice: effect on mortality, tumors and tissue levels. *J. Nutr.* 83: 239-250 (1964).

Schroeder, H.A.; Mitchener, M.; and Nason, A.P. Life-term effects of nickel in rats: survival, tumors, interactions with trace elements and tissue levels. *J. Nutr.* 104: 239-243 (1974).

Schroeder, H.A. and Mitchener, M. Life-term effects of mercury, methyl mercury, and nine other trace metals on mice. *J. Nutr.* 105: 452-458 (1975).

Sunderman, F.W.; McCully, K.S.; and Rinehimer, L.A. Negative test for transplacental carcinogenicity of nickel subsulfide in Fischer rats. *Res. Commun. Chem. Pathol. Pharmacol.* 31: 545-554 (1981).

**APPENDIX B**

**U.S. AND FOREIGN MINING, MILLING, AND SMELTING OPERATIONS**

**TABLE 2-2: U.S. AND FOREIGN MINING, MILLING, AND SMELTING OPERATIONS**

| <b>FACILITY AND LOCATION</b>                                      | <b>MATERIAL PROCESSED</b>                    | <b>TYPE OF PROCESS</b>                      | <b>PRODUCT</b>                                     |
|---|--|---|--|
| Bindura<br>Zimbabwe   | Sulfide ore                                  | Electrolytic                                | Cathode  |
| Cerro Matoso<br>Colombia  | Laterite ore                                 | Pyrometallurgical                           | FeNi shot  |
| China<br>Jinchuan   | Sulfide ore                                  | Electrolytic                                | Cathode  |
| Codemin<br>Brazil   | Laterite ore                                 | Pyrometallurgical                           | FeNi shot  |
| Cubaniquel<br>Punta Gorda, Cuba<br>Nicaro, Cuba                   | Laterite ore                                 | Hydrometallurgical<br>Ammonical leach       | NOS*   |
| Empress Nickel (Rio Tinto)<br>Zimbabwe                            | Sulfide ore                                  | Electrolytic                                | Cathode  |
| Eramet-SLN<br>Doniambo , New Caledonia<br>Sandouville , France    | Laterite ore<br>Sulfide matte                | Pyrometallurgical<br>Electrolytic           | FeNi shot<br>Cathode                               |
| Falconbridge<br>Kristiansand, Norway<br>Bonao, Dominican Republic | Sulfide matte<br>Laterite ore                | Electrolytic<br>Pyrometallurgical           | Cathode<br>FeNi cones                              |
| Fenimak<br>FYROM  | Laterite ore                                 | Pyrometallurgical                           | FeNi   |
| Impala<br>South Africa  | Sulfide concentrate                          | Hyrometallurgical                           | Briquettes   |
| INCO<br>Sudbury, Canada<br>Thompson, Canada<br>Clydach, Wales     | Sulfide ore<br>Sulfide matte<br>oxide sinter | Carbonyl<br>Electrolytic<br>Carbonyl        | Pellets, powder, NOS<br>Cathode<br>Pellets, powder |
| Korea Nickel<br>Korea   | Oxide sinter                                 | Pyrometallurgical                           | Utility Slugs                                      |
| Korea Nickel<br>Korea   | Oxide sinter                                 | Pyrometallurgical                           | Utility nickel                                     |
| Larco<br>Greece   | Laterite ore                                 | Pyrometallurgical                           | FeNi shot  |
| Morro do Niquel<br>Brazil   | Laterite ore                                 | Pyrometallurgical                           | FeNI shot  |
| Nippon Yakin<br>Japan   | Laterite ore                                 | Pyrometallurgical                           | FeNi shot  |
| Outokumpu<br>Finland  | Sulphide matte                               | Electrolytic<br>Hydrometallurgical          | Cathode<br>Briquettes                              |
| Pacific Metals<br>Japan   | Laterite ore                                 | Pyrometallurgical                           | FeNi shot  |
| PT Aneka Tambang<br>Indonesia                                     | Latrerite ore                                | Pyrometallurgical                           | FeNi shot  |
| Queensland Nickel<br>Australia                                    | Laterite ore                                 | Hydrometallurgical                          | Rondelles  |
| Russian Federation<br>Norilsk<br>Severonikel                      | Sulfide ore<br>Oxide sinter                  | Electrolytic<br>Carbonyl, Pyrometallurgical | Cathode<br>Pellets, FeNi                           |
| Rustenburg<br>South Africa  | Sulfide concentrate                          | Electrolytic                                | Cathode  |
| Sherritt Gordon<br>Canada   | Sulfide concentrate                          | Hydrometallurgical                          | Briquettes<br>Powder                               |

**TABLE 2-2: U.S. AND FOREIGN MINING, MILLING, AND SMELTING OPERATIONS**

| FACILITY AND LOCATION                      | MATERIAL PROCESSED                  | TYPE OF PROCESS                   | PRODUCT         |
|--|-------------------------------------|-----------------------------------|-----------------|
| Sumitomo<br>Niihama, Japan<br>Hyuga, Japan | Sulfide concentrate<br>Laterite ore | Electrolytic<br>Pyrometallurgical | Cathode<br>FeNi |
| Taiwan Nickel<br>Taiwan                    | Oxide sinter                        | Pyrometallurgical                 | Utility nickel  |
| Tocantins<br>Brazil                        | Sulfide ore                         | Electrolytic                      | Cathode         |
| Tokyo Nickel<br>Japan                      | Oxide sinter                        | Pyrometallurgical                 | NOS             |
| Ukraine Republic                           | Laterite ore                        | Pyrometallurgical                 | FeNi            |
| Western Mining<br>Australia                | Sulfide ore                         | Hydrometallurgical                | Briquettes      |

**APPENDIX C**

**REVIEW OF THE MANUSCRIPT BY ANDERSEN *ET AL.* TITLED "EXPOSURE TO NICKEL COMPOUNDS AND SMOKING IN RELATION TO INCIDENCE OF LUNG CANCER AMONG NICKEL REFINERY WORKERS."**

**The Andersen *et al.* (1996) Study of Kristiansand Workers  
and the Assessment of Carcinogenic Risks Associated  
with Exposure to Soluble Nickel**

S.K. Seilkop [Statistician for the ICNCM study (1990)]  
August 20, 1996

The recent paper by Andersen *et al.* (1996) has stimulated concern in the European community over the impending regulatory classification of nickel chloride. This concern primarily relates to the lung cancer risks in Kristiansand nickel refinery workers. In considering the classification of nickel chloride, as well as the reclassification of nickel sulfate that has also been proposed, it is important to address several questions: 1) What is our current understanding of the mechanisms of carcinogenicity for nickel compounds, particularly those that are water soluble? 2) Do the Andersen *et al.* lung cancer results from Kristiansand workers differ from those of previous studies of these workers or from those found in other epidemiologic cohorts exposed to soluble nickel? 3) How does the Andersen *et al.* smoking analysis contribute to our understanding of human health risks associated with soluble nickel exposure? and 4) How do we use the available data in assessing and managing cancer risks associated with exposure to these compounds?

