DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR NICKEL

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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Peer reviewers for the third pre-public draft of the Toxicological Profile for Nickel were:

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NOTE: Peer reviewer comments are written next to "COMMENTS:" in unformatted text. Any italicized text following the comment is added for clarification purposes. Any page and line numbers that were added by the Reviewers have been kept, but often will not align with the appropriate text.

Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: Yes, the general effects associated with nickel exposure in humans described here is accurate. Some of these effects, such as contact dermatitis, and carcinogenicity have more substantive and a plethora of literature to support them. Other effects have not been consistently seen across human or animal studies.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: Some of the effects observed only in animals are likely to be of concern to humans but may probably not be relevant to humans. For example, whilst some chronic animal bioassay studies have observed pheochromocytomas associated with nickel exposure, the relevance of this effect to humans is questionable.

RESPONSE: *No revisions were suggested.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain

COMMENT: Yes, the exposure conditions have been described as adequately as possible.

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT: For the acute inhalation, the rationale for not deriving an MRL, although several studies were identified with NOAELs or LOAELs, is not clear and could be buttressed with a few clear, concise sentences. Acute regulatory standards have been derived in various jurisdictions using various health effects associated with acute exposure to nickel, for example, acute REL using immunological effects.

RESPONSE: *The reviewer's specific suggestions in a later comment regarding the derivation of an acute inhalation MRL are addressed in detail. Text was added to the worksheet to further support the rationale for not deriving a MRL and the Buxton et al. (2021) study was also added to the Tox Profile.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT: Yes, I agree with the studies selected to derive the intermediate and chronic inhalation MRLs, the use of MPPD model and the uncertainty factors employed.

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: The interspecies UF of 3 and human variability UF of 10 are appropriate.

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: To the point under question (4), I think an acute MRL could be derived with the available database. In appendix A, in table A-1 and under the rationale for not deriving an acute inhalation MRL, several studies that identified NOAELs/LOAELs are described. For the acute MRL derivation, the effects of nickel on another system (eg, immune system) beside the respiratory system could be selected. A guideline-compliant 24-h inhalation exposure and T-dependent antibody response study which identified a NOAEC for immunomodulatory effects was recently published (https://pubmed.ncbi.nlm.nih.gov/34644513/). Moreover, a BMD_{10} for inflammation gene expression of 0.06 mg Ni/m^3, a NOAEC for lung inflammation of 0.03 mg Ni/m^3, and/or a NOTEL (No Observed Transcription Effect Level) for gene expression of 0.03 mg Ni/m^3 is observed in Efremenko et al and other studies following inhalation exposure to nickel sulfate hexahydrate.

RESPONSE: *The toxicology data from Buxton et al. (2021) was added to the LSE database for acute inhalation. The study examined immune response in female mice and reported a NOAEL of 0.08 mg Ni/m^3 as nickel chloride hexahydrate, which was the highest concentration tested in the study. The text in the MRL worksheets was updated to reflect the inclusion of this study into the database. With consideration of the Buxton et al. (2021) study, the acute-duration inhalation database for immune effects was not considered suitable for MRL derivation due to lack of evidence of immune toxicity at low concentrations in rats, which appear more sensitive to the effects of nickel than other laboratory animals. Additionally, evidence of respiratory toxicity in mice are limited and a NOAEL value of 0.44 mg Ni/m^3 was identified. The peer-reviewer also noted data from Efremenko et al. (2014) for MRL consideration. The NOAEL for the gene expression change is not an endpoint considered for MRL derivation. In the same study, lung histology was also examined, however these results cannot be used to derive a MRL since the lung was only evaluated in the control group and the highest exposure group (the other exposure level groups were excluded). Thus, the lung histology data from Efremenko et al. (2014) are lacking a dose-response relationship for acute respiratory effects.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: Yes, the conclusions for each health effect seem appropriate and supported by the evidence.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Yes, the studies identified, the study limitations and other study parameters were adequately addressed without providing unnecessarily long information on each study.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: Yes. Where necessary, I have made some edits and comments on these studies in the main document.

RESPONSE: *The reviewer's recommended edits were reviewed and incorporated as appropriate. Responses to annotated comments are provided in the section 'Annotated Comments in the Profile.'*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: Yes, the rodent species (rats and mice) used in most of the studies were appropriate.

RESPONSE: *No revisions suggested.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT: The doses/concentrations at which health effects were observed in both human and animal studies have been stated appropriately.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: Yes, I have noted a few studies in the main document. Another important study, which is not published yet but is in prepublication stage in the Journal of Applied Toxicology, is titled "Comparative pulmonary and genotoxic responses to inhaled nickel subsulfide and nickel sulfate in F344" by Oller A. et al. This study report has been used in various regulatory submissions; if it's published before the ATSDR Toxicological Profile of Nickel is finalized, I suggest that it's included.

RESPONSE: *The study by Oller et al. (2022) was added to the profile into relevant health effect sections in Chapter 2 as follows:*

Section 2.4:

> "Oller et al. (2022) reported increased incidence of alveolitis, proteinosis, and perivascular/peribronchiolar inflammation in Fischer-344 rats exposed to 0.04 mg Ni/m^3 as nickel subsulfide for 13 weeks (6 hours/day, 5 days/week). The incidence and severity of lung lesions at 3 and 13 weeks of exposure showed that increases in both are concentration dependent. Rats exposed under similar conditions to nickel sulfate hexahydrate showed similar concentration-dependent results in pulmonary lesions (Oller et al. 2022). At a NOAEL of 0.03 mg Ni/m^3 as nickel sulfate hexahydrate, there was no difference between the exposed rats and controls for incidence of lung inflammation or lesions, or changes in lung weight. At 0.11 mg Ni/m^3 as nickel sulfate hexahydrate, the incidence of alveolitis, perivascular/peribronchiolar inflammation, and bronchiolar epithelial degeneration and apoptosis was high. In addition, increases in LDH levels in bronchoalveolar lavage fluid (BALF) were significant at 0.11 mg Ni/m^3 as nickel sulfate hexahydrate (Oller et al. 2022). Comparison of lesions showed that the incidence and severity of perivascular/peribronchiolar lesions and alveolar type II cell hyperplasia was higher in rats exposed to nickel subsulfide (Oller et al. 2022)."
>
> "In the study by Oller et al. (2022), one group of rats was exposed to a high dose of nickel sulfate hexahydrate (0.44 mg Ni/m^3) but died within the first week of exposure, and the death were attributed to respiratory toxicity. Rats showed labored breathing and nasal discharge; gross necropsy showed severe pulmonary edema as the likely cause of death (Oller et al. 2022)."
>
> "In isolated lung cells from rats exposed to concentrations ≤0.22 mg Ni/m^3 as nickel sulfate hexahydrate, DNA damage was not increased after 3 weeks but appeared to increase after 13 weeks (Oller et al. 2022). Exposure to nickel subsulfide showed DNA damage increased with exposure concentration regardless of duration (Oller et al. 2022)."

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: I have suggested a study already (https://pubmed.ncbi.nlm.nih.gov/34644513/) that may be relevant to the derivation of an acute inhalation MRL. The yet-to-be-published Oller et al study I mentioned above may also prove pertinent to the MRLs.

RESPONSE: *The studies by Buxton et al. (2021) and Oller et al. (2022) were both added to the Tox Profile LSE data and discussed within related text. These studies were also reviewed as part of the larger database for consideration of deriving an inhalation MRL, however no MRLs were ultimately derived based on the data from these studies.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Yes, I think where necessary, the NOAELS and/or LOAELs have been identified.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: Generally, yes. Since this categorization is a bit subjective, there are some effects that can be considered "serious." For example, immune effects that results in decreased ability to clear bacteria from lungs may lead to severe morbidity and can be considered "serious" but has been categorized as "less serious" in table 2-1 (Adkins et al., 1979a).

RESPONSE: *ATSDR's guidelines for classifications of LOAELs and serious LOAELs is outlined in the introduction to Chapter 2 in the Tox Profile as follows:*

> *"LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction."*

The reviewer's comment notes the classification of decreased ability to clear bacteria from lungs as a LOAEL stating it should be considered a serious LOAEL (SLOAEL). The data from Adkins et al. (1979) were reviewed and the categorization of immune LOAELs were changed to SLOAELs. The study indicates that the increased susceptibility to bacterial infection, and decreased ability to clear bacteria from the lungs of mice resulted in significant increases in mortality and in incidence of sepsis. Since these immune effects directly resulted in higher death rates within 24 hours after exposure compared to controls, these effects are classified as serious LOAELs. Increased susceptibility to infection alone may otherwise be classified as a LOAEL per ATSDR's Guidance Document for the Developmental of Toxicological Profiles since this effect is reversible. The text in Section 2.14 and data for Adkins et al. (1979) in Table A-2 were revised to the following:

> *"A concentration-related increase in susceptibility to Streptococci infection was seen in mice exposed to nickel chloride (≤ 0.5 mg Ni/m^3) for 2 hours and then infected either immediately or after a 24-hour recovery period (Adkins et al. 1979c). Increased susceptibility was indicated by*

an exposure-related increase in mortality and decrease in relative mean survival time in exposure groups when compared to simultaneously infected non-nickel exposed controls (Adkins et al. 1979c). Increased mortality and reduced survival time were also observed following a 2-hour exposure to 0.46 mg Ni/m^3 as nickel sulfate (Adkins et al. 1979b). An additional group of mice, exposed to 0.66 mg Ni/m^3 as nickel chloride, developed septicemia from the Streptococcal infection and had a reduced ability to clear the inhaled bacteria 96 hours after infection (Adkins et al. 1979a)."

Table 2-1. Levels of Significant Exposure to Nickel – Inhalation (mg Ni/m^3)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Adkins et al. 1979a									**Nickel chloride**
9	MOUSE (CD-1) 113F	2 hours	0, 0.66	BI CS	Immuno			0.66	Decreased ability to clear bacteria from lungs resulting in a significant increase in mortality (>20% higher than controls) and increased incidence of sepsis
Adkins et al. 1979b									**Nickel sulfate**
10	MOUSE (CD-1) 120F	2 hours	0, 0.46	BI CS	Immuno			0.46	Increased susceptibility to Streptococcal infection resulting in a significant increase in mortality (21% higher than controls) and reduced mean survival time (2 days less than controls)
Adkins et al. 1979c									**Nickel chloride**
11	MOUSE (CD-1) 80-160F	2 hours	0, 0.288, 0.292, 0.369, 0.5, 0.51	BI CS	Immuno	0.37		0.5	Increased susceptibility to Streptococcal infection resulting in a significant increase in mortality (26% higher than controls) and reduced mean survival time (2.73 days less than controls)

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: It is difficult to exhaustively discuss all possible mechanisms of action in a "profile" document. The mechanisms of action discussed in this document seem appropriate.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: The conclusions seem appropriate, but where I may have a different interpretation, I have noted that in the document.

RESPONSE: *Responses to the reviewer's comments in the Tox Profile document are provided in the 'Annotated Comments in the Profile' section.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: The ADME discussions are appropriate and relevant for this document.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT: There is a paper describing PBPK model of rat and occupational exposure to nickel that have not been described: https://pubmed.ncbi.nlm.nih.gov/29903311/ This article is in Chinese but there is an English version; I'm not familiar with it though.

RESPONSE: *This study was captured in the literature review for nickel conducted in 2020. An English version of the full article could not be located to determine if the study should be included in the discussion on PBPK models in Chapter 3. Should an English version be found, it will be reviewed for consideration in future updates to this profile. Future updates of this profile will include a search and review of all available pharmacokinetic/pharmacodynamic models and supporting data.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: I would say yes. However, there is an important publication on animal-to-human extrapolations that I expected to see under section 3.1.6 (https://pubmed.ncbi.nlm.nih.gov/25055843/). So this section could be expanded a bit more.

RESPONSE: *This study is discussed in Appendix A of the toxicological profile. The approach using the Multiple Particle Path Dosimetry model was adapted to derive the provisional intermediate and chronic-*

duration inhalation MRLs for nickel. A brief description of the study provided by the reviewer was added to Section 3.1.6, as follows:

> *"Oller et al. (2008) describes an approach to derive human equivalent concentrations from rat studies, accounting for differences in respiratory tract deposition and clearance. Deposition fractions in the respiratory tract of rats and human were calculated using the Multiple Path Particle Dosimetry (MPPD) model; this approach was similarly done in calculating human equivalent concentrations to derive inhalation MRL values (See* ***Error! Not a valid bookmark self-reference.****)."*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: Not that I am aware of. It appears all relevant literature on susceptible populations have been captured.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: Yes and yes. There is discussion of children and other populations susceptible to nickel exposure here and in section 5.7.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT: The biomarkers of exposure in the urine, hair/nails, milk, feces are specific to nickel. I made a comment on the last sentence in section 3.3.1 about a non-specific biomarker of exposure described in the document.

RESPONSE: *The reviewer's comment on Section 3.3.1 is addressed in the annotated comments section.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT: Yes. There are not adequate or enough biomarkers of effect for nickel exposure. This is an area where more data will be helpful.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: Yes. However, what is lacking in the general literature is information on the interaction between nickel and the organic substances that those working in the production and use industries are exposed to.

RESPONSE: *A data need was added to the profile in Section 6.2, as follows:*

> *"As noted in Section 3.4, there are many reported interactions with nickel including interactions that may occur in occupational settings with nickel exposure, including those that may elevate toxicity. Literature on the impact of co-exposures that are likely to occur in occupational settings would be useful."*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: Yes, the text discusses the mechanisms underlying these interactions with other substances. For example, the text discusses how other metal substances, such as iron, magnesium, interact with nickel.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: The major nickel substances have been described in table 4-1. There are other nickel substances not described but they are of low economic and toxicological importance.