**1. What is our current understanding of the mechanisms of carcinogenicity for nickel compounds?**

In evaluating cancer risks associated with nickel compounds, the importance of considering all of the available epidemiologic, animal study, and mechanistic information was recognized by the International Committee on Nickel Carcinogenesis in Man, which concluded its report (ICNCM, 1990) with the following statement:

"Other information to help refine our understanding of human health risks associated with nickel exposure is on the horizon. For example, animal carcinogenesis studies using inhalation as the route of exposure for nickel subsulfide, high temperature nickel oxide, and nickel sulfate hexahydrate are currently underway, and it will be of great interest to see if they support our findings. In addition, future work that improves our understanding of the mechanisms of nickel carcinogenesis may help to unify and explain the results of our findings in conjunction with animal experimentation."

Since the time of the ICNCM report, the animal experimentation to which this passage refers has been completed. Two-year bioassays of rats and mice conducted by the National Toxicology program of the U.S. showed distinctly different carcinogenic risks for the three nickel compounds (NTP, 1996a, 1996b, 1996c). While nickel subsulfide gave clear evidence of producing increased rates of lung tumor incidence in both sexes of rats (but not mice), the results for nickel oxide were less definitive, with some evidence of increased lung cancer rates in both sexes of rats, and equivocal evidence in female mice. For nickel sulfate hexahydrate, there was no evidence to suggest that the compound was carcinogenic in either rats or mice.

The complete pathological examinations and evaluations of lung lavage fluid that were performed in these studies provide insight into disparities between nickel compounds with respect to acute toxicity and inflammatory response. While there is evidence of cytotoxicity and inflammatory response in lung tissue for all three nickel compounds, the severity of this response appears to be related to nickel solubility (Benson *et al.*, 1989). Thus, when effects produced by each compound at equivalent nickel aerosol concentrations are compared, they are consistently strongest for nickel sulfate and weakest for nickel oxide, while nickel subsulfide exhibits an intermediate response.

Persistent inflammatory and cytotoxic responses often induce cell proliferation, which has also been observed in the lung epithelia of rodents exposed to either nickel oxide or nickel subsulfide (Oberdörster *et al.*, 1995). While cell proliferative response has not yet been examined in animals exposed to water soluble nickel compounds, it is likely to be even stronger, given the evidence of a higher degree of acute

respiratory toxicity for nickel sulfate than for nickel oxide or subsulfide. Cell proliferation contributes to the carcinogenic process, as it is required to convert repairable DNA lesions into non-repairable mutations, whether these DNA lesions are directly produced by a compound, or whether they are indirect lesions produced by oxygen radicals (Swenberg, 1995). Cell proliferation is also involved in clonal expansion of initiated cell populations, thereby increasing the probability of a second mutational event that leads to malignancy. Thus, through increased cell proliferative activity, nickel compounds can potentially act as promoters of genotoxic events induced by the nickel compounds themselves or by other substances. Based on the disparities in respiratory toxicity of the compounds, the strength of this promotional effect would appear to be directly related to water solubility, with the soluble compounds likely to be the strongest promoters.

The potential for nickel to be delivered to the target cell nucleus also appears to vary with solubility. Endocytosis is considered to be the primary mechanism for delivery of nickel compounds to the cell nucleus (Costa et al., 1981). Although nickel oxide and nickel subsulfide are readily endocytized, soluble compounds are not (Sunderman Jr., et al., 1987). One might surmise that solubility through diffusion would afford greater access to the cell nucleus. However, the Ni(II) ion is believed to cross the cell membrane using the Mg(II) ion transport system, and in the cell must compete with millimolar levels of Mg(II). Furthermore, soluble nickel compounds such as nickel sulfate are rapidly cleared (Benson et al., 1995). Thus, an efficient mechanism for delivery of soluble nickel compounds to the cell nucleus does not appear to exist. Because of the absence of a delivery mechanism, soluble nickel would not be expected to be a carcinogen *per se*; this has been corroborated in the NTP two-year bioassays for nickel sulfate (NTP, 1996c). The cell proliferative activity that it is likely to induce would, however, place it in the category of a potential cancer promoter. In contrast, less soluble compounds that are readily endocytized and which also have been demonstrated to induce increased cell proliferation would be more likely to act as complete carcinogens.

**2. Does the Andersen et al. (1996) paper indicate a lung cancer response that differs from that which was found by the ICNCM or from those found in other epidemiologic cohorts?**

When the Kristiansand workers were studied by the International Committee on Nickel Carcinogenesis in Man (ICNCM, 1990), they were followed-up through 1984. Lung and nasal cancer risks were based primarily on mortality. Andersen et al. have extended the follow-up of these workers through 1993 and conducted their analysis on the incidence of lung and nasal cancer cases (some of whom may still be living). As much of the cohort was hired prior to 1960, a considerable amount of follow-up (at least 24 years) had already occurred for much of the cohort at the time of the ICNCM report, the results that Andersen et al. obtained would be expected to be similar to those reported by the ICNCM.

This is indeed the case. The ICNCM evaluated lung and nasal cancer risk both on a process basis (e.g., electrolysis vs. roasting and smelting) and like Andersen et al., with respect to cumulative exposure to different nickel compounds. The Committee's report concluded that workers in two areas (electrolysis, roasting and smelting) had increased lung and nasal cancer risks. However, it also indicated that the electrolysis department, where the predominant nickel exposure was to soluble compounds, had appreciably higher lung cancer risks than the roasting and smelting area, where the primary exposure to nickel was in oxidic and sulfidic form. The ICNCM interpreted the results of its cumulative exposure analysis (summarized in Figure 1a) as giving evidence of an association between soluble nickel and lung cancer risk, but it also discussed the possibility of an interaction between soluble and oxidic nickel exposure. This was suggested by the disparities in risk attributable to "high" cumulative exposure to oxidic nickel ( $\geq 15 \text{ mg Ni m}^3 \text{ year}$ ) levels at different levels of cumulative exposure to soluble nickel. In particular, at the lowest and highest levels of soluble nickel exposure, differences in risk between "high" and "low" oxidic nickel exposure were relatively small ( $< 1.25$ -fold increase in high relative to low). However, when the exposures to soluble nickel were more moderate ( $5\text{-}14 \text{ mg Ni m}^3 \text{ year}$ ), there was an appreciably larger (more than two-fold) increased risk for men exposed to high levels of oxidic nickel relative to the risk in men exposed to low oxidic levels. Based on additional evidence of an interaction that was suggested by cross-classified cumulative exposure analyses of workers at the Clydach (Wales) refinery before 1930, the ICNCM concluded that "there was an indication that soluble nickel in some way played a role in accentuating risk associated with exposure to other nickel compounds." This response is

clearly consistent with the animal and mechanistic experimental evidence suggesting a promotional role for soluble nickel.

The results that Andersen *et al.* obtained were characterized with a Poisson regression model, based on cross-classified soluble and oxidic nickel exposures. Although this model is useful in showing general trends in response relative to each of these forms of nickel individually, it does not provide sufficient detail to explore the possibility of an interaction between soluble nickel and oxidic nickel. In presentations made in a NiPERA sponsored research workshop (June, 1996) and at a European Union meeting on the classification and labelling of dangerous substances, Andersen provided the summary data (Table 1) on which the Poisson regression model was based. The dose-response function derived from these data (Figure 1b) has the same general features as those found by the ICNCM (Figure 1a). Both sets of data provide evidence that soluble nickel plays a strong role in the induction of lung cancer in Kristiansand workers, but there is also evidence that its role is one of enhancement of other risks. In particular, there is the same indication that the level of risk associated with "high" oxidic nickel exposure is dependent on the level of soluble nickel exposure. Specifically, for cumulative soluble nickel exposure of less than 1 mg/m<sup>3</sup> year, the difference between SIR's for "high" and "low" oxidic nickel exposure is approximately 1.0; however, for cumulative soluble nickel exposure of 1-4 mg/m<sup>3</sup> year, this difference is more than twice as large. The Andersen *et al.* data also suggest a similar enhancement in lung cancer risk at the highest level of soluble nickel exposure (≥15 mg/m<sup>3</sup> year, thus providing additional support to the ICNCM hypothesis of an interaction between soluble and oxidic nickel.

As well as lending strength to this hypothesis, the Andersen *et al.* study facilitates a better understanding of the dose-response function for soluble nickel at low levels of oxidic nickel exposure. While the ICNCM study did not find evidence of increased lung cancer risk in men exposed to less than 5 mg/m<sup>3</sup> year soluble nickel and less than 15 mg/m<sup>3</sup> year oxidic nickel, this result was highly uncertain because of the small amount of available data (Figure 1a, 50% confidence interval for SMR≈ 50 - 400). The evaluation of lung cancer risk was also complicated by its apparent enhancement in unexposed workers (SMR=183). The additional follow-up in the Andersen *et al.* study provides risk estimates that are more precise, thereby permitting a clearer definition of the dose-response curve and improved insight with respect to the ICNCM results. In particular, the SIR for workers with less than 1 mg/m<sup>3</sup> year cumulative exposure to both soluble and oxidic nickel (Table 1, SIR= 1.8, 95% C.I.= 1.6-2.0, depicted as "unexposed" in Figure 1b) is virtually identical to that of unexposed workers in the ICNCM report. The estimated lung cancer risk for workers with the same level of soluble nickel exposures and less than 15 mg/m<sup>3</sup> year oxidic nickel is slightly higher, but statistically comparable (SIR=2.0, 95% CI=1.9-2.3). At this level of oxidic nickel exposure, there is also no evidence of additional risk when soluble exposure is increased to 1-4 mg/m<sup>3</sup> year (SIR=2.1, 95% C.I.=1.9-2.5). Thus, the Andersen *et al.* study produces evidence to suggest that exposure to soluble nickel at 1-4 mg/m<sup>3</sup> year and oxidic nickel of less than 15 mg/m<sup>3</sup> year did not add to the apparent background lung cancer risk in Kristiansand workers. The increased SIR's in workers exposed to these levels of soluble and oxidic nickel are probably not work-related, and are due to other causes. The most likely of these, cigarette smoking, is discussed in Section 3 below.

Evidence of an absence of increased lung cancer risks for low-level soluble nickel exposure (and low levels of oxidic nickel) in Kristiansand workers is consistent with evidence from other epidemiologic data. Specifically, the ICNCM found little, if any evidence to suggest that Clydach workers who were exposed to low levels of soluble nickel and oxidic nickel (SMR=196) had increased lung cancer risk relative to those workers who were unexposed (SMR=166). The contention that low level soluble nickel in the absence of high nickel oxide exposure does not produce increased lung cancer risk is also supported by the ICNCM analysis of INCO's Port Colborne electrolysis workers. For the 2,747 men in this operation who had less than five years in sintering operations, there was no evidence of a gradient of lung cancer risk with years worked in electrolysis, and only a marginally increased risk overall (SMR=137). That these workers showed a much lower risk than those at Kristiansand was attributed by the ICNCM to either a lower level of soluble nickel than found at Kristiansand and/or a seven-fold lower level of insoluble exposure. The Andersen *et al.* study facilitates a higher degree of understanding of the Port Colborne data. Based on the environmental estimates for the two facilities, nearly all of the Port Colborne workers would be found in the lowest two exposure categories of the bottom curve in Figure 1b. Thus, the absence of risk in the Port

Colborne workers is consistent with the Kristiansand dose-response function derived from the Andersen *et al.* data.

### **3. How does the Andersen *et al.* smoking analysis contribute to our understanding of human health risks associated with soluble nickel exposure?**

One of the difficulties with the ICNCM study, as well as other epidemiological investigations of lung cancer in nickel workers, has been the absence of smoking data. Smoking is widely accepted as the principal cause of lung cancer. Furthermore, "blue collar" workers, such as those in nickel refineries, typically have higher smoking prevalence than the general population. Thus, using national mortality (or incidence) rates to calculate Standardized Mortality Ratios (or Standardized Incidence Ratios) for such workers often results in upwardly biased estimates of lung cancer risk.