RESPONSE: *No revisions were suggested.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT: Yes. Initially, it was obvious that the hydrated forms of some of the substances have not been described, but it later became clear that data related to some of the hydrated forms have been summarized in the tables.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: Since this is a document with a focus on the US, the information provided seems appropriate.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: I do not have additional information to provide on this section. I think the textual information is appropriate.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: The information provided in the document is adequate but not unnecessary nor over-detailed.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: The use of "nickel" to refer to all nickel substances or just nickel metal is not consistent and sometimes confusing. If a study identifies the nickel species, it should be clearly indicated. Other than that, I found the information here adequate.

RESPONSE: *Several chapters of the profile were reviewed for consistency. When available, the specific nickel substance discussed is stated.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: I agree with the textual information here.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT: I have added some studies to the document. However, I'm also aware of some studies, such as extended one generation reproductive toxicity study with nickel metal, that are either ongoing or have just been completed.

RESPONSE: *All references provided by the reviewer were reviewed and their inclusion is detailed in other comments. The ongoing studies the reviewer mentioned were not located to include in the Ongoing Studies section. Future updates of this profile will include a search and review of all available ongoing studies.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT: Yes, the general data needs identified seem appropriate. Additional area of data needs pertains to the mode of action of nickel carcinogenesis and the mechanisms underlying other health effects of nickel exposure.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT: Yes. I think there is no overt bias in the presentation of data needs.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT: There are different US State regulatory agency values (eg, Cal-OEHHA 1-hour REL) that could be included, unless it will make the information too much.

RESPONSE: *Information on regulatory values is focused on national-level US regulatory agencies. The information included is in accordance with ATSDR's Guidance for the Preparation of Toxicological Profile.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT: No specific comments on the appendices.

RESPONSE: *No revisions were suggested.*

Annotated Comments on the Profile

COMMENT: Page 1, Line 8: This is important use to emphasize due to the growing importance of EVs. Reviewer inserted: "such as Electronic Vehicle batteries"

RESPONSE*: Reviewer inserted: "such as electronic vehicle batteries" into the following sentence in section 1.1: "Alloys are used in medical devices such as dental appliances and tools, orthopedic implants, birth control implants, and cardiovascular prosthesis; batteries, such as Electronic Vehicle batteries; and equipment and parts for chemical plants, petroleum refineries, jet engines, power generation facilities, and offshore installations."*

This edit was incorporated into the Tox Profile.

COMMENT: Page 1, Line 12-13: This study is not reporting tolerable upper intake level for Ni, therefore statement cannot be verified as written here. Perhaps this is the appropriate reference: Nielsen F. (2021). Nickel. Adv Nutr 12(1): 281-282 doi: 10.1093/advances/nmaa154

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7849985/#bib12

RESPONSE: *The reference was edited to the study suggested by the reviewer. The sentence now states the following:*

> *"The Tolerable Upper Intake Level for nickel is 1.0 mg/day as soluble salts for adults 19 years and older and varies for children aged 1 to 13 years (Nielsen 2020)."*

COMMENT: Page 1, Line 19-20: Please note that this is not in reference to the US population specifically but to the global general population, so a clarification may be necessary since this section is on "overview and U.S. exposures."

RESPONSE: *In response to the reviewer's comment, these statements were reviewed for accuracy and the following is now stated regarding nickel allergy prevalence:*

> *"According to the Cleveland Clinic, nickel allergy and sensitivity, typically observed as contact dermatitis, is estimated to affect over 10% of the U.S. population (Cleveland Clinic 2018). Studies indicate that the prevalence of nickel allergy is between 11-16% (Alinaghi et al. 2019; Uter et al. 2003) and is more prevalent among females (Thyssen and Menne 2010)."*

COMMENT: Page 1, Line 26: Please check reference and link. Could not verify numbers because I could not locate reference on EPA website and link doesn't direct to actual site.

RESPONSE: *The reviewer is referring to the following sentence: "In ambient air in 2020, the mean nickel concentration across 22 U.S. sites ranged from 0.000078 to 0.16 µg/m^3 (EPA 2020)." These values*

were obtained from the data set provided on the cited EPA change and the range is not available in a report or study to cite. The citation was updated to link to the data download page rather than the homepage.

COMMENT: Page 1, Line 30: 27.67 ug/g in Table 5-13

RESPONSE: *The reviewer is referring to the following sentence: "Nickel is also present in tobacco products and e-cigarettes at concentrations ranging from 1.19 to 16.8 µg/g in cigarettes and smokeless tobacco products, and up to 22,600 µg/L in e-cigarette liquid (see* ***Error! Not a valid bookmark self-reference.****)."*

The maximum value in the range was updated to 27.67 ug/g upon re-review of Table 5-13.

COMMENT: Page 3, Line 2-4: Consider adding something like "However, these workers were also exposed to other metals, and cigarette smoking may have been a confounder."

RESPONSE: *The following sentence was added per the reviewer's comment:*

> *"However, these workers were also exposed to other metals and cigarette smoking may also be a confounder."*

COMMENT: Page 3, Line 23: Remove. This reference reported respiratory effects of Nickel in dogs but not in rats.

RESPONSE: *The reviewer is referring to the Ambrose et al. 1976 citation in the following sentence: "Oral doses of nickel compounds in rats as low as 5.75 mg Ni/kg/day also induce respiratory effects including emphysema, bronchiectasis, irregular respiration, pneumonitis, increased lung weight, and altered lung enzyme levels (Ambrose et al. 1976; American Biogenics Corporation 1988; Obone et al. 1999; Oller and Erexson 2007:; RTI 1988a, 1988)." The Ambrose citation was deleted from the list of references since the study did not report respiratory effects for rats that was included in the LSE table.*

COMMENT: Page 3, Line 24: I don't think this study describes lung effects of Ni.

RESPONSE: *The reviewer is referring to the Oller and Erexson reference in the following sentence: "Oral doses of nickel compounds in rats as low as 5.75 mg Ni/kg/day also induce respiratory effects including emphysema, bronchiectasis, irregular respiration, pneumonitis, increased lung weight, and altered lung enzyme levels (American Biogenics Corporation 1988; Obone et al. 1999; Oller and Erexson 2007; RTI 1988a, 1988)."*

The Oller and Erexson study was kept in the list of citations as irregular respiration was a reported health effect of nickel exposure.

COMMENT: Page 4, Line 7-9: Consider including: "an updated, guideline-compliant study in mice exposed to nickel chloride for 24-h showed no impaired immune function (Buxton et al., 2021)" https://pubmed.ncbi.nlm.nih.gov/34644513/

RESPONSE: *The study by Buxton et al. 2021 was added to the Tox Profile in Chapter 2 and the LSE data. Additionally, a sentence was added in the first section similar to the reviewer's recommendation as follows:*

> *"However, a recent study in mice exposed 24-hours to concentrations up to 0.0801 mg Ni/m^3 reported no exposure-related immunosuppressive effects (Buxton et al. 2021)."*

COMMENT: Page 5, Figure 1-1: Insert referenced studies as footnotes? That will make it possible for the reader to check which studies reported these health effects and corresponding concentrations. Same comment applies to Figure 1-2 below.

RESPONSE: *The references were not added to the table as the figure aims to summarize the LSE data which can be found in detail in Table 2-2 and 2-3.*

COMMENT: Page 6, Line 4: Since table is showing only effects in animals, why not then remove the reference to humans?

RESPONSE: *This figure includes effects in humans at the lowest concentration range under Acute exposure hence this footnote is included.*

COMMENT: Page 8, Figure 1-3: Using superscript numbers/letters to each health effect, insert references as footnotes? Otherwise, it's difficult to reconcile these effects and LOAELs with corresponding studies. Same applies to Figure 1-4

RESPONSE: *References were not added since these figures aim to provide an overview of the database and data details are provided in the LSE tables in Tables 2-2 and 2-3.*

COMMENT: Page 11, Line 24-25: Dermal exposure route only presented in Table 2-3 without an accompanying figure

RESPONSE: *Data from dermal exposure route studies are only presented in tables and not figures as the units of results are presented vary widely across studies and may not be appropriate to compare on the same axis.*

COMMENT: Page 13, Figure 2-1: This figure is confusing. If each count represents a study, then there are more animal studies evaluating health effects than human studies. But most studies investigate more than one health effect. This figure needs clarification, otherwise the numbers don't add up.

RESPONSE: *An edit was made to the footnote of this figure to now state the following:*

"Includes studies discussed in Chapter 2. A total of 204 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints."

COMMENT: Page 14, Table 2-1: Consider "Levels of Significant Exposure to Nickel in Animal Studies - Inhalation". That lets the reader immediately know this table is referring to animal studies.

RESPONSE: *The table title was kept as is for consistency with previously published Tox Profiles and in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: Page 14, Table 2-1: Parameters monitored could be taken out and table will still read well. I'm not sure it adds much to the table.

RESPONSE: *Parameters monitored are presented in all health effects tables for Tox Profiles and provide readers context on study methodology in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: Page 58, Table 2-2: Consider "Levels of Significant Exposure to Nickel in Animal Studies - Oral" That lets the reader immediately know this table is referring to animal studies.

RESPONSE: *The table title was kept as is for consistency with previously published Tox Profiles and in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: Page 84, Line 19: *Reviewer suggested insertion of "and non-metallic substances" to the following sentence in section 2.2:* "However, all of the workers were exposed to other metals (arsenic, uranium, iron, lead, chromium) and non-metallic substances, so it cannot be concluded that nickel was the sole causative agent."

RESPONSE: *The reviewer's suggested edit was inserted and now states the following:*

"However, all of the workers were exposed to other metals (arsenic, uranium, iron, lead, chromium) and non-metallic substances, so it cannot be concluded that nickel was the sole causative agent."

COMMENT: Page 84, Line 25: I don't see a "Hirano et al. 1994a', so delete 'b'

RESPONSE: *It was confirmed that there is no need to state Hirano et al. 1994b. This reference was updated to Hirano et al. 1994 in all sections where it is referenced.*

COMMENT: Page 85, Line 2: *Reviewer suggested insertion of "hexahydrate" into the following sentence of section 2.2*: "At 1.4 mg Ni/m3 as nickel sulfate hexahydrate, all mice and no rats died, and at 7.33 mg Ni/m3 as nickel subsulfide, all mice and only 2 of 10 rats died following exposure for 6 hours/day, 5 days/week, for up to 12 exposures (NTP 1996a, 1996b, 1996c)."

RESPONSE: *The reviewer's suggestion was inserted into the sentence.*

COMMENT: Page 85, Line 16: This is a noteworthy deficiency of this study. It limits making interpretation of the deaths observed here.

RESPONSE: *This comment refers to the following sentence in section 2.2*: *"Lung lesions including edema, hyperemia, and hemorrhage were the principal causes noted however no controls were used in this study." The study used data on the same species from previous carcinogenic studies as the comparative control group. This is still a noteworthy deficiency and was added to the text, as follows:*

> *"Lung lesions including edema, hyperemia, and hemorrhage were the principal causes noted. A major study deficiency was the lack of control animals, the study instead compared exposure groups to data of same-species controls from previous carcinogenic studies (Hueper 1958)."*

COMMENT: Page 85, Line 21-22: Throughout document, the strain of rodent (rat/mice) is sometimes stated but other times not stated. Consider being consistent, if possible. *Reviewer suggested the addition of "Swiss albino" into the following* sentence: "Oral LD50 values of 116 and 136 mg Ni/kg as nickel acetate in Fischer-344 female rats and male Swiss-albino mice, respectively have been reported for soluble nickel compounds (Haro et al. 1968)"

RESPONSE: *Chapter 2 was reviewed to ensure that strains are mentioned at least in the first instance where a study is discussed and additionally stated as appropriate. The reviewer's suggestion to add the mice strain was added in addition to other edits throughout.*

COMMENT: Page 86, Line 22 *: Reviewer suggested the change of "metallic nickel" to "nickel oxide" in the following sentence*: "Acute continuous exposure of 23.6 mg Ni/m3 as nickel oxide for 12 days in a 16-day period did not affect body weight in Fischer- 344 rats and B6C3F1 mice of both sexes (NTP 1996a)."

RESPONSE: *The reviewer's suggested edit was included.*

COMMENT: Page 86, Line 25-26: Please doublecheck NTP studies: *this is referring to Ni subsulfide and sulfate; no metallic Ni study by NTP. *For Ni subsulfide, significant change in final body weight was observed from 2.5 mg/m3 (1.83 mg Ni/m3) group. These changes are to reflect the above points. *Reviewer changed the following sentence to reflect these comments* "Subsequent studies from the National Toxicology program observed significant decreases in body weight (22-28%) of Fischer-344 rats after 12 days of continuous exposure to 0.7 and 1.83 mg Ni/m^3 nickel sulfate hexahydrate and nickel subsulfide, respectively (NTP 1996b, 1996c)"

RESPONSE: *These sentences on body weight effects reported from the NTP were reviewed and edited incorporating the reviewer's suggested edits as follows:*

> *"Acute continuous exposure of 23.6 mg Ni/m^3 as nickel oxide for 12 days in a 16-day period did not affect body weight in Fischer-344 rats and B6C3F1 mice of both sexes (NTP 1996a). Subsequent studies from the National Toxicology program observed significant decreases in body*

weight (22-28%) of Fischer-344 rats after 12 days of continuous exposure to 0.7 to 1.83 mg Ni/m^3 nickel sulfate hexahydrate and nickel subsulfide (NTP 1996b, 1996c)."

COMMENT: Page 86, Line 28: Similar study? The reference is to Ni subsulfide. Revise this sentence to indicate substance and dose or delete. I couldn't find a 14% weight change in male mice exposed to Ni subsulfide in the 16-day study.

RESPONSE: *The comment is in response to the following sentence in section 2.3: "Male and female B6C3F1 mice exposed for a similar duration to 1.4 mg Ni/m^3 of nickel sulfate appeared emaciated (NTP 1996c) while a similar study observed male mice body weight decrease by 14% (NTP 1996b)" The sentence was edited to include the dose and substance. Final body weight was 14% less in the exposed group compared to controls based on the data reported in Table 19 of the NTP (1996b) study. The sentence now states the following:*

"Male and female B6C3F1 mice exposed for a similar duration to 1.4 mg Ni/m^3 of nickel sulfate appeared emaciated (NTP 1996c) while a similar study observed male mice final body weight from exposure to 3.65 mg Ni/m^3 of nickel subsulfide was 14% less than controls (NTP 1996b)."

COMMENT: Page 88, Line 10*: Reviewer suggests the insertion "of nickel sulfate hexahydrate" to the following sentence:* "… and in rats treated with 75 mg Ni/kg/day of nickel sulfate hexahydrate for 2 years in diet (Ambrose et al. 1976)"

RESPONSE: *The reviewer's suggested edit was added.*

COMMENT: Page 88, Line 19 : *Reviewer suggested the change from "male mice" to "rats" in the following sentence*: "In rats, no body weight changes were reported following a 6-week exposure to 5 mg Ni/kg/day as nickel acetate in feed (Whanger 1973)"

RESPONSE: *The reviewer's suggested edit was added.*

COMMENT: Page 88, Line 24: *Reviewer suggested the change from "(Stephen Adeyemi et al. 2017) to (Ayedemi et al. 2017).*

RESPONSE: *The citation was changed to "Adeyemi et al. 2017" to align with proper formatting conventions throughout the document, and in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: Page 88, Line 28: *Reviewer suggests the change of "nickel chloride" to "nickel sulfate"*

RESPONSE: *The reviewer's suggested edit was made to correct to the proper form of nickel.*

COMMENT: Page 91, Line 4: Reviewer suggests change of "sulphur" to "sulfur"

RESPONSE: *The reviewer's suggestion was incorporated to reflect American spelling conventions and in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: Page 96, Line 24: *Reviewer suggested change from "cobalt sulfate heptahydrate" to "nickel sulfate"*

RESPONSE: *The sentence was edited for clarity to now state the following:*

> *"At similar lower concentrations of exposure to 0.00017 mg Ni/m^3 as nickel sulfate in ApoE mice, exposure induced microcirculatory dysfunction indicated by increases in adherent and rolling monocytes in the microcirculation after a 3-month continuous exposure (5 days/week, 6 hours/day) (Xu et al. 2012)."*

COMMENT: Page 120, Line 8-11: This study describes hepatotoxic effects of Ni without mentioning nephrotoxic effects. Wrong reference or delete this.

RESPONSE: *The reviewer is referring to the following sentences: "Cellular changes were observed in liver sections of rats exposed to 0.7585 mg Ni/m^3 as nickel sulfate for 21 days (Stephen Adeyemi et al. 2017). These changes included swollen renal tubules, necrosis, and nephritis, and further there was a 12% decline in kidney-to-body weight ratio and increases in plasma creatinine and urea (Stephen Adeyemi et al. 2017)." These sentences are referencing the wrong Adeyemi study and were corrected from Adeyemi et al. (2017) to Adeyemi and Elebiyo (2014).*

COMMENT: Page 126, Line 19-21: Consider adding something like "Similarly, chronic exposure to metallic nickel at 0.4 mg Ni/m3 in male rats caused a significant increase in incidence of pheochromocytomas; the authors noted that the pheochromocytomas were secondary to lung toxicity of nickel exposure (Oller et al., 2008)

RESPONSE: *The summary of this study for endocrine effects was edited incorporating the reviewer's suggested edits and further review of the study as follows:*

> *"Chronic exposure to metallic nickel at 0.4 mg Ni/m^3 in male rats resulted in relative adrenal gland weight 89% higher than controls and correlated with increased incidence of pheochromocytomas (Oller et al. 2008). However, the authors noted that the pheochromocytomas were secondary to lung toxicity of nickel exposure. In female rats exposed to 0.4 mg Ni/m^3, the incidence of angiectasis in the adrenal glands was greater than controls (Oller et al. 2008)."*

COMMENT: Page 130, Line 1-2: What is the immunologic response in this study?

RESPONSE: *The reviewer is referring to the following sentence: "Springborn Laboratories (2000a) observed no gross necropsy changes in the spleen or thymus of rats following 16 to 18 week daily exposures to doses of 0.22 to 2.2 mg Ni/kg/day as nickel sulfate hexahydrate; Springborn Laboratories (2000b) exposed one generation of rats parents to 2.2 to 16.6 mg Ni/kg/day for approximately 8 weeks." The text after the semicolon is an error and was deleted, the sentence now states the following:*

"Springborn Laboratories (2000a) observed no gross necropsy changes in the spleen or thymus of rats following 16-to-18-week daily exposures to doses of 0.22 to 2.2 mg Ni/kg/day as nickel sulfate hexahydrate."

COMMENT: Page 133, Line 5: Please note that this is a preliminary qualitative report. Subsequent large epidemiological study of the Russian cohort was launched following this report, resulting in a series of publications. To make this complete, please review the following studies here and under developmental effects:

https://pubmed.ncbi.nlm.nih.gov/18165195/

https://pubmed.ncbi.nlm.nih.gov/18365800/

https://pubmed.ncbi.nlm.nih.gov/16539171/

https://onlinelibrary.wiley.com/doi/abs/10.1002/ajim.20609

RESPONSE: *A summary of these studies was added to Section 2.16 Reproductive. Upon review, these studies did not appear to evaluate the same cohort of workers as in the Chaschin study, but rather examined a population of women who worked at similar facilities. The following sentences were added:*

"Several epidemiological studies examined the association between maternal occupational exposure to water soluble nickel at the start of pregnancy and the risk of varying fetal outcomes among a population living near a large complex of nickel, copper, and cobalt refineries operating in the Kola Peninsula (Vaktskjold 2006, 2007, 2008a, 2008b). Maternal occupation and birth outcomes were obtained from the Kola Birth Registry and used to categorize nickel exposure based on job (Vaktskjold 2006, 2007, 2008a). Exposure did not affect the risk of birthing a small for gestation age newborn (Vaktskjold et al. 2007), delivering a newborn with a genital malformation (Vaktskjold et al. 2006), or delivery of a newborn with musculoskeletal defects (Vaktskjold et al. 2008a). The adjusted odds ratio for per unit increase in maternal occupational exposure to water soluble nickel and birthing a small-for-gestation age (SGA) newborn is 0.84 (95% CI: 0.75-0.93) (Vaktskjold et al. 2007). The adjusted odds ratio for nickel-exposed women delivering a newborn with a genital malformation is 0.81 (95% CI: 0.52–1.26), and that for an undescended testicle is 0.76 (95% CI 0.40–1.47) (Vaktskjold et al. 2006). The adjusted odds ratio for per unit increase in maternal nickel exposure category and musculoskeletal defects is 0.96 (95% CI: 0.76–1.21) (Vaktskjold et al. 2008a). In a case-control study of the same population, workers of facilities within and outside of the refinery complex self-reported pregnancy outcomes and employment history (Vaktskjold et al. 2008b). There was no significant association between maternal occupational exposure to water soluble nickel in early pregnancy and the risk of spontaneous abortion; the adjusted odds ratio is 1.14 (95% CI: 0.95 – 1.37) (Vaktskjold et al. 2008b)"

COMMENT: Page 145, Line 8: *Reviewer suggested the addition of "as a nickel powder" in the following sentence in section 2.19:* "In contrast, Wistar rats exposed to concentrations up to 1 mg Ni/m3 for 24 months, 6 hours/day, 5 days/weeks, did not show increased incidence of respiratory tract neoplasms but other signs of lung toxicity were present (Oller et al. 2008)."

RESPONSE: *The reviewer's suggested edited was added and the sentence now states the following:*

"In contrast, Wistar rats exposed to concentrations up to 1 mg Ni/m^3 as a nickel powder for 24 months, 6 hours/day, 5 days/weeks, did not show increased incidence of respiratory tract neoplasms but other signs of lung toxicity were present (Oller et al. 2008)."

COMMENT: Page 145, Line 12: *Reviewer suggested the addition of "metal" to the following sentence:* "However, this same study found that incidence of benign and malignant adrenal gland pheochromocytoma in male rats and cortical adenoma/carcinomas in females were concentration-dependent to nickel exposure and were particularly significant at 0.4 mg Ni/m3 for both sexes (Oller et al. 2008)."

RESPONSE: *The reviewer's suggested edited was added and the sentence now states the following:*

"However, this same study found that the incidence of benign and malignant adrenal gland pheochromocytoma in male rats and cortical adenoma/carcinomas in females were concentration-dependent to nickel metal exposure and increased tumor incidence was significant at 0.4 mg Ni/m^3 for both sexes (Oller et al. 2008)."

COMMENT: Page 145, Line 16: *Reviewer suggested the addition of "nickel subsulfide" to the following sentence*: "Significant increases in the incidence of benign or malignant pheochromocytoma in the adrenal medulla were also observed in males at 0.11 or 0.73 mg Ni/m3 and females at 0.73 mg Ni/m3 (NTP 1996b).

RESPONSE: *The reviewer's suggested edited was added and the sentence now states the following:*

"Significant increases in the incidence of benign or malignant pheochromocytoma in the adrenal medulla were also observed in males at 0.11 or 0.73 mg Ni/m^3 and females at 0.73 mg Ni/m^3 nickel subsulfide (NTP 1996b)."

COMMENT: Page 146, Line 8: Review here Heim et al (2007) study on oral exposure to nickel sulfate hexahydrate and cancer incidence.https://pubmed.ncbi.nlm.nih.gov/17692353/

RESPONSE: *The results from the Heim et al. (2007) for other noncancer effects are included in the LSE data and discussed in other chapters of the Profile. The cancer findings were added to this section as follows:*

"Similarly, neoplastic and non-neoplastic findings in Fischer-344 rats exposed for 2 years to doses up to 11.16 mg Ni/kg/day were not related to nickel exposure and were similar to the control group (Heim et al. 2007)."

COMMENT: Page 157, Line 10-11: This review does not indicate association between occupational NiNP inhalation exposure and lung and nasal cancer. Either provide appropriate references for these effects or remove the lung and nasal cancer.

RESPONSE: *The comment is in response to the following sentence: "Occupational NiNPs inhalation is associated with increased risk of lung fibrosis, as well as lung and nasal cancer (Genchi et al. 2020)."*

The review notes higher incidence among workers for nasal and lung cancer; the sentence was edited to state that high incidence was reported, and not to suggest an association, as follows:

> *"Occupational NiNPs inhalation is associated with increased risk of lung fibrosis, and high incidence of lung and nasal cancer is also reported (Genchi et al. 2020)."*

COMMENT: Page 160, Line 29: Distinguish between the use of NiNPs to refer to NPs of nickel metal and NPs of nickel compounds, such as NiO NPs.

RESPONSE: *The section was reviewed throughout to ensure that for each study, the proper Ni NP form is stated. The reviewer commented on the specific sentence on the Shinohara et al. 2017 study which was edited as follows:*

> *"In a study of intratracheal exposure in rats, spherical NiO NPs dissolved less readily in artificial lysosomal fluid and had lower pulmonary clearance rates than wire-shaped NiO NPs, suggesting that wire-shaped NiNPs may be more readily absorbed by the lungs. The smallest NiO NPs also had the highest absorption and distribution rates (Shinohara et al. 2017)."*

COMMENT: Page 161, Line 10-14: Looks like direct quotes from the article. Reword or quote appropriately?

RESPONSE: *The comment is in response to the following sentence*: *"Translocation of NiO NPs from the lungs to the thoracic lymph nodes increased in a time- and dose-dependent manner for three spherical and irregular shaped NiNPs, but not for the wire-like NiO NPs (Shinohara et al. 2017). Thirty-five percent of the wire-like NiO NPs were excreted in the first 24 hours after administration; excretion was 0.33–3.6% in that period for the spherical and irregular-shaped NiO NPs". This sentence was rephrased as follows:*

> *"In a study by Shinohara et al. (2017) pulmonary clearance rate constants were estimated using a one-compartment model in rats which demonstrated that the shape of NiNPs had an effect on the clearance. Spherical and irregular shaped NiO NPs showed time- and dose-dependent increases in translocation from lungs to the thoracic lymph nodes, but wire-like NiO NPs did not (Shinohara et al. 2017)."*

COMMENT: Page 161, Line 25-27: This is repeated from above, so consider deleting.

RESPONSE: *The comment is in response to the following sentence: "In the study of differently shaped NiNPs administered intratracheally to rats, wire shaped NiO particles were excreted much more quickly than spherical and irregular particles (35% after 24 hours vs 0.33 3.6%) (Shinohara et al 2017)." This sentence was edited since it discusses excretion relevant to this paragraph and repeated information from the previous paragraph was deleted, and the sentence now states the following:*

> *"In a study of differently shaped NiNPs administered intratracheally to rats, wire-shaped NiO NP were excreted in urine much more quickly (35% 24-hours after administration) than spherical and irregular particles (0.33-3.6% 24-hours after administration) (Shinohara et al. 2017)."*

COMMENT: Page 163, Line 16: Reviewer suggest the change of values from 0.13 and 0.14 to 11 and 13, respectively.

RESPONSE: *The comment is in response to the following sentence: "Following a single acute exposure to either NiO or Ni_3S_2, Benson et al. (1994) reported total respiratory tract fractional depositions of 0.13 and 0.14 for NiO and Ni_3S_2, respectively in F344/N rats." Upon review of Benson et al. (1994) no changes were made to the current text and the values were kept as 0.13 and 0.14 for total respiratory tract deposition for nickel oxide and nickel subsulfide, respectively. These values are reported in the study both in Table 3 and in-text on page 175 in Benson et al. (1994). The peer-reviewer is referring to the values of 0.11 and 0.13 for "fractions of the inhaled NiO and Ni_3S_2 aerosols that deposited in the respiratory tract." Review of Benson et al. (1994) indicates that 0.11 and 0.13 are in error since these values are not reported in the study text or tables and deposition fractions for the respiratory tract. We opted to report the values that are consistently reported within the study.*

COMMENT: Page 171, Line 21-23: Reviewer suggest the deletion of the following sentence: "Studies in animals are limited. Following oral intubation of nickel chloride in rats, 94–97% had been excreted in the feces and 3–6% had been excreted in the urine after 24 hours

RESPONSE: *This sentence repeats from the preceding sentence in error and was deleted.*

COMMENT: Page 183, Line 17-21: Whilst this is a valid point, it's not a biomarker of exposure, at least not unique to nickel exposure. For example, some microRNAs are modulated by nickel exposure, but it's difficult to ascribe changes in these miRNAs to only nickel exposure; thus, these cannot be used alone as biomarkers of exposure. Please consider removing this or adding an explanation statement.