In the ICNCM study, there was evidence that Kristiansand workers smoke more than the general population. This was based on an SMR of 183 in workers who had no exposure to nickel. Unfortunately, the "ever" vs. "never" smoking data in the Andersen *et al.* paper do not provide a complete picture of the smoking patterns of Kristiansand workers relative to that of the general population. Based on the expected number of cases in Table 6, the primary information about smoking patterns that can be inferred is that the proportion of "ever" smokers in the cohort is approximately 80%, which is similar to the rate of "ever" smokers during the same period in other industrialized countries (e.g., Canada). The paper does not, however, have information about the prevalence and intensity of cigarette consumption during the follow-up period. If this information was available, it could be used to evaluate the validity of assuming that the increased lung cancer risk in unexposed Kristiansand workers found by ICNCM is due to greater cigarette consumption. Nonetheless, Andersen *et al.* found that workers exposed to both soluble and oxidic nickel at less than 1 mg/m<sup>3</sup> year had a significantly increased SIR of 1.8 (p<0.01), which was virtually identical to the SMR for unexposed workers examined by the ICNCM. That this increase is not due to nickel exposure is supported by a statistically significant increased lung cancer risk (SIR=2.0, two-sided p=0.011) for workers with less than 15 years since first exposure in the refinery (Andersen *et al.*, Table 3). Since the latency period for lung cancers is generally believed to be more than 15 years, cancers identified earlier than 15 years from time of first employment at Kristiansand are likely to have been induced by smoking that was begun prior to when workers started working at the refinery. Thus, there is evidence of a two-fold smoking-induced increase in the background lung cancer risk for Kristiansand workers. The same two-fold increase in workers with exposure to low levels of nickel therefore appears to be attributable to workers' smoking habits, and not to nickel exposure.

The primary importance of the Andersen *et al.* analysis of lung cancer and smoking behavior in nickel workers is in its contribution to the understanding of the mechanisms for nickel carcinogenesis. Most importantly, the strong evidence of synergy between cigarette smoking and nickel exposures in inducing lung cancer provided by Andersen *et al.* corroborates experimental evidence suggesting that nickel compounds act as "promoters" of genotoxic events arising from exposure to initiators or complete carcinogens (such as tobacco smoke).

The animal evidence that soluble nickel is likely to be a more powerful promoter than insoluble compounds is also supported by the Andersen *et al.* data. The absence of evidence of carcinogenicity in the NTP study of nickel sulfate hexahydrate suggests that humans exposed to soluble nickel alone would not experience increased lung cancer risk. The Andersen *et al.* Kristiansand data appear to contradict this, with evidence of a dose-related risk above 5 mg/m<sup>3</sup> year cumulative soluble nickel exposure, even when the level of oxidic nickel exposure is low (figure 1b). Furthermore, there is an indication of a soluble nickel dose-related increase when cumulative oxidic nickel exposure is less than 1 mg/m<sup>3</sup> year (Table 1). Unlike laboratory animals in the NTP studies, however, workers' lungs at the Kristiansand refinery were exposed to soluble nickel in the presence of a substance that has been demonstrated to contain powerful initiators (*i.e.*, tobacco smoke). Thus, the association between soluble nickel exposure and lung cancer risk found in Kristiansand workers conforms to the anticipated promotional response. Furthermore, the weaker lung cancer response to oxidic nickel exposure might also be anticipated. There is an increasing body of evidence that that carcinogenic process is extremely sensitive to changes in cellular kinetics, particularly through enhanced cell turnover induced by cytotoxicity. As discussed above,

oxidic nickel exhibits less toxicity in lung tissue than soluble nickel, and would therefore not be expected to enhance the effect of smoking as strongly as would soluble nickel.

It should be noted that while the lung cancer response to soluble nickel exposure in Kristiansand workers can be reasonably attributed to the promotion of smoking-induced cancers, and this response might be elicited by other nickel compounds as well, it is clearly not the only response that is likely to be associated with exposure to less soluble compounds. The animal, mechanistic study, and epidemiologic data suggest that these compounds induce genetic or epigenetic effects as well as cell proliferative activity. However, in conjunction with the epidemiologic data, animal and mechanistic study data strongly suggest that lung cancer risks associated with soluble nickel exposure are dependent upon the concomitant presence of other substances with the potential to initiate the carcinogenic process.

#### **4. Implications for Risk Assessment and Risk Management**

The evidence that water soluble nickel compounds are promoters rather than complete carcinogens has important implications for regulatory and industrial personnel engaged in risk assessment and risk management for these compounds. As a respiratory irritant, soluble nickel might be expected to produce an inflammation dose-response curve which exhibits strong non-linearity or a threshold effect. This is based on the fact that one of the roles of respiratory epithelial cells is to maintain a protective barrier against inhaled pathogens and toxic chemicals. When exposed to low levels of a respiratory toxicant, epithelial cells are capable of performing this protective role. However, at sufficiently high concentrations the epithelial barrier can be breached, thereby exposing underlying cells to risk of direct exposure to air contaminants, and the influx of inflammatory cells which may release powerful chemical mediators (Butterworth *et al.*, 1995). This "all or nothing" response induces a strongly non-linear or threshold dose-response function for cell proliferative activity. As a consequence, a promoter acting through increased cell proliferation exhibits either non-linearity or a threshold in dose-related enhancement of cancer risks associated with exposure to complete carcinogens (such as tobacco smoke). The epidemiologic data from Kristiansand refinery workers provides evidence of such a threshold in lung cancer risk for men exposed to soluble nickel at relatively low levels of oxidic nickel exposure (lower curve in Figure 1b). The understanding of the likely origin of this threshold response provides confidence that if airborne concentrations of soluble nickel are sufficiently low, there is no substantive risk that exposure to these compounds will promote the carcinogenic activity of other hazards.