RESPONSE: *This comment is in response to the following sentence: "A recent study shows that exposure to nickel induced epithelial-mesenchymal transition (EMT) as a crucial step in the pathogenesis of several lung diseases. This leads to a persistent downregulation of E-cadherin expression in human lung epithelial cells and that the EMT remained irreversible postexposure (Zhang et al. 2022)." This sentence was edited to note that this biomarker is not unique to nickel exposure and now states the following:*

> *"This is not a biomarker of exposure unique to nickel and cannot be used alone as a biomarker of nickel exposure."*

COMMENT: Page 211, Line 25: Reviewer suggested adding the word "processes" to the following sentence: "Nickel is naturally present in the earth's crust, and natural sources/processes will also release nickel to the soil."

RESPONSE: *The reviewer's suggested edit was added.*

COMMENT: Page 211, Line 27: Reviewer suggested adding the word "anthropogenic" to the following sentence: "The major sources of anthropogenic nickel release to soil are industrial waste materials, lime, fertilizer, and sewage sludge (McIlveen and Negusanti 1994)."

RESPONSE: *The reviewer's suggested edit was added.*

COMMENT: Page 244, Line 12: *Reviewer suggested the insertion of "these workers had inconsistent use of personal protective equipment" in the following sentence:* **"**Hughson et al. (2010) measured dermal and inhalable nickel exposure in workers in primary nickel production and primary nickel user industries, including workers involved in front-end refinery processes, electrowinning/electrolysis, packing solid nickel metal products, packing nickel compounds, packing nickel metal powders, powder metallurgy, and stainless-steel production; these workers had inconsistent use of personal protective equipment."

RESPONSE: *The reviewer's suggested edit was added.*

COMMENT: Page 244, Line 24- 25: *Reviewer suggested the insertion of the following sentence:* **"**The authors concluded that the exposures to nickel likely resulted from direct skin contact with items rather than from airborne dust deposition."

RESPONSE: *This sentence was added upon review of the Julander et al. (2010) study.*

COMMENT: Page 245, Line 3: More cobalt not nickel? I think that was what the study found

RESPONSE: *The comment was in response to the following sentence: "A study of dental technicians in Sweden found that technicians exposed to CoCr in a two-hour period without handwashing had more nickel on the skin than non-exposed technicians (Kettelarij et al. 2016)." This study was reviewed to confirm the study details and the text was edited. The study found similar levels of nickel in all participants. The sentence now read as follows:*

> *"A study of dental technicians in Sweden compared dental technicians exposed to cobalt-chrome via work tasks, such as preparing prostheses and metal constructions for dental crowns, to non-exposed technicians aiming to quantify exposure to nickel, cobalt, and chromium (Kettelarij et al. 2016). The study authors reported that nickel was found on all participants both after 2 hours of exposure with no handwashing and at the end of the workday indicating exposure might be attributed to use of tools and materials that release nickel."*

COMMENT: Page 245, Line 6-10: I couldn't access the study, so please check these numbers again. Something seems off about the skin Ni levels in exposed vs non-exposed workers.

RESPONSE: *The comment was in response to the following sentence: "Before work, the median concentrations of nickel on the skin were 0.014 µg/cm3 in exposed technicians and 0.026 µg/cm3 in non-exposed technicians (Kettelarij et al. 2016). After 2 hours of work without hand washing, concentrations had increased to 0.0.57 µg/cm3 for exposed individuals and 0.012 µg/cm3 for non-exposed individuals (Kettelarij et al. 2016). At the end of the day, the median concentrations were 0.018 µg/cm3 in exposed individuals and 0.014 µg/cm3 in non-exposed individuals". The numbers reported from the study were confirmed and these sentences were rewritten for clarity to now state the following:*

> *"Before work, the median concentrations of nickel on the skin were 0.014 µg/cm^3 in exposed technicians and 0.026 µg/cm^3 in non-exposed technicians, then increased to 0.0.57 µg/cm^3 in exposed technicians and 0.012 µg/cm^3 for non-exposed technicians after 2 hours of work with no hand washing (Kettelarij et al. 2016). At the end of the day, the median concentrations were 0.018 µg/cm^3 in exposed technicians and 0.014 µg/cm^3 in non-exposed technicians (Kettelarij et*

al. 2016). Nickel was found in 4 of 10 air samples taken during this study at concentrations ranging from 0.48 to 3.7 µg/m^3 and metal urine concentrations were normal (Kettelarij et al. 2016).”

COMMENT: Page 250, Line 31: Reviewer suggested the insertion of “sulfate, nickel, chloride” to the following sentence: “No studies on the neurotoxicity of nickel in humans following dermal exposure were located. Neurological effects (giddiness, weariness) were reported in individuals accidentally exposed to nickel sulfate, nickel chloride and boric acid in drinking water (Sunderman et al. 1988).”

RESPONSE: *The reviewer’s suggested edit was added.*

COMMENT: Page 251, Line 15-19: Reviewer suggested the insertion of the following sentence: “Follow-up large epidemiological studies to clarify the observed effects in these workers suggested that female refinery workers exposed to soluble nickel did not result in adverse outcomes for male newborns with genital malformations (Vaktskjold et al., 2006), spontaneous abortions (Vaktskjold et al., 2008), small-for-gestational-age newborns (Vaktskjold et al., 2007), or congenital musculoskeletal effects (Vaktskjold et al., 2008).

RESPONSE: *The studies recommended by the reviewer were added to Chapter 2 under the Reproductive health effects section. The studies were also added to Chapter 6 as the reviewer suggests. The recommended edit was incorporated into the text with edits and the text now states:*

“Large epidemiological studies of the population from this area (Kola Peninsula) suggest that female refinery workers exposed to soluble nickel did not result in adverse outcomes for male newborns with genital malformations (Vaktskjold et al., 2006), spontaneous abortions (Vaktskjold et al., 2008b), small-for-gestational-age newborns (Vaktskjold et al., 2007), or congenital musculoskeletal effects (Vaktskjold et al., 2008a).”

COMMENT: Page 251, Line 17: https://pubmed.ncbi.nlm.nih.gov/16539171/

RESPONSE: *This reference was added into Chapters 2 and 6 of the profile as Vaktskjold et al. 2006.*

COMMENT: Page 251, Line 18: https://pubmed.ncbi.nlm.nih.gov/18365800/

RESPONSE: *This reference was added into Chapters 2 and 6 of the profile as Vaktskjold et al. 2008b.*

COMMENT: Page 251, Line 18: https://pubmed.ncbi.nlm.nih.gov/18165195/

RESPONSE: *This reference was added into Chapters 2 and 6 of the profile as Vaktskjold et al. 2007.*

COMMENT: Page 251, Line 19 : https://pubmed.ncbi.nlm.nih.gov/18655106/

RESPONSE: *This reference was added into Chapters 2 and 6 of the profile as Vaktskjold et al. 2008a.*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: I agree

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: The effects of nickel observed in animals only may not be totally devoid of concern in humans at least from the concept of One Health.

RESPONSE: *No revisions were suggested.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain

COMMENT: I agree

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT: I agree

RESPONSE: *No revisions were suggested.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT: I understand that the factor of 30 is likely to be the usual 10-fold for within human variability and a 3-fold for toxicodynamic differences between the experimental animal and humans. The reason a 3-fold is used, instead of the usual 10-fold between the experimental animal and humans, is likely due to the fact that the experimental animal concentration has been adjusted to the human equivalent concentration (referred to as HEC) using US EPA dosimetric adjustment methods (EPA, 1994). When this is done, the usual 10-fold between the experimental animal and humans is reduced to 3-fold because the kinetic differences between the experimental animal and humans is reduced to 1-fold.

RESPONSE: *For the derivation of the provisional intermediate- and chronic-duration inhalation MRLs, an uncertainty factor of 3 was applied for species extrapolation for the reason provided by the reviewer. The concentrations in rats were converted to human equivalent concentrations.*

QUESTION (Subset of preceding question): Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: I agree for the same reason stated in (5) above

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: I have no comments

RESPONSE: *No revisions were suggested.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: Yes, I agree with the conclusions

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: Yes

RESPONSE: *No revisions were suggested.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT: Yes. The detailed information on dose-response effects of vast species of animals (mice, rats, dogs etc.) and human should be considered convincing that adequate attention has been given to the dose-response relationships for both human and animal data

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: No

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: No

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: All through the document, I didn't see any information as I have highlighted here under in green 'The induction of oxidative stress by nickel and subsequent generation of ROS tend to stimulate cell signaling pathways by creating an intracellular low-oxygen microenvironment which ultimately elicits hypoxia-inducible factor-1α(HIF-1α) transcription factor and which modulates hypoxia gene expressions. In the same vein, Ni^{2+} via ROS may imitate cellular hypoxia without necessarily inducing HIF-1 dependent genes (Salnikow et al 1994). All in all, the ability of ability to activate hypoxia-inducible factor-1α(HIF-1α) transcription factor may at least be due to its capacity to substitute Fe^{2+} in oxygen transport and formation nonfunctional hemoglobin (Das et al 2019).

Reference

Salnikow, K., Gao, M., Voitkun, V., Huang, X. and Costa, M., 1994. Altered oxidative stress responses in nickel-resistant mammalian cells. *Cancer research*, *54*(24), pp.6407-6412.

Das, K.K., Reddy, R.C., Bagoji, I.B., Das, S., Bagali, S., Mullur, L., Khodnapur, J.P. and Biradar, M.S., 2019. Primary concept of nickel toxicity–an overview. *Journal of basic and clinical physiology and pharmacology*, *30*(2), pp.141-152'.

RESPONSE: *A discussion on potential mechanism of action for the induction of tumors was added to Section 2.19 Cancer, incorporating the references and text provided by the reviewer and adding additional detail, as follows:*

> *"Nickel-induced alterations in gene expression may be mediated by activated transcription factors and is shown to alter several transcription factors including hypoxia-inducible transcription factor (HIF-1) and activated transcription factor (ATF-1) (Kasprzak et al. 2003). Nickel exposure is associated with accumulation of HIF-1 which is involved in the regulation of hypoxia-inducible genes involved in cell transformation, tumor promotion, and progression, angiogenesis, altered metabolism, and apoptosis (Salnikow et al. 2003). HIF-1α, one of the HIF-1 subunits, is over-expressed in both primary and metastatic tumors, and is induced in response to hypoxia and exposure to nickel (Li et al. 2004; Salnikow et al. 2000). Both soluble and insoluble nickel compounds have also been shown to induce Cap43 (also called NDRG1) gene expression, a tumor marker, which requires HIF-1α activation (Costa et al. 2003; Li et al. 2004; Salnikow et al. 2000, 2003). Nickel (II) via reactive oxygen species (ROS) can imitate cellular hypoxia without activating HIF-1 dependent genes (Salnikow et al 1994). The ability of nickel to activate HIF-1α transcription factors may be attributed to nickel's capacity to substitute iron (II) in oxygen transport and formation of non-functional hemoglobin (Das et al. 2019). Nickel-transformed rat and mice cells show that the induction of ATF-1 transcription factor down-regulates thrombospondin-1 (TSP-1) expression (Kasprzak et al. 2003; Salnikow et al. 1997). TSP-1 suppresses angiogenesis; thus, the suppression of TSP-1 stimulates tumor growth."*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT: Yes**.**

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT: Yes. The serum and urine nickel levels drawn from various epidemiological and occupational exposure settings tend to justify serum and urine nickel levels (though toenails and hair levels are also helpful) as most specific indicators of body burden of nickel.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT: There seem to a paucity of data of biomarkers of effect since the only literature cited about 30 years ago (Frenkel et al. 1994) and the recommendation for new biomarkers of effect (possibly oxidized DNA product. In the light of this, the biomarker of effect is not specific.

RESPONSE: *The literature review conducted in 2020 did not identify studies on specific biomarkers of effect for nickel to include in the Biomarkers of Effect section.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: Yes, there is an adequate discussion of the interactive effects. No, the discussion does not focus on effects at hazardous waste sites The discussion of the interactive effects of nickel with other substances is adequate in my opinion

RESPONSE: *No revisions were suggested.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: No comment provided.

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: No. It looks like all available information in literature is contained therein

RESPONSE: *No revisions were suggested.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes**.**

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT: Yes. There is need for specific biomarkers of effect and human or animal data on the toxicokinetic properties of nickel in children or immature animals or studies etc.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT: Yes.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT: No.

RESPONSE: *No revisions were suggested.*

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT: No comment provided.

RESPONSE: *No revisions were suggested.*

Annotated Comments on the Profile

No annotated comments on the profile were received from the reviewer.

Comments provided by Peer Reviewer #3

ATSDR Charge Questions and Responses

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: I agree with the effects as reported in the text.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT: Effects seen in animals, especially respiratory effects, are relevant for humans despite differences in pharmacokinetics and physiology (rodent versus human).

RESPONSE: *No revisions were suggested.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain

COMMENT: Exposure conditions have been adequately described.