**References**

- Andersen, Aa., Engeland, A., Berge, S.R., Norseth, T. (1996) Exposure to nickel compounds and smoking in relation to incidence of lung and nasal cancer among nickel refinery workers. Unpublished manuscript.
- Benson, J.M., Burd, D.G., Cheng, Y.S., Hahn, F.F., Haley, P.J., Henderson, R.F., Hobbs, C.H., Pickrell, J.A., and Dunnick, J.K. (1989). Biochemical responses of rats and mouse lung to inhaled nickel compounds. *Toxicology* 57: 255-266.
- Benson, J.M., Barr, E.B., Bechtold, W.E., Cheng, Y.S., Dunnick, J.K., Eastin, W.E., Habbs, C.H., Kennedy, C.H., Maples, K.R. (1995). Fate of inhaled nickel oxide and nickel subsulfide in F344/N Rats. *Inhalation Toxicology* 6:167-183.
- Butterfield, B.E., Conolly, R.B., Morgan, K.T. (1995) A strategy for establishing mode of action of carcinogens as a guide for approaches to risk assessments. *Cancer Letters* 93:129-146.
- Costa, M., Abbracchio, M.P., Simmons-Hanson, J. (1981). Factors influencing the phagocytosis, neoplastic transformation, and cytotoxicity of particulate nickel compounds in tissue culture systems. *Toxicol. Appl. Pharmacol.* 60: 313-323.
- ICNCM (1990). Report of the International Committee on Nickel Carcinogenesis in Man, *Scandinavian Journal of Work, Environment & Health*, Volume 16, number 1(special issue), February, 1990, 82 pp.
- NTP (1996a). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Oxide (CAS No. 1313-99-1) in F344/N Rats and B6C3F<sub>1</sub> Mice (Inhalation Studies). NTP Technical Report 451.
- NTP (1996b). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Subsulfide (CAS No. 12035-72-2) in F344/N Rats and B6C3F<sub>1</sub> Mice (Inhalation Studies). NTP Technical Report 453.
- NTP (1996c). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Sulfate Hexahydrate (CAS No. 10101-97-0) in F344/N Rats and B6C3F<sub>1</sub> Mice (Inhalation Studies). NTP Technical Report 454.
- Oberdörster, G., Baggs, R.B., Finkelstein, J. (1995). Pulmonary retention and effects of inhaled NiO and Ni<sub>3</sub>S<sub>2</sub> in rats and mice: indicators of maximum tolerated dose: Fifeh COMTOX Symposium on Toxicology and Clinical Chemistry of Metals. Vancouver, BC Canada (July, 1995), page 26.
- Sunderman, F.W. Jr., Hopfer, S.M., Knight, J.A., McCully, K.S., Cecutti, A.G., Thornhill, P.G., Conway, K., Miller, C., Patierno, S.R., Costa, M. (1987). Physicochemical characteristics and biological effects of nickel Oxides. *Carcinogenesis* 8(2): 305-313.
- Swenberg, J.A. (1995). Bioassay design and MTD setting: old methods and new approaches. *Reg Toxicol. and Pharm.* 21:44-51.

Table 1

(Results from the Norwegian study, not included in the paper)

**NUMBERS OF NEW CASES OF LUNG CANCER AMONG 4902 MALE NICKEL REFINERY WORKERS,  
BY CUMULATIVE EXPOSURE TO NICKEL COMPOUNDS; FOLLOW-UP, 1953-92**

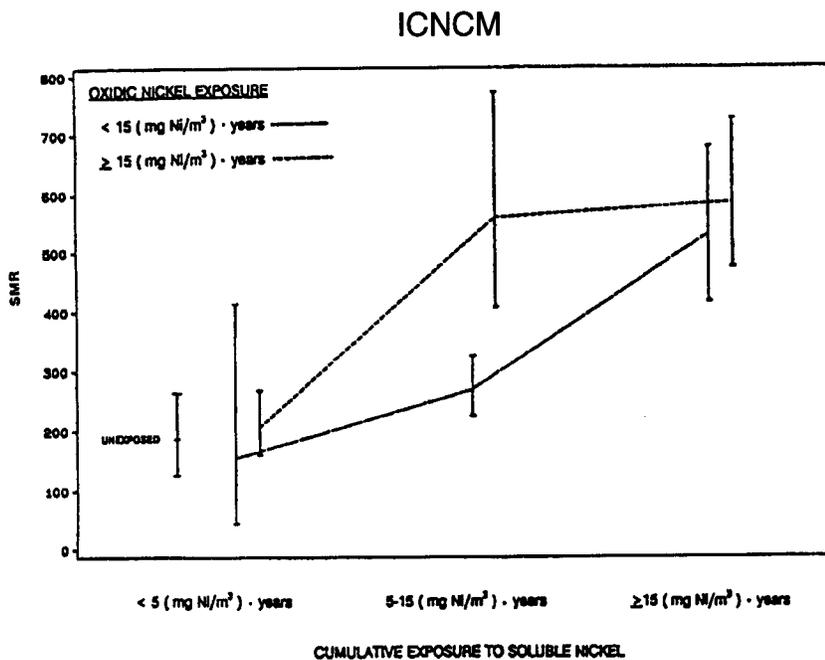
| Soluble nickel compounds (mg/m <sup>3</sup> ) | Cumulative Exposure to Oxidic Nickel (mg/m <sup>3</sup> ) |       |       |       |        |       |      |       |       |       |
|---|---|-------|-------|-------|--------|-------|------|-------|-------|-------|
|   | < 1   |       | 1 - 4 |       | 5 - 14 |       | ≥ 15 |       | TOTAL |       |
|   | O   | SIR   | O     | SIR   | O      | SIR   | O    | SIR   | O     | SIR   |
| < 1   | 40  | 1.8** | 2     | 1.4   | 17     | 3.3** | 33   | 2.9** | 92    | 2.3** |
| 1-4   | 15  | 2.6** | 13    | 1.8   | 2      | 2.0   | 5    | 4.6*  | 35    | 2.3** |
| 5-14  | 3   | 4.3   | 10    | 2.2*  | 8      | 6.5** | 1    | 1.8   | 22    | 3.1** |
| ≥ 15  | 1   | 13.3  | 16    | 5.6** | 27     | 8.2** | 8    | 9.2** | 52    | 7.3** |
| Total   | 59  | 2.0** | 41    | 2.6** | 54     | 5.0** | 47   | 3.4** | 201   | 2.9** |

O, number of observed cases; SIR, standardized incidence ratio.

\* p < 0.05

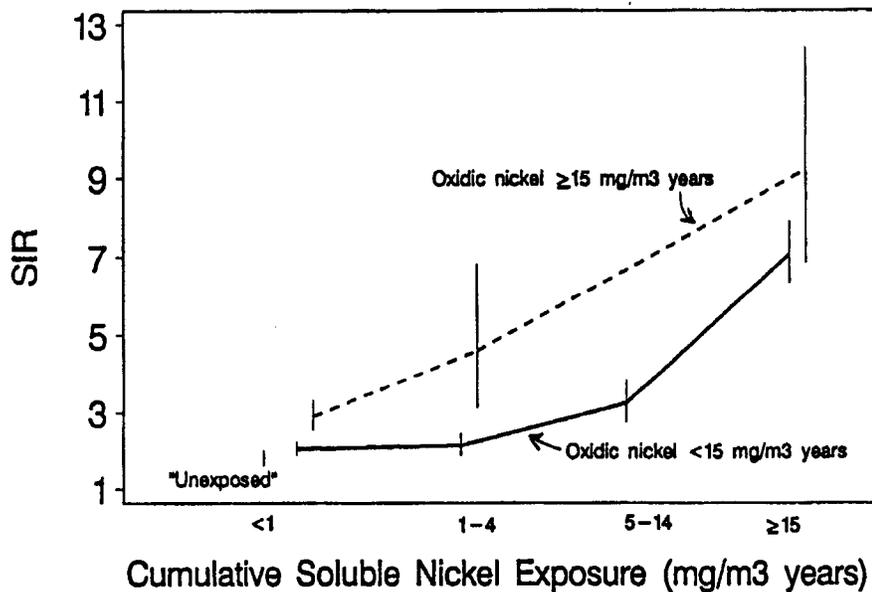
\*\* p < 0.01

Figure 1  
Lung Cancer Risks<sup>1</sup> by Cumulative Exposure to Soluble and Oxidic Nickel



(a)

Andersen et al.<sup>2</sup>



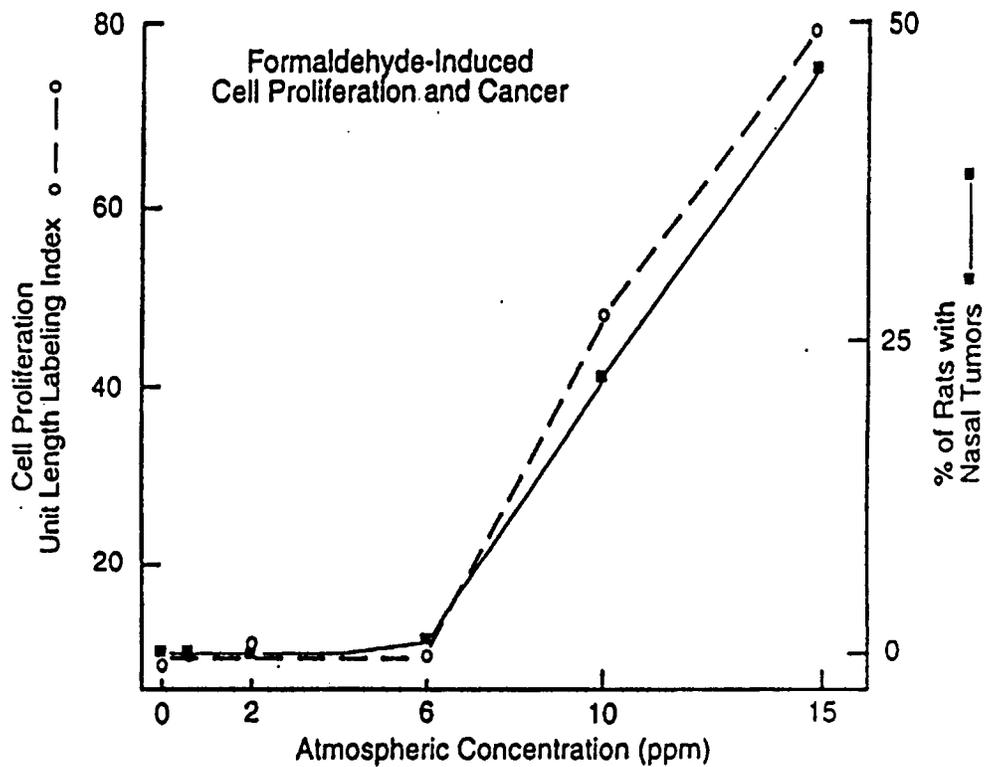
(b)

<sup>1</sup> SMR's and SIR's plotted with 50% confidence limits.

<sup>2</sup> Note: SIR for category with cumulative soluble nickel exposure of 5-14 mg/m<sup>3</sup> years and cumulative oxidic nickel ≥ 15 mg/m<sup>3</sup> year is not plotted as it is based on a single lung cancer case.

Figure 2

Threshold Response in Cell Proliferation and Tumor Incidence  
for Rats Exposed to Formaldehyde (Butterworth *et al.*, 1995)



**APPENDIX D**

**DETAILED COMMENTS REGARDING THE FINNISH REFINERY STUDIES**

**CONCOMITANT EXPOSURES - SULFURIC ACID MIST AND INSOLUBLE NICKEL**

While the authors of the RoC Background Document have noted the presence of sulfuric acid mist in the Outokumpu tankhouse during the period critical to the development of the lung and nasal cancers, not enough attention is given to this detail.<sup>1</sup> IARC (1992) classified inorganic mists containing sulfuric acid as being carcinogenic to humans. This conclusion was based on studies that showed an association of sulfuric acid mist exposures with nasal sinus, laryngeal, and lung cancers in workers in various manufacturing operations. The nasal sinus cancers are of particular interest in that they occurred mainly in workers manufacturing isopropanol, a compound that has not been demonstrated to be carcinogenic in laboratory animals through any routes of exposure tested (inhalation, subcutaneous injection, skin painting, and diet) (Weil *et al.*, 1952; Van Esch, 1960; NIOSH, 1976; Burleigh-Flayer *et al.*, 1997).<sup>2</sup> Others, in addition to IARC, have also concluded that nasal cancers seen in isopropanol workers result from the strong acid process utilized in such plants (Solskolne *et al.*, 1984; Lynch *et al.*, 1979).

It is essential to note this, since it appears that the generation of strong acid mists in the Outokumpu tankhouse has always been a problem that the company has strived to control.<sup>3</sup> Communications from Outokumpu indicate that strong inorganic acid mists containing sulfuric acid measured in 1966 ranged from 0.2 to 1.2 mg/m<sup>3</sup>, with a mean and median of 0.6 mg/m<sup>3</sup>. The mean concentration for such samples taken over the 1970s was even higher (0.8 mg/m<sup>3</sup>). Although such concentrations of mists containing sulfuric acid were believed to be safe back then, the safety of such concentrations may be questionable in light of the IARC Report that showed an association of respiratory cancers with sulfuric acid mists at or below such concentrations.