RESPONSE: *No revisions were suggested.*

Minimum Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT: There are multiple rodents students showing a LOAEL for acute respiratory effects including one from the NTP. These data should be sufficient to derive an MRL using an uncertainty factor to account for NOAEL to LOAEL adjustment. It is unclear why this approach was not used. If using this approach results in an MRL higher than that derived for an intermediate duration MRL – and this is the reason why ATSDR did not pursue this approach then extending the intermediate duration MRL to also represent an acute MRL remains rational. Not deriving oral MRLs also illustrates several inherent weaknesses in ATSDRs approach (e.g., the document states: Serious developmental and reproductive effects are observed at the lowest doses tested in experimental animal studies thus precluding MRL derivation from these end points due to the ATSDR policy of not deriving MRLs from serious LOAELs – yet skeletal anomalies may (or may not be a serious effect). Likewise a NOAEL to LOAEL adjustment is also not considered for the intermediate oral MRL. The ATSDR also states that: In nickel-sensitized individuals, allergic dermatitis occurred from ingesting a single challenge dose ≥0.058 mg Ni/kg as nickel sulfate (Gawkrodger et al. 1986; Hindsén et al. 2001; Jensen et al. 2003). Jensen et al. (2003) noted that the results should not be extrapolated to larger populations. It appears that the ATSDR did not consider

either a meta-analytic approach or weight of evidence approach to evaluate these studies. In essence the document states repeatedly that allergic dermatitis is a well recognized effect seen in humans; however, despite being well recognized an MRL can't be developed. That makes little scientific sense since the human data are coherent and involve virtually identical exposures.

Finally, the ATSDR does not derive a chronic oral MRL stating that: The EPA derived an oral reference dose of 0.02 mg Ni/kg/day for nickel based on a rat study by Ambrose et al. (1976). This study was not used to derive an oral chronic MRL due to concerns of study quality…. The study quality was deemed insufficient for derivation of a MRL by ATSDR as the high mortality among controls does not allow for accurate interpretation of the results. This is not a study quality issue per se. ATSDR has provided no evidence of a high risk of bias/study quality – likewise the ATSDR has not provided any indication of what an "acceptable" mortality rate in controls would be for a chronic rodent study.

RESPONSE: *A respiratory LOAEL of 0.44 mg Ni/m^3 has been identified in several acute inhalation studies. Serious respiratory effects were observed at this concentration precluding derivation. MRLs may be derived based on LOAELs but not for serious LOAELs. The intermediate-duration MRL was not applied as the acute-duration inhalation MRL. The exposure duration from the critical study is not comparable to an acute-duration inhalation MRL and there is no evidence that the point-of-departures between the databases for both durations would be similar.*

For the oral acute-duration studies, several studies reported serious developmental effects in animals at 10.29 mg/kg/day including skeletal anomalies and significantly low body weight in offspring (El Sekily et al. 2020; Saini et al. 2014b). In El Sekily et al. (2020) the number of live-birth pups decreased significantly at all exposure doses of nickel compared to controls (exact rates not provided). At the lowest nickel exposure level tested of 10.29 mg Ni/kg/day, skeletal anomalies included increased incidence of incomplete ossification of skull bones, vertebrae, ribs, sternum, bones of forelimbs and hindlimbs among several other skeletal anomalies. These results taken together were deemed a SLOAEL for developmental effects. Additionally, serious reproductive effects are reported at 11.4 mg Ni/kg/day in two studies that did not test lower doses (Saini et al. 2013, 2014a). Taken together, the database does not identify a LOAEL for reproductive and developmental effects that would be protective for derivation of an MRL.

The reviewer also notes several acute-duration studies identifying doses for allergic dermatitis. As stated in the worksheet, these studies were not selected due to small sample sizes that may not accurately represent the larger population. The limitation noted by Jensen et al. (2003) states "Of course, an extrapolation of the results found among a low number of individuals, as in this study, to a larger number would be statistically incorrect, and the outcome of a study involving the estimated number of individuals might still show no statistical significance." The study conducted an analysis and found that 36 volunteers would be needed for each exposure group to reach statistical significance. Among the three studies reporting allergic dermatitis, no more than 10 volunteers were exposed to a dose (Gawkrodger et al. 1986; Hindsen et al. 2001; Jensen et al. 2003). Given these limitations, a meta-analytic approach with these data to calculate a POD would not be appropriate for MRL derivation.

For the intermediate-duration oral MRL the, the lowest dose tested in animals is a renal SLOAEL of 0.036 mg Ni/kg/day as nickel sulfate from Dahdouh et al. (2016). This study only had one experimental group and reports renal dysfunction indicated by significant change in serum urea, creatinine, and uric acid. Histology findings in the kidneys of control mice were normal while mice exposed to 0.036 mg Ni/kg/day showed "nickel-induced proximal tubule degeneration and tubular necrosis, multiple foci of hemorrhage and inflammation with the presence of lymphocytes in interstitial tissue." Taken together,

this exposure level was deemed a SLOAEL for renal effects and thus not appropriate as a LOAEL for MRL derivation.

For the chronic-duration oral MRL, the Ambrose et al. (1976) study in rats was not considered for MRL derivation as survival was poor among all groups, especially among controls of both sexes and male rats exposed to the highest dose. Study authors did not attribute mortality to nickel exposure. ATSDR determined that this study alone would not be sufficient to derive an MRL in consideration of the high mortality in exposure groups that limits the interpretation of the bodyweight effect.

Additional detail was added and text was clarified on justifications for not deriving an MRL in Appendix A worksheets for the acute-duration inhalation MRL, acute-duration oral MRL, intermediate-duration oral MRL, and chronic-duration oral MRL.

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

COMMENT: I agree with the proposed MRL values that were developed.

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT: I agree with the uncertainty factors that were used: 3 for interspecies extrapolation with dosimetric adjustments and 10 for human variability

RESPONSE: *No revisions were suggested.*

QUESTION (Subset of preceding question): Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT: Science policy decisions (e.g., as mentioned above) need to be referenced in a transparent way for the reader to find the relevant documentation.

RESPONSE: *A citation for the EPA's IRIS assessment for nickel soluble salts, relevant to the chronic-duration oral MRL worksheet, was added to the text as follows:*

> *"The EPA derived an oral reference dose of 0.02 mg Ni/kg/day for nickel based on a rat study by Ambrose et al. (1976) (IRIS 1994)."*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT: Chapter 2 adequately reflects the findings.

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Descriptions were adequate; however, the ATSDR would benefit from streamlining their approaches (e.g., decreased reliance of text – increased reliance on tables)

RESPONSE: *No revisions were suggested.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT: Descriptions were adequate.

RESPONSE: *No revisions were suggested.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT: Literature from appropriate species were cited and used.

RESPONSE: *No revisions were suggested.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT: Adequate attention was paid to dose-response relationships.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT: I am not aware of missing studies.

RESPONSE: *No revisions were suggested.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT: I am not aware of other studies that could be used to derive an MRL.

RESPONSE: *No revisions were suggested.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: No changes suggested.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT: As I indicated earlier skeletal changes in rodent teratology studies may (or may not) represent a serious effect. Categorization of these effects should be transparent. For example, some skeletal changes represent a variation versus a malformation (ICH document): Malformation: Permanent structural deviation that generally is incompatible with or severely detrimental to normal postnatal development or survival. Variation: Structural change that does not impact viability, development, or function (e.g., delays in ossification), which can be reversible and is found in the normal population under investigation. Reversible or minor manifestations of developmental toxicity (e.g., changes in fetal weight, skeletal variations) by themselves are of minimal concern from a risk assessment perspective – this these would fail to meet the threshold of a serious effect (see also: Carney & Kimmel, BDR(B) 1007;80:473-496).

RESPONSE: *The reviewer is referring to their earlier comment on page 35 in response to the first question on MRLs, specifically regarding the classification of LOAEL and SLOAEL values for developmental effects in rodent studies. A general overview on the categorization of serious and less serious effects is provided in the introduction of Chapter 2 as follows:*

> *"LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction."*

ATSDR's Guidance for the Preparation of Toxicological Profiles includes the categorization of developmental effects which follows guidance similar to that quoted by the peer-reviewer. Variations (structural abnormalities) such as delayed ossification or supernumerary ribs are considered LOAELs while serious skeletal anomalies include permanent malformations such as spina bifida which are

classified as SLOAELs. In reviewing the developmental database in response to the peer-reviewer's comment, data from two studies currently in the Tox Profile were updated for consistency in the classification of developmental effects. Saini et al. (2014a) reported increased incidence of skeletal anomalies at 11.35 mg/kg/day (specifically, delayed bone ossification which is considered a variation and the effect is reversible). This effect was incorrectly categorized as a SLOAEL, and in conducting updates was changed to a LOAEL. The data from Saini et al. (2014b) were also updated. The dose of 10.29 mg/kg/day was incorrectly categorized as a SLOAEL for exposure-related reduction in offspring body weight, however since the effect is not expected to impact viability or function, this was changed to a LOAEL value. In the same Saini et al. (2014b) study, the dose of 20.59 mg/kg/day was categorized as a SLOAEL for increased incidence offspring mortality.

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: Addressed adequately.

RESPONSE: *No revisions were suggested.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: Conclusions are adequate.

RESPONSE: *No revisions were suggested.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

Toxicokinetics:

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: Descriptions are adequate

RESPONSE: *No revisions were suggested.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT: Relevant data has been presented.

RESPONSE: *No revisions were suggested.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT: Discussions are adequate

RESPONSE: *No revisions were suggested.*

Children and Other Populations that are Unusually Susceptible:

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT: Descriptions are adequate

RESPONSE: *No revisions were suggested.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT: I agree with choices.

RESPONSE: *No revisions were suggested.*

Biomarkers of Exposure and Effect:

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT: Some biomarkers (e.g., toenail, deciduous teeth) have not been included for completeness.

RESPONSE: *The discussion of results from Salcedo-Bellido (2021) was expanded and an additional study was discussed in the Biomarkers of Exposure section, as follows:*

> *"Like hair, toenails may also provide evidence of past exposure. Exposure to nickel has been monitored by assessing the content of nickel in toenails, and a systematic review found that nickel levels in toenails may indicate exposure occurring 7-12 months before measurement (Salcedo-Bellido et al. 2021). In a study of 47 welders in Massachusetts, nickel levels in toenails and welding hours are not significantly associated (Grashow et al. 2015). However, study authors reported that nickel levels and welding hours 7 to 9 months prior to measurement approached statistical significance."*

No studies were located discussing environmental exposure and nickel levels in primary teeth.

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT: No chemical specific biomarkers of effect are available.

RESPONSE: *No revisions were suggested.*

Interactions with Other Chemicals:

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT: Discussions are adequate

RESPONSE: *No revisions were suggested.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT: Discussions are adequate

RESPONSE: *No revisions were suggested.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT: Values have been spot checked and appear correct.

RESPONSE: *No revisions were suggested.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT: Information on different chemical forms has been provided.

RESPONSE: *No revisions were suggested.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT: Appears to be complete.

RESPONSE: *No revisions were suggested.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: Discussions are adequate – not aware of missing information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT: Discussions are adequate – not aware of missing information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT: Discussions are adequate – not aware of missing information.

RESPONSE: *No revisions were suggested.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT: I agree with the selection of the populations.

RESPONSE: *No revisions were suggested.*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT: I am not aware of missing information.

RESPONSE: *No revisions were suggested.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT: Data needs have not been prioritized – instead represent a "wish list" of data without consideration of which data may be most useful for the hazard assessment/risk assessment.

RESPONSE: *The data needs section was reviewed, and minor updates were made throughout. Edits were especially made regarding acute-duration MRL needs as follows:*

*"**Acute-Duration MRLs.** The acute-duration inhalation animal database was not adequate for the derivation of an acute-duration inhalation MRL. No human studies evaluated acute-duration inhalation exposure. Several studies in animals evaluated the respiratory system, identifying it as the most sensitive endpoint to nickel toxicity. Multiple rat studies identified 0.43 to 0.44 mg Ni/m^3 as the LOAEL for respiratory toxicity as lung lesions, including alveolitis and lung inflammation, were seen following 5-12 days of exposure (Benson et al. 1995b; Efremenko et al. 2014; NTP 1996c). The lungs were not evaluated in studies where lower concentrations were tested on animals; therefore, a concentration-response cannot be established. While immune function was evaluated at lower concentrations of 0.08 and 0.369 mg Ni/m^3 (Adkins et al. 1979a, 1979b, 1979c; Buxton et al. 2021; Graham et al. 1978), these studies did not evaluate the respiratory system. Studies evaluating the lung following exposure to lower concentrations of nickel in rats would be useful to establish a concentration-response relationship, especially given acute-duration exposure to high levels of nickel in air is of concern in occupational studies, as evidenced by several case studies documenting acute toxicity. Few studies in humans examining oral exposure to nickel have reported allergic dermatitis, however these studies examine nickel-sensitized individuals and the small sample sizes do not allow for statistically correct extrapolation to a larger population. (Gawkrodger et al. 1986; Hindsén et al. 2001; Jensen et al. 2003). Oral exposure studies examining allergic dermatitis using larger sample groups would elucidate whether incidence is significant among a larger population. Several experimental studies in animals suggest reproductive and developmental toxicity following oral exposure, however these data indicate toxicity at doses lower than those tested (El Sekily et al. 2020; Saini et al. 2014b; Sobti and Gill 1989). Studies examining reproductive and developmental outcomes from oral exposure to nickel are needed to establish a NOAEL for these endpoints."*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT: I did not recognize bias in this discussion.

RESPONSE: *No revisions were suggested.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT: I am unaware of missing citations.

RESPONSE: *No revisions were suggested.*

QUESTION: Are there any that should be removed? Please explain.

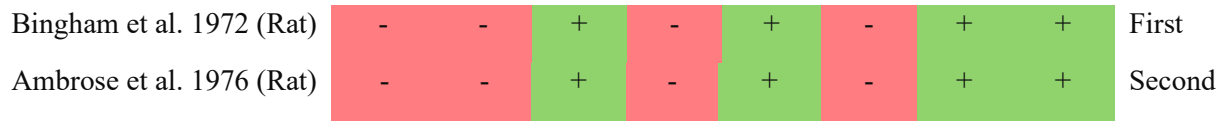
COMMENT: No changes suggested.

RESPONSE: *No revisions were suggested.*

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT: Appendix A. Not clear how risk of bias assessments were translated into the RoB tiers. For example, Bingham et al. 1972 (Rat) is assigned to Tier I while Ambrose et al. 1976 (Rat) is assigned to Tier II despite having identical RoB results.



It's not clear why ATSDR has used an approach for the initial rating of study confidence that is different from OHAT. The domains used for study quality in this step have been incompletely described, criteria used to develop a Yes or No assessment are unavailable. Difficult to separate Sufficient number of animals in a group from Adequate data for statistical analyses. These domains are overlapping. How is sufficient defined? adequate data defined?

RESPONSE: *Several animal studies were incorrectly placed in the first tier when they should have been in the second tier including Bingham et al. (1972). The criteria for how the tiers are established precede table C-7 as follows:*

> *"**First Tier.** Studies placed in the first tier received ratings of "definitely low" or "probably low" risk of bias on the key questions **AND** received a rating of "definitely low" or "probably low" risk of bias on the responses to at least 50% of the other applicable questions.*
>
> ***Second Tier.** A study was placed in the second tier if it did not meet the criteria for the first or third tiers.*
>
> ***Third Tier.** Studies placed in the third tier received ratings of "definitely high" or "probably high" risk of bias for the key questions **AND** received a rating of "definitely high" or "probably high" risk of bias on the response to at least 50% of the other applicable questions."*

Several animal studies had been placed in the first tier as the key question had been incorrectly counted when determining if at least 50% of responses (excluding key questions) received a rating of definitely low or probably low. Table C-8 and subsequent tables were updated.

The text describing the initial confidence rating are available in Section C.6.1. Changes to this text will be considered by ATSDR. Table C-10 and C-11 were additionally edited to include additional text that describes the key features for human-controlled exposure studies and experimental animal studies, as follows:

Table C-2. Key Features of Study Design for Human-Controlled Exposure Studies

A comparison group was used or the subjects served as their own control
A sufficient number of subjects were tested (i.e., 10 or more subjects)
Appropriate methods were used to measure outcomes (i.e., clinically-confirmed outcome versus self-reported)
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis

Table C-3. Key Features of Study Design for Experimental Animal Studies

A concurrent control group was used
A sufficient number of animals per group were tested (i.e., 3 or more animals for acute exposure, 10-20 animals for intermediate exposure, 50 or more animals for chronic exposure)
Appropriate parameters used to assess a potential adverse effect (i.e., clinical, gross, and histopathological outcomes were assessed. If an endpoint was not amendable to a clinical assessment then we did not downgrade the confidence in a study for not including it)
Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis (i.e., the statistical procedures used were presented in the paper and they were appropriate for the data)

Annotated Comments on the Profile

COMMENT: Page 1, Line 11: Identify organization – e.g., is this EFSA?

RESPONSE*: This comment is in response to the following sentence: "The Tolerable Upper Intake Level for nickel is 1.0 mg/day as soluble nickel salts for adults 19 years and older but varies for children (Whitehead et al. 2015)." The National Academies was added as the organization and the source for this data, and additional detail was added. The revised text now states the following:*

> *"The Tolerable Upper Intake Level for nickel reported by the National Academies of Sciences, Engineering, and Medicine (NASEM) is 1.0 mg/day as soluble salts for humans 14 years and older, 0.6 mg/day for 9 to 13 years old, 0.3 mg/day for 4 to 8 years of age, and 0.2 mg/day for children 1 to 3 years old (NASEM 2019)."*

COMMENT: Page 4, Line 7: Include route of exposure – here and elsewhere

RESPONSE*: This comment is in response to the following sentence: "Impaired immune response is seen in studies of mice exposed to nickel compounds for 2 hours (Adkins et al. 1979a, 1979b, 1979c; Graham et al. 1978), rats exposed for 4 months (Spiegelberg et al. 1984), and mice exposed for 65 days (Haley et al. 1990)." The route being discussed was added and checked that it is stated elsewhere in this paragraph. The sentence now reads as follows:*

> *"Impaired immune response is seen in inhalation exposure studies of mice exposed to nickel compounds for 2 hours (Adkins et al. 1979a, 1979b, 1979c; Graham et al. 1978), rats exposed for 4 months (Spiegelberg et al. 1984), and mice exposed for 65 days (Haley et al. 1990)."*

COMMENT: Page 7, Line 6: It's unknown whether it's sensitive at all – or is equally sensitive as the respiratory system? FIG 1-3 is also inconsistent with this statement and could confuse the reader.

RESPONSE*: This comment is in response to the following sentence: "Therefore, it is undetermined if the cardiovascular system is sensitive to nickel toxicity." These statements were deleted as they do not provide meaningful detail to figure 1-3. These sentences were originally added to detail why the cardiovascular LOAEL wasn't used for MRL derivation.*

COMMENT: Page 90, Line 28: On admission?

RESPONSE*: This comment is in response to the following sentence: "The patient's urine nickel levels were 692 µg/L (reference value: <10 mcg/L) (Bowman et al. 2018)." The urine levels were measured on admission and the sentence was edited to state this as follows:*

> *"The patient's urine nickel levels on admission were 692 µg/L (reference value: <10 mcg/L) (Bowman et al. 2018)."*

COMMENT: Page 90, Line 31: Reviewer suggests change from "X- ray of the chest" to "chest radiograph"

RESPONSE*: This comment is in response to the following sentence: "The patient immediately developed a persistent strong cough and a chest radiograph taken three days later showed reticular opacities in middle and lower lung fields, while a CT scan of the chest showed bilateral non-segmental ground-glass opacities." The reviewer's revision was incorporated.*

COMMENT: Page 93, Line 25: could be confusing – state i.e., pneumonia

RESPONSE*: This comment is in response to the following sentence: "Chronic exposure to nickel (6 hours/day, 5 days/week for 2 years) resulted in chronic active lung inflammation (or pneumonia) in rats and mice at 0.06 mg Ni/m3 as nickel sulfate,…" The reviewer's recommended edit was incorporated, and the sentence now states:*

> *"Chronic exposure to nickel (6 hours/day, 5 days/week for 2 years) resulted in chronic active lung inflammation (e.g., pneumonia) in rats and mice at 0.06 mg Ni/m^3 as nickel sulfate, in rats at 0.11 mg Ni/m^3 and higher as nickel sulfide (NTP 1996b; Ottolenghi et al. 1975), in mice at 0.44*

mg Ni/m^3 and higher as nickel subsulfide (NTP 1996b), in rats at 0.2 mg Ni/m^3 and higher as nickel oxide (NTP 1996a; Tanaka et al. 1988), and in mice at 1 mg Ni/m^3 as nickel oxide (NTP 1996a)."

COMMENT: Page 95, Line 22: Were lung weights normalized to bw? Unlikely that changes in bw would confound the lung pathology reported.

***RESPONSE**: This comment is in response to the following sentence*: *"In a multigeneration study (RTI 1988a, 1988b), increased lung weights were observed in rats provided with nickel chloride in the drinking water at 55 mg Ni/kg/day, and an increase in cellular infiltration of the lungs was observed at 20 mg Ni/kg/day. This study is confounded by decreased food and water intake observed in exposed animals." The study reported an increase in both relative and absolute lung weight. The sentence was edited to state that the change was seen in relative lung weight and the detail on the confounder was deleted, the sentence now states:*

"In a multigeneration study (RTI 1988a, 1988b), increased relative lung weights were observed in rats provided with nickel chloride in the drinking water at 55 mg Ni/kg/day, and an increase in cellular infiltration of the lungs was observed at 20 mg Ni/kg/day."

COMMENT: Page 95, Line 28: Reword this since it implies multiple tests were performed

***RESPONSE**: This comment is in response to the following phrase: "scratch test." The sentence was rephrased for conciseness and now states the following:*

"Scratch tests and intradermal tests performed on a patient diagnosed with nickel-related asthma resulted in respiratory distress indicated by a more severe response to the tests when compared to the results from non-asthmatic controls (McConnell et al. 1973)."

COMMENT: Page 96, Line 24: only gave two exposure concentrations for mice even though three forms were used

***RESPONSE**: This comment is in response to the following sentence: "Microscopic examinations of the hearts of Fischer-344 rats and B6C3F1 mice exposed to nickel sulfate, nickel subsulfide, or nickel oxide for 12 6-hour exposures over 16 days did not reveal any changes at concentrations as high as 23.6, 12.2, or 7.33 mg Ni/m3, respectively, in rats and 1.4, or 23.6 mg Ni/m3, respectively, in mice (NTP 1996a, 1996b, 1996c)." These sentences were revised as only two of the NTP studies should be referenced here. The text now read as follows:*

"Microscopic examinations of the hearts of Fischer-344 rats exposed to nickel oxide, nickel subsulfide, or nickel sulfate for 12 6-hour exposures over 16 days did not reveal any changes at concentrations as high as 23.6, 7.33, or 12.2 mg Ni/m^3, respectively (NTP 1996a, 1996b, 1996c). Similarly, no changes were observed in B6C3F1 mice exposed to nickel oxide or nickel sulfate at concentrations as high as 23.6 or 1.4 mg Ni/m^3, respectively (NTP 1996a, 1996c)."

COMMENT: Page 97, Line 2: Which study?

RESPONSE*: This comment is in response to the following sentence: "Cardiovascular effects of exposure to any form of nickel for any duration did not show an effect in rats and mice of different strains except ApoE-/- mice." The references for this sentence were added and the sentence now states the following:*

> *"Overall, cardiovascular effects of exposure to any form of nickel for any duration did not show an effect in rats and mice of different strains except ApoE$^{-/-}$ mice (Ying et al. 2013; Xu et al. 2012)."*

COMMENT: Page 113, Line 18: describe changes (e.g., changes mentioned Line 32 without description)

RESPONSE*: The description of the effects was added to the beginning of the description of this study and now states the following:*

> *"Discolored gastrointestinal contents, ulcerative gastritis, and enteritis were observed in rats that died following treatment by gavage with 25 mg Ni/kg/day as nickel chloride hexahydrate for up to 91 days (American Biogenics Corporation 1988)."*

COMMENT: Page 114, Line 15: Also lack of a clear dose-response

RESPONSE*: This comment is in response to the following sentence: "The biological significance of a decrease in hematocrit level in the absence of hemoglobin or erythrocyte alterations is not known." The detail recommended by the reviewer was added and the text now states the following:*

> *"The biological significance of a decrease in hematocrit level in the absence of hemoglobin or erythrocyte alterations is not known and lacks a clear dose-response."*

COMMENT: Page 118, Line 10: indicate if this is absolute or normalized organ weights

RESPONSE*: This comment is in response to the following sentence: "Because histological changes in the liver were not observed in these studies and decreases in body weight gain were often observed at the same dose levels, the significance of the liver weight changes is unclear." The study reported normalized weight (liver-to-body weight ratio changes); this detail was added to the text as follows:*

> *"Because histological changes in the liver were not observed in these studies and decreases in body weight gain were often observed at the same dose levels, the significance of the changes on liver-to-body weight ratio changes are unclear."*

COMMENT: Page 118, Line 19: Same concern about potential oral exposure?

RESPONSE*: This comment is in response to the following sentence: "In this study, there was no indication that the rats were prevented from licking the nickel from the skin; therefore, these effects could have resulted from oral exposure." This concern would apply to both dermal studies referenced in this paragraph. The paragraph was edited to clarify this as follows:*

"Increased Mg2+ ATPase activity was observed in the livers of guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 15 or 30 days (Mathur and Gupta 1994). Acid phosphatase and glucose-6-phosphatase activities were increased only after 30 days of treatment. In both of these studies, there was no indication that the animals were prevented from licking the nickel from the skin; therefore, these effects could have resulted from oral exposure."

COMMENT: Page 118, Line 24: Estimated or measured exposure?

***RESPONSE**: This comment is in response to the concentration in the following sentence: "Marked tubular necrosis was observed in the kidneys of a man who died of adult respiratory distress syndrome 13 days after a 90-minute exposure to a very high concentration (382 mg/m3) of metallic nickel of small particle size (<1.4 µm) (Rendall et al. 1994)." The study authors estimated the exposure using a simulation; this detail was added to the text as follows:*

"Marked tubular necrosis was observed in the kidneys of a man who died of adult respiratory distress syndrome 13 days after a 90-minute exposure to a very high concentration, simulated by study authors to be 382 mg/m^3 of metallic nickel of small particle size (<1.4 µm) (Rendall et al. 1994)."

COMMENT: Page 119, Line 7: Some caution here since excretion may vary despite both sexes having similar exposures

***RESPONSE**: This comment is in response to the following sentence: "Although the average exposure concentration was the same for women and men, women were more highly exposed as indicated by urine concentrations of 10.3 µg Ni/g creatinine in women compared to 5 µg Ni/g creatinine in men." No references could be located to support a statement that nickel urinary excretion would differ by sex. Generally, creatinine excretion is expected to be higher in men than woman, but this statement does not seem relevant here. The text was edited to indicate that women may have been more highly exposed, as follows:*

"Although the average exposure concentration was the same for women and men, women may have been more highly exposed as indicated by urine concentrations of 10.3 µg Ni/g creatinine in women compared to 5 µg Ni/g creatinine in men."

COMMENT: Page 119, Line 13 : Time frame ?

***RESPONSE**: This comment is in response to the "voids" in the following sentence: "Sanford and Nieboer (1992) noted that elevated urinary β2-microglobulin levels were found in spot urine samples of three workers; however, when the levels were averaged over three or more voids, the average levels were within the normal range." Multiple 24-hour urine samples were collected from each participant, but the study does not state exactly how many. The sentence was edited as follows:*

"Sanford and Nieboer (1992) did not find significant alterations in urinary β2-microglobulin levels in nickel refinery workers with urine nickel levels of less than 60 µg/L. Multiple 24-hour urine collections were collected from each participant. Sanford and Nieboer (1992) noted that elevated urinary β2-microglobulin levels were found in spot urine samples of three workers;

however, when the levels were averaged over three or more voids (multiple samples from a participant), the average levels were within the normal range."

COMMENT: Page 119, Line 26: blood urea (BUN)?