As noted in the RoC Background Document, there is also evidence that nickel exposures in the electrolytic part of the refinery for much of the period relevant to the induction of respiratory cancer were to both soluble and insoluble forms of nickel. The critical exposure period for the induction of both the nasal and lung cancers seen in these workers would have been in the 1960s through the early 1970s when the nickel refinery was first put into operation and engineering “bugs” were being eliminated from the system.<sup>4</sup>

With respect to soluble nickel, documentation from Outokumpu indicates that during the first 15 years of the refinery’s operation, only 11 stationary samples were taken in the tankhouse and this occurred during a two-day period in November of 1966. Both the mean and median concentrations of these samples were 0.5 mg Ni/m<sup>3</sup>; concentrations up to 0.8 mg Ni/m<sup>3</sup> were reported. No samples were taken previous to this point, and the next set of samples taken in the tankhouse were not until 1976. With respect to insoluble nickel exposures, concentrations in grinding and leaching (a part of the electrolysis hall until 1973) were reported to be as high as 2 mg Ni/m<sup>3</sup>. Therefore, it is clear that exposures in the 1960s-early 1970s were, at least in a number of instances, higher than those taken between 1979-1981. The use of the 1979-1980 exposure measurements (reported to be below 0.5 mg Ni/m<sup>3</sup>) as the basis for the analysis of cancer mortality by the authors of the study is, therefore, potentially misleading.

<sup>1</sup> It should be noted that the authors of the paper failed to mention the presence of these acid mists.

<sup>2</sup> Nasal cancers have also been seen in phosphate fertilizer workers exposed to sulfuric acid mists (Hagmar *et al.*, 1991).

<sup>3</sup> Anode hoods and gas channels to draw off oxygen and electrolyte mists were installed when the refinery opened, but problems were encountered when salts accumulated in the channel. Mist suppression was reported as being problematic. Because of these problems, the hoods were removed in 1976 and the anodes were enclosed in polyester bags in an attempt to prevent misting from the surface of the electrolyte. In a continuing effort to reduce mists, the bags were replaced with polyurethane balls in 1980.

<sup>4</sup> The two workers with “confirmed” nasal cancers had retired by 1982. Nasal cancer latency in nickel refinery workers in other cohorts has been observed to be at least 15 years, with some latency periods of 30 years or more being reported. Therefore, if nickel was the causative agent of the nasal cancers in the Finnish workers, it would have been the early exposures (1960s) that contributed to these cancers. Likewise, with respect to lung cancer, it appears that all 6 lung cancers observed in the nickel refinery workers came from workers with 20+ years of latency, raising the possibility that these cancers, too, occurred in workers who were exposed to higher concentrations of soluble and insoluble nickel compounds, as well as acid mists, in the early years of the refinery’s operation.

**ESTABLISHMENT OF NASAL CANCER CASES - TIMING, DIAGNOSIS, OTHER WORK HISTORY**

Nasal cancer is sufficiently rare that a single spontaneous case in a small cohort can produce significance by conventional statistical criteria ( $p=0.05$ ), and two cases will achieve a very high level of significance ( $p > 0.001$ ). However, to infer a causal link from such a small number of cases, without a more thorough examination of them is simply not good science. This is particularly true in an instance such as this where the total number of workers presenting with nasal cancers is small and, therefore, it would not be difficult to examine these cancers on a case-by-case basis. Specifically, as nasal cancer is known to be associated with other occupations (e.g., carpentry work), it is important to investigate previous work experience. An evaluation of the timing of the occurrence of the cancer relative to this work experience assists in developing a more firmly grounded assessment of the most likely origin of the cancer.

Information obtained from the company on the four potential nasal cancers reported reveals that two of the workers were previously employed in carpentry work prior to their work in the nickel refinery at Harjavalta. While this does not rule out a possible role for nickel exposures contributing to these nasal cancers, it does call into question the precise etiologic cause of the cancers. The possibility that the carpentry work could have caused these nasal cancers should be noted, particularly because nasal cancers have such a long latency period. Further, the type of work that these workers were involved in at the refinery should be noted as certain jobs (e.g. maintenance, cleaning) are likely to result in exposures that are higher than the norm.

In addition, while it is not unreasonable for the authors to note the nasopharyngeal cancer seen in one female worker relative to her exposure to nickel in the refinery, the fact that the origin of this cancer (nose or pharynx) is uncertain may also be of some relevance, as other nasal cancers in nickel refinery workers have originated in the nasal sinuses. It would be helpful to reexamine the pathology of all the nasal sinus cancers observed in the Finnish cohort to determine their precise origin. If most of the purported "nasal sinus" cancers prove to be of uncertain origin (*i.e.* possibly pharyngeal in nature), the results from this cohort would differ from any other nickel cohort studied (see attached Table). This would require a much more rigorous examination of the exposures and processes involved in this refinery (e.g. electrowinning versus electrolyses) that might set it apart from other refineries. All of these factors should be discussed in the RoC Background Document as they may have a profound influence on inferences drawn from this study.

**COMPARABILITY OF NASAL CANCERS IN THE FINNISH STUDIES TO THOSE OF OTHER STUDIES**

In the Norwegian studies, while there was some evidence linking nasal cancer to soluble nickel exposures, the evidence was much stronger for oxidic nickel (Andersen *et al.*, 1996). In contrast to the situation at Outokumpu, no nasal cancers have occurred in Kristiansand workers first employed since 1956, nor have there been any excess nasal cancer in workers who have been employed in Clydach during a comparable time period.<sup>5</sup> It is also worth noting that many of the Kristiansand electrolysis workers had exposures to soluble nickel that were higher than those reported at Outokumpu. Furthermore, the exposures of sulfuric acid mist were lower at Kristiansand than at Outokumpu. This again raises the possibility that sulfuric acid mist or the combination of soluble nickel with sulfuric acid mists induced the nasal cancers in Outokumpu workers. In short, the very large nasal cancer risk in Outokumpu workers is inconsistent with that found in other nickel refinery workers with a comparable (or higher) degree of soluble nickel exposure. This weakens the evidence that soluble nickel was the putative agent, and strongly suggests that exposures to other carcinogenic agents (e.g., sulfuric acid mist) may have played a causal or contributory role.