***RESPONSE**: This comment is in response to the "voids" in the following sentence "Changes in urea are reported in 21 and 28 day studies in male rats exposed to concentrations of 0.8 and 0.178 mg Ni/m3 as nickel oxide, respectively (Weischer et al. 1980)." Serum urea was measured, this detail was added to the text as follows:*

"Changes in serum urea are reported in 21 and 28 day studies in male rats exposed to concentrations of 0.8 and 0.178 mg Ni/m^3 as nickel oxide, respectively (Weischer et al. 1980)."

COMMENT: Page 120, Line 8: Reviewer suggests the change from "liver" to " kidney

***RESPONSE**: The comment is in response to the following sentence: "Cellular changes were observed in kidney sections of rats exposed to 0.7585 mg Ni/m3 as nickel sulfate for 21 days (Adeyemi et al. 2016)." The reviewer's suggested change was incorporated for accuracy.*

COMMENT: Page 120, Line 14: See previous comment – were the designs similar so oral exposure may have occurred?

***RESPONSE**: The comment is in response to the following sentence: "Increased Mg2+ ATPase activity was observed in the kidneys of guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 30 days (Mathur and Gupta 1994)." The concern on potential oral exposure occurring was added to this paragraph which now states the following:*

"No gross or microscopic lesions were observed in the kidneys of rats treated dermally with ≤100 mg Ni/kg/day as nickel sulfate for 15 or 30 days (Mathur et al. 1977). Increased Mg^{2+} ATPase activity was observed in the kidneys of guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 30 days (Mathur and Gupta 1994). No adverse effect was noted at 15 days, and dermal nickel exposure had no effect on kidney acid phosphatase or glucose-6-phosphatase activities. In these studies, there was no indication that the animals were prevented from licking the nickel from the skin; therefore, the animals could have been orally exposed."

COMMENT: Page 127, Line 17: For nickel? Cobalt?

***RESPONSE**: The comment is in reference to the following sentence: "Exposure levels were not reported." The text describing the study were rephrased as workers were chosen because they had asthma resulting from cobalt exposure. The text now sates the following:*

"Similar but less-pronounced effects were observed in eight workers with hard metal asthma attributed to cobalt exposure and who then underwent a bronchial provocation challenge to nickel sulfate (Shirakawa et al. 1990). A relationship between nickel and cobalt sensitization is

further supported by the finding that nickel-reactive IgE antibodies were observed in all of the workers (Shirakawa et al. 1990)."

COMMENT: Page 131, Line 24: I'd double check this since IHC evidence of recovery is occurring faster than expected for basal cell division and replacement of olfactory neurons that were presumed lost (atrophy was reported)

RESPONSE*: This comment is in reference to the following sentence: "Carnosine, a neurochemical marker, was reduced in the olfactory epithelium following 12 days of exposure but was back to control levels by exposure day 16, suggesting adaptation to nickel exposure." The study by Evans et al. (1995) was reviewed and the text was updated for accuracy. The atrophy that occurred in the septal olfactory epithelium recovered 12 days into the recovery period when exposure ceased. The study authors don't explicitly state that carnosine levels in the epithelium recovered, only those levels in the olfactory bulb did. This recovery was attributed to a potential defense mechanism likely in the nasal mucosa. The text was edited and states the following:*

"Exposure for 6 hours/day for 16 days to cobalt sulfate heptahydrate in male Long-Evans rats at 0.635 mg Ni/m^3 resulted in histological changes including decreased bipolar receptor cells and atrophy in the septal olfactory epithelium (Evans et al. 1995). However, no changes of olfactory function were noted following completion of behavioral studies for olfactory absolute threshold (odor detection) and discrimination. Thinning (atrophy) of the epithelium appeared normal after 12 days of recovery, and carnosine, a neurochemical marker, was reduced in the olfactory epithelium only at 12 days of exposure. Carnosine levels in the olfactory bulb were reduced up to the 12^{th} day of exposure and returned to control levels by the 16^{th} exposure day. Study authors attributed the recovery of carnosine levels during the exposure period to a defensive response against continued exposure (Evans et al. 1995)."

COMMENT: Page 134, Line 24: Possibly Cite: Rehm S, White TE, Zahalka EA, Stanislaus DJ, Boyce RW, Wier PJ. Effects of food restriction on testis and accessory sex glands in maturing rats. Toxicol Pathol. 2008 Jul;36(5):687-94?

RESPONSE: *This comment is in reference to the following sentence: "Although it was not discussed in the report, the final body weights of males exposed for 28 days appear to be lower than control body weights; this may have contributed to the histological findings." This reference was added as it is relevant to the point suggested here that reduction in food intake may affect reproductive development. The causes of body weight reduction are not discussed in Kakela et al. (1999). The text now states:*

"Although it was not discussed in the report, the final body weights of males exposed for 28 days appear to be lower than control body weights; this may contribute to the histological findings in the maturing rats (Rehm et al. 2008)."

COMMENT: *Reviewer suggests the insertion of the phrase* "in rats exposed to 0, 1.3 , 6.8, or 31.6 mg Ni/kg/day, respectively," into the following sentence: "In the first litter, the percentages of dead pups per litter at postnatal day 1 were 1.7, 3.1, 0, and 13.2% in rats exposed to 0, 1.3 , 6.8, or 31.6 mg Ni/kg/day,

respectively, (statistically significant at the high dose only); no significant alterations were observed in the number of dead pups at postnatal day 21."

***RESPONSE**: The reviewer's edit was incorporated into the text.*

COMMENT: *Reviewer suggests the insertion of the phrase*: "in rats exposed to 0, 1.3 , 6.8, or 31.6 mg Ni/kg/day respectively" into the following sentence: "In the second litter, the number of litters with dead pups at birth (2, 7, 6, and 10%; statistically significant at high dose only), the percentages of dead pups per litter at postnatal day 1 (1.0, 4.3, 4.6, and 8.8%; statistically significant at all three dose levels), and the percentage of dead pups at postnatal day 21 (12.5, 13.4, 19.4, and 29.2%; significant at high dose only) were increased in rats exposed to 0, 1.3 , 6.8, or 31.6 mg Ni/kg/day respectively.

***RESPONSE**: The reviewer's edit was incorporated into the text.*

COMMENT: Page 140, Line 6: presence or absence of developmental effects may not include number of pups – if there was no effect on litter size then I would explicitly state that

***RESPONSE**: There was no significant dose related effect on embryo and fetal toxicity, added additional language to clarify as follows:*

> *"No adverse developmental effects, including no effect on litter size, were observed in the cesarean delivered F2b rats, suggesting that the nickel-induced decrease in live litter size occurred postnatally."*

COMMENT: Page 141, Line 32 : Reactive ?

***RESPONSE**: The comment is in reference to the following sentence : "No effects on figure eight maze reactive locomotor activity levels were observed in the offspring of mice treated by gavage at 45.3 mg Ni/kg/day as nickel chloride on gestation days 8–12 (Gray et al. 1986)." The figure 8 test was used to test locomotor activity. The text was edited for clarity as follows:*

> *"No effect on locomotor activity was observed following a figure 8 maze test in the offspring of mice treated by gavage at 45.3 mg Ni/kg/day as nickel chloride on gestation days 8–12 (Gray et al. 1986)."*

COMMENT: Page 141, Line 19: not clear just state 23.6 hr as was used earlier in the document

***RESPONSE**: The comment is in reference to the following sentence: "Wistar rats continuously exposed to 0.385 mg Ni/m^3 as nickel oxide for 28 days (23.6/24 hours) (Weischer et al. 1980)." The text was changed to state "(23.6 hours/day)"*

COMMENT: Page 142, Line 2 : indicate if both sexes were used in these studies since you mention a possible sex difference following nickel inhalation

RESPONSE*: The sex evaluated in each study was added and the paragraph now states the following:*

> *"Two studies reported significant alterations in serum glucose levels in rats exposed to nickel chloride. A significant decrease in blood glucose levels was observed in female rats administered 8.6 mg Ni/kg/day via gavage for 91 days (American Biogenics Corporation 1988). In contrast, Weischer et al. (1980) reported a significant increase in blood glucose levels in male rats administered 0.23 mg Ni/kg/day via drinking water for 28 days. In both studies, significant decreases in body weight gain (20% and higher) were also observed at the same dose effect levels. Thus, it is difficult to asse ss whether this is a direct effect of nickel or secondary to the effect on body weight."*

COMMENT: Page 142, Line 7: The other Mathur study had concerns about possible oral exposure – could that also have happened in this study?

RESPONSE*: The comment is in reference to the citation in the following sentence: "Blood glucose levels were significantly increased in guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 15 or 30 days (Mathur and Gupta 1994)." The limitation of potential oral exposure was added to this paragraph which now states the following:*

> *"Blood glucose levels were significantly increased in guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 15 or 30 days (Mathur and Gupta 1994). There was no indication that the animals were prevented from licking the nickel from the skin; therefore, these effects could have resulted from oral exposure."*

COMMENT: Page 143, Line 2-4: Not needed

RESPONSE*: Reviewer suggests deletion of the following sentence: "While these studies are only suggestive of an association several occupational studies indicate that respiratory cancer risk is increased by nickel exposure." The sentence was deleted in agreement with the reviewer's suggestion.*

COMMENT: Page 144, Line 9: high? highly exposed?

RESPONSE*: The comment is in reference to the citation in the following sentence: "Redmond (1984) and Arena et al. (1998) reported significant increases in lung cancer risks among high nickel alloy production workers as compared to the U.S. population." This wording was an error and was changed to state exposed workers as follows:*

> *"Redmond (1984) and Arena et al. (1998) reported significant increases in lung cancer risks among exposed nickel alloy production workers as compared to the general U.S. population."*

COMMENT: Page 144, Line 25: Is this needed? Who performed the revised meta-analysis (no citation provided)

RESPONSE*: The phrase was deleted, and the previous sentences provide citations for the studies discussed. The text now reads as follows:*

"However, the Ojajärvi et al. (2000) meta-analysis has been criticized (Seilkop 2001) for excluding a study of nickel mining and smelting workers (Shannon et al. 1991) and a study of nickel alloy production workers (Arena et al. 1998). The addition of these studies lowered the meta-analysis ratio from 1.9 (95% confidence interval 1.2–3.2) to 1.3 (95% confidence interval 0.9–1.9)."

COMMENT: Page 145, Line 12: *Reviewer suggests insertion of "increased tumor incidence" into the following sentence:* **"**However, this same study found that the incidence of benign and malignant adrenal gland pheochromocytoma in male rats and cortical adenoma/carcinomas in females were concentration-dependent to nickel exposure and increased tumor incidence were significant at 0.4 mg Ni/m^3 for both sexes (Oller et al. 2008)."

RESPONSE*: The reviewer's edit was incorporated into the text.*

COMMENT: Page 147, Line 13: clarify if the workers = controls or the Ni-exposed workers

RESPONSE*: The comment is in reference to the following sentence: "Workers in a welding factory exposed to high concentrations of nickel (0.340-10.129 mg/m3) showed significant increases in chromosomal aberrations relative to unexposed controls, though the workers were co-exposed to chromium and PAHs (Borsk? et al. 2003)." In the study, controls were co-exposed to chromium and PAHs, and the text was edited as follows:*

"Workers in a welding factory exposed to high concentrations of nickel (0.340-10.129 mg/m^3) showed significant increases in chromosomal aberrations relative to unexposed controls, though the controls were co-exposed to chromium and PAHs (Borsk? et al. 2003)."

COMMENT: Page 147, Line 20: Collected from who? Workers?

RESPONSE*: The comment is in response to the following sentence: "In a metaphase analysis of human lymphocytes, positive evidence of genotoxicity was observed (Arrouijal et al. 1992)." Samples were collected from all study participants and the text was edited as follows:*

"In a metaphase analysis of human lymphocytes from nickel-hypersensitized and nickel-unsensitized subjects, positive evidence of genotoxicity was observed (Arrouijal et al. 1992)."

COMMENT: Page 148, Line 11: again ID source; controls?

RESPONSE*: The comment is in response to the "lymphocytes" in following sentence: "Micronuclei formation was observed in one in vitro study of human lymphocytes (Arrouijal et al. 1992). More detail on the study was added and now states the following:*

"Increased micronuclei formation was observed in one in vitro study of human lymphocytes from nickel-unsensitized subjects, and the effect was dose-dependent and 50% greater than in nickel-sensitized subjects (Arrouijal et al. 1992)."

COMMENT: Page 148, Line 13: cell line? primary? if primary collected from who?

RESPONSE*: The comment is in response to the "cells" in the following sentence: "No evidence of increased micronuclei formation was found in studies of human bronchial epithelial cells (Gliga et al. 2020), human colon cancer cells (Kim and Seo 2011) and Chinese hamster V79 cells (Buxton et al. 2020; Nordin et al. 2018." Detail on the cell line was added and the text now states the following:*

> *"No evidence of increased micronuclei formation was found in several studies including an immortalized human bronchial epithelial cell line (BEAS-2B) (Gliga et al. 2020), human colon cancer cells (Kim and Seo 2011), and Chinese hamster V79 cells (Buxton et al. 2020; Nordin et al. 2018)."*

COMMENT: Page 148, Line 15: Provide Ni exposure

RESPONSE*: The comment is in response to the following sentence: "In mouse lung and nasal mucosal cells, DNA damage consisted of fragmentation (Mayer et al. 1998)." Detail on the nickel exposure was added and the text now states the following:*

> *"In mice exposed to single nose-only inhalation doses of nickel subsulfide, DNA damage in lung and nasal mucosal cells consisted of fragmentation (Mayer et al. 1998)."*

COMMENT: Page 148 , Line 22: Ni exposure ?