<sup>5</sup> Since 1950, two nasal cancer deaths have occurred in Clydach workers (Draper *et al.*, 1994). One was in a worker recruited in 1964 at the age of 63 who worked for the company for less than two years; it is questionable whether his nasal cancer can be attributed to his brief employment at Clydach. The second nasal cancer occurred in a worker who was hired in 1953 and worked for the company for 11 years. This worker was involved in cleaning one of the old Mond reducing towers being used for experimental nickel powder production. In this activity, his exposure to inorganic nickel compounds would have been considerably higher than that of the other workers, and he had no soluble nickel exposure as it was not believed to have been present in the Mond reducers.

***THE POTENTIAL ROLE OF SMOKING IN THE FINNISH LUNG CANCERS***

As seen in the Norwegian cohort, a higher prevalence of smoking in the Finnish refinery workers could be a possible explanatory factor for the increased lung cancer rates seen in this study. Although the increased rate of lung cancer in non-refinery workers at Outokumpu (SIR=1.48 for those with 20+ years of latency) is not elevated statistically, it suggests the possibility of increased smoking prevalence in the workforce at Outokumpu that would bias the reported results. The contention that this is true is strengthened by the significantly elevated SIR for lung cancer in Outokumpu smelter workers (SIR=2.00) and the absence of duration of exposure-related lung cancer response in these workers (or those in the refinery).

Epidemiologists generally believe that relative risks for lung cancer in excess of approximately 1.5 are unlikely to be due to differential patterns of cigarette smoking. Evidence from Kristiansand nickel refinery workers, however, challenges this view. Specifically, workers with little, if any nickel exposure at Kristiansand exhibited enhanced lung cancer risks (SMR=183 in Report of the ICNCM, 1990; SIR=1.8 in Andersen *et al.*, 1996) which can be logically attributed to the abnormally high proportion of smokers at Kristiansand (>80%) that can be derived from Table 6 of Andersen *et al.* (1996). A similar smoking prevalence in Outokumpu workers would inflate the true baseline lung cancer risk to the level observed in smelter workers, which is statistically consistent with that of unexposed workers. At a background rate comparable to that of Kristiansand (SIR=1.8), the increase in lung cancer risk in the Finnish refinery workers can be reasonably attributed to chance alone ( $p=0.11$ ). Furthermore, there is no compelling statistical evidence to differentiate lung cancer risk in smelter workers from that of refinery workers ( $p=0.27$  for workers with 20+ years latency,  $p=0.08$  overall).

To adequately interpret the increased lung cancer incidence at Outokumpu, it is essential to obtain a better characterization of cigarette smoking prevalence in the Finnish workers. The Kristiansand study strongly suggests that smoking prevalence in refinery workers may far exceed that of a national or even regional reference population which is used to derive SIRs. It is not at all far-fetched to assume that a similar situation exists with the Outokumpu workers. Consequently, the lung cancer SIR's in this study may be severely upwardly biased, and this possibility should be addressed in the discussion of the results of the study.

***EVIDENCE THAT SOLUBLE NICKEL IS A PROMOTER, AND NOT A COMPLETE CARCINOGEN***

The lack of elevated tumor rates in the NTP animal bioassay on nickel sulfate (NTP, 1996) supports the theory that soluble nickel, alone, is not a complete carcinogen and that the effects of soluble nickel seen in humans may be due to its promotional characteristics. This reflects the ICNCM Report's conclusion that soluble nickel enhanced the respiratory cancer risks associated with exposure to other nickel compounds (both sulfidic and oxidic forms), and should be clearly stated in the RoC Background Document.

The role of soluble nickel as a promoter rather than a complete carcinogen is derived from epidemiologic data from the Clydach refinery before 1930, comparisons of Port Colborne to Kristiansand electrolysis workers, in Kristiansand workers alone, (see above comments), as well as the most recent theories regarding the carcinogenic mechanisms and bioavailability of different nickel species (Oller *et al.*, 1997). A discussion of this information needs to be included in this report to allow the reader to examine the results of the analysis of the Finnish workers in the context of the current scientific understanding of soluble nickel's role in carcinogenesis.

## REFERENCES

- Burleigh-Flayer, H.; Garman, R.; Neptun, D.; Bevan, C.; Gardiner, T.; Kapp, R.; Tyler, T.; and Wright, G. (1997). Isopropanol vapor inhalation oncogenicity study in Fischer 344 rats and CD-1 mice. *Fund. Appl. Toxicol.*, 36, 95-111.
- Draper, M. H.; Morgan, L. G.; Metcalf, L.M.; Duffus, J. H.; Park, M. V.; Johns, P. (1994). Study of the evolution of the nickel refinery processes and the chemical nature of some of their historical process materials and contemporary environmental dusts at the INCO nickel refinery works at Clydach, Wales, U.K. Report to Nickel Producers Environmental Research Association.
- Hagmar, L.; Bellander, T.; Andersson, C.; Linden, K.; Attewell, R.; and Moller, T.(1991). Cancer morbidity in nitrate fertilizer workers. *Int. Arch. Occup. Environ. Health*, 63, 63-67.
- Lynch, J.; Hanis, N.M.; Bird, M.G.; Murray, K. J.; and Walsh, J.P. (1979). An association of upper respiratory cancer with exposure to diethyl sulfate. *J. occup. Med.*, 21, 333-341.
- NIOSH (1976). Criteria for a recommended standard. Occupational exposure to isopropyl alcohol. p.54. U.S. DHEW, PHS, CED, Rockville, MD.
- NTP (1996c). NTP Technical Report on the Toxicology and Carcinogenicity of Nickel Sulfate Hexahydrate (CAS No. 10101-97-0) in F344/N Rats and B6C3F<sub>1</sub> Mice (Inhalation Studies). NTP Technical Report 454.
- Solskolne, C.; Zeighami, E.; Hanis, N.; Kupper, L.; Herrmann, N.; Amsel, J., Mausner J. ; and Stellman, J. (1984). Laryngeal cancer and occupational exposure to sulfuric acid. *Am. J. Epidemiol.*, 120,358-369.
- Van Esch, G. (1960). Suitability of a rapid test for carcinogenic properties of chemical compounds with the aid of a promoter substance. *Verslag. Medel. Betreffende Volksgezondheid.*, 186-189.
- Weil, C.; Smyth, H.; Nale, T. (1952), Quest for a suspected industrial carcinogen. *Industrial Hygiene and Occupational Medicine*, 5, 535-547.