RESPONSE*: The comment is in response to the following sentence: "Two studies observed significant increases in DNA double-strand breaks in mouse sperm cells (Domshlak et al. 2005; Doreswamy et al. 2004)." Detail on the nickel exposure was added and the text now states the following:*

> *"Two studies observed significant increases in DNA double-strand breaks in mouse sperm cells following intraperitoneal administration to either nickel sulfate or nickel chloride (Domshlak et al. 2005; Doreswamy et al. 2004)."*

COMMENT: Page 148, Line 23: For consistency, this should go first as a separate paragraph

RESPONSE*: Per the reviewer's suggestion, a separate paragraph was created beginning with the following sentence:*

> *"Evidence from in vivo studies in humans has been mixed. DNA oxidative damage was observed in nickel smelting workers and correlated with length of employment (Cheng et al. 2019)."*

COMMENT: Page 158, Line 8: These parameters are not clear – indicate inflammation occurred in lungs

RESPONSE*: This comment refers to the following sentence: "Male F344 rats received 4 doses of 2 mg/kg/bw as intratracheal instillations which caused pulmonary injury, inflammation and increased the parameters indicating the processing the foreign material (Senoh et al. 2017)." The text was rephrased, and detail was added that particles were found in the lungs; the text now states the following:*

"Male Fischer-344 rats received NiO NPs as 4 doses of 2 mg/kg/bw as intratracheal instillations which caused pulmonary injury and inflammation, and NiO particles were detected in the lung and lung associated lymph nodes (Senoh et al. 2017)."

COMMENT: Page 158, Line 23: Inhalation?

RESPONSE*: This comment refers to "instillation" the following sentence: "A 4-week intratracheal instillation of 0.1-3 mg NiNPs in male Wistar rats caused pulmonary inflammation (Mizuguchi et al. 2013; Ogami et al. 2009)." The exposure was not inhalation but rather intratracheal instillation for both studies; the text was edited to state the following:*

"A 4-week intratracheal instillation of 0.1-3 mg NiO NPs in male Wistar rats caused pulmonary inflammation (Mizuguchi et al. 2013; Ogami et al. 2009)."

COMMENT: Page 158, Line 24-25: *Reviewer suggests deleting* "Similar pulmonary effects were observed in C57BL/6 mice when exposed to 50 µg NiNPs, and" *of the following sentence* "Similar pulmonary effects were observed in C57BL/6 mice when exposed to 50 µg NiNPs, and a dose-dependent increase in acute lung inflammation and injury was seen in mice after exposure to 50 µg NiNPs via intratracheal instillation (Mo et al. 2019).

RESPONSE*: The reviewer's edits were incorporated, and the text now states the following:*

"A dose-dependent increase in acute lung inflammation and injury was seen in C57BL/6 mice after exposure to 50 µg NiNPs via intratracheal instillation (Mo et al. 2019). "

COMMENT: Page 159, Line 6: feeding survival rate?

RESPONSE*: The comment refers to the following sentence: "Developmental toxicity was observed in the pups with a significant decrease in birth survival rate and feeding survival rate (Kong et al. 2014)". The authors measured survival rates at birth and during the feeding period; the text was edited as follows:*

"Developmental toxicity was observed in the pups with a significant decrease in survival rates at birth and during feeding (Kong et al. 2014)."

COMMENT: Page 160, Line 14 : System ?

RESPONSE*: The comment is in response to the following sentence: "A comet assay revealed that 62, 125, 250 and 500 µg/mL NiO NPs induced a significant increase in DNA damage (De Carli et al. 2018)". Detail was added on the cell line used and the text now states the following:*

"A comet assay of V79 cells revealed that 62, 125, 250 and 500 µg/mL NiO NPs induced a significant increase in DNA damage (De Carli et al. 2018)."

COMMENT: Page 160, Line 15-16: *Reviewer suggests the deletion of* " Immortalized human bronchial epithelial cells (BEAS 2B) cells" *from the following sentence* "Immortalized human bronchial epithelial cells (BEAS 2B) cells exposed to NiNPs induced genotoxic effects and increased oxidized stress at doses as low as 1 µg/ml after 48 hours (Di Bucchianico et al. 2018)."

RESPONSE*: The reviewer's edits were incorporated, and the text now states the following:*

> *"Exposure to NiNPs induced genotoxic effects and increased oxidized stress in immortalized human bronchial epithelial (BEAS-2B) cells at doses as low as 1 µg/ml after 48 hours (Di Bucchianico et al. 2018)."*

COMMENT: Page 160, Line 27: Which NiNP?

RESPONSE*: The comment is in response to the following sentence: "Dose-dependent genotoxicity was observed in D. melanogaster after 24 hours (Alaraby et al. 2018)." The study is discussing nickel nano materials, this detail was added as follows:*

> *"Dose-dependent genotoxicity to nickel nanomaterials was observed in D. melanogaster after 24 hours (Alaraby et al. 2018)."*

COMMENT: Page 161, Line 29: in what media – urine? feces?

RESPONSE*: The comment is in response to "excreted" in the following sentence: "In a study of differently shaped NiNPs administered intratracheally to rats, wire-shaped NiO particles were excreted much more quickly than spherical and irregular particles (35% after 24 hours vs 0.33-3.6%) (Shinohara et al. 2017)." The media was urine, and the detail was added as follows:*

> *"In a study of differently shaped NiNPs administered intratracheally to rats, wire-shaped NiO NP were excreted in urine much more quickly (35% 24-hours after administration) than spherical and irregular particles (0.33-3.6% 24-hours after administration) (Shinohara et al. 2017)."*

COMMENT: Page 162, Line 24: Not absolute – and changes in UF range – e.g., could say predominantly deposit…

RESPONSE*: The comment is in response to the following sentence: "Deposition of particulates greater than 2.5 µm occurs in the nasopharyngeal area, whereas particulates less than 2.5 µm are deposited in the bronchioalveolar region of the lungs." The reviewer's edit was incorporated, and the text now states the following:*

> *"Deposition of particulates greater than 2.5 µm predominantly occurs in the nasopharyngeal area, whereas particulates less than 2.5 µm are predominantly deposited in the bronchioalveolar region of the lungs."*

COMMENT: Page 163, Line 2-3: *Reviewer suggests deletion of the following sentence:* **"**There appears to be good correlation between air exposure to soluble nickel and urinary nickel levels in workers."

RESPONSE*: The sentence was deleted in agreement with the reviewer's suggestion.*

COMMENT: Page 163, Line 18: General comment – the document is inconsistent in using chemical names versus chemical formula (even in the same paragraph)

RESPONSE*: Various sections in the profile especially in Chapter 3 were edited for consistency on the use of chemical name or chemical formula.. Generally, abbreviations for the nickel form are only used when stating the concentration or dose except in the nickel nanoparticle section where abbreviations are used throughout. Other sections may use the formula as appropriate, otherwise the chemical name is stated.*

COMMENT: Page 163, Line 19: use same units

RESPONSE*: The comment is in reference to the following sentence: ""Fractional deposition of nickel chloride was reported to be 0.107 for an acute exposure and 6.9% for repeated exposures in male Sprague-Dawley rats (Menzel et al. 1987). The units were standardized from percentage to fraction, and the text now states the following:*

> *"Fractional deposition of nickel chloride was reported to be 0.107 for acute single exposures and 0.069 for repeated exposures in male Sprague-Dawley rats (Menzel et al. 1987)."*

COMMENT: Page 163, Line 24: provide species

RESPONSE*: The comment is in reference to the "nickel" in the following sentence: "Hirano et al. (1994) reported almost complete absorption into the lung tissue following deposition into the lungs of nickel sulfate 12 hours post inhalation." The species was added, and the text now states the following:*

> *"Hirano et al. (1994) reported almost complete absorption into the lung tissue of Wistar rats following nickel sulfate deposition into the lungs 12 hours post inhalation."*

COMMENT: Page 164, Line 5-6: *Reviewer suggested the insertion of "inconsistently" into the following sentence:* **"**Diamond et al. (1998) calculated oral nickel absorption in humans using data from several studies and found that absorption was inconsistently affected by fasting."

RESPONSE*: The reviewer's suggested edit was incorporated.*

COMMENT: Page 165, Line 4: As originally written it implies diffusion only

RESPONSE*: The comment is in reference to the following sentence edited by the reviewer: "…however, the carriers become saturated at concentrations greater than 10 mg Ni/L and nickel absorption also occurs via passive diffusion" Reviewer suggests the insertion of "also occurs via passive diffusion". Suggestion has been incorporated.*

COMMENT: Page 165, Line 11-12: This sentence is unclear – both ends are 5 min and 96 hr – but also increased with longer duration?

RESPONSE*: This comment is in response to the following sentence: "The reported dermal absorption rates were low at both ends of the exposure duration and higher as the exposure duration increased." The Hostynek et al. (2001b) study was reviewed, and the text was updated for clarity as follows:*

> *"In another study using sequential tape stripping on the skin of human volunteers, Host?nek et al. (2001b) measured dermal absorption of nickel ions after exposing the skin to nickel metal powder at exposure durations of 5 minutes, 30 minutes, 3 hours, 24 hours, and 96 hours. Dermal absorption rates increased with exposure duration, but the amount of nickel removed after 10-20 strips was similar across durations."*

COMMENT: Page 165, Line 23: This result warrants additional discussion – why so high? form of Ni?

RESPONSE*: This comment is in reference to the following sentence: "Norgaard (1955) conducted an experiment using radiolabeled nickel sulfate which showed that a nickel absorption rate of 55-77% through the human skin in vivo under occlusion within 24 hours" The study looks at nickel resorption on skin following exposure for 24 hours while wrapped in foil. The 55-77% rate was incorrectly attributed to absorption. Additionally, the study authors noted differences in resorption when a different material was used to cover the exposed skin area. The main takeaway of this study was that resorption appeared similar between the nickel-hypersensitized individuals and normal individuals; therefore, the text was edited as follows:*

> *"Norgaard (1955) conducted an experiment using radiolabeled nickel sulfate which showed that nickel resorption is similar between individuals with and without nickel-hypersensitivity"*

COMMENT: Page 170, Line 11: Worth mentioning negligible biliary excretion e,g., Marzouk A, Sunderman FW Jr. Biliary excretion of nickel in rats. Toxicol Lett. 1985 Sep;27(1-3):65-71.

RESPONSE*: The recommended study was incorporated into the text as follows:*

> *"In rats administered doses of nickel chloride, biliary excretion was negligible (<0.5%) 24-hours after injection (Marzouk and Sunderman Jr. 1985)."*

COMMENT: Page 171, Line 12: this model is not a traditional PBPK model as physiologic parameters are not incorporated.

RESPONSE*: The reviewer is referring the following header in Section 3.1.5: "Oral PBPK Models (Sunderman et al. 1989b and Dede et al. 2018)"*

The header was changed to state the following:

> *"Oral Toxicokinetic Model (Sunderman et al. 1989b and Dede et al. 2018)"*

COMMENT: Page 193, Line 16-17: *Reviewer suggests the deletion* of "the majority of nickel in the United States is used in stainless and alloy steel and nickel-containing alloys (USGS 2021)" *from the following sentence* "Nickel is primarily used for stainless and alloy steels, nonferrous alloys and superalloys, and electroplating; the majority of nickel in the United States is used in stainless and alloy steel and nickel-containing alloys (USGS 2021)

RESPONSE*: The sentence was edited in agreement with the reviewer's suggestion and the text now states the following:*

> *"Nickel is primarily used for stainless and alloy steels, nonferrous alloys and superalloys, and electroplating (USGS 2021)."*

COMMENT: Page 194, Line 8: why report a negative value? the LOD is likely more informative here

RESPONSE*: The comment is in reference to the "-0.0004" value in the following sentence. "Nickel is naturally present in soil and sediment, and in food. According to the FDA's Total Diet Study, the concentration of nickel in U.S. foods range from -0.0004 to 3.2 mg/kg (FDA 2017b)." The negative sign was a typo, and this text has been corrected as follows:*

> *"According to the FDA's Total Diet Study, the average concentration of nickel in various U.S. foods ranges from 0.0004 to 3.2 mg/kg (FDA 2017a)."*

COMMENT: Page 217, Line 23: Put into context e.g., United States: a substance is considered to be not bioaccumulative if it has a BCF less than 1000

RESPONSE*: The comment is in reference to the following sentence in Other Media Section 5.4.1: "When the authors also included data for exposure concentrations outside the range of 5–50 µg/L, a BCF value of 157±135 was obtained." A reference was located where the EPA generally considers BCF >1000 in fish to be of concern for accumulation. Nickel does not bioaccumulate in aquatic organisms and this is stated at the beginning of this section. The EPA reference was added to the beginning of this section to provide context on the BCF values, as follows:*

> *"It has been reported that nickel is not accumulated in significant amounts by aquatic organisms (Birge and Black 1980; Zaroogian and Johnson 1984). The EPA considers bioconcentration factors (BCF) greater than 1,000 to be of concern for bioaccumulation in fish (EPA 2020b). BCF values for nickel calculated in fish and other aquatic organisms are reported to be well below 1,000. The mean bioconcentration factor (BCF) for three carnivorous fish was 36. The concentration of nickel in mussels and oysters treated with 5 µg nickel/kg of seawater for 12 weeks averaged 9.62 and 12.96 µg nickel/g, respectively, on a dry weight basis (Zaroogian and Johnson 1984). When these data are adjusted for controls and the nickel concentration in tissue is expressed on a wet weight basis, the BCF for the mussels and oysters is ≈100. After 2 weeks in flowing seawater, 58 and 38% of the tissue nickel was lost from the mussel and oyster, respectively. No significant loss of nickel occurred during the remainder of the 28-week depuration period. In the work of McGeer et al. (2003), BCFs for nickel in various aquatic organisms (e.g., algae, arthropods, mollusks, and fish) was assessed based on whole-body metal concentrations and exposure concentrations that were obtained from the literature. For exposure concentrations within the range of 5– 50 µg/L nickel in water, mean BCF values of 106±53 (1*

standard deviation) was obtained for all organisms. When the authors also included data for exposure concentrations outside the range of 5–50 µg/L, a BCF value of 157±135 was obtained. The authors noted that the BCF values were inversely correlated with the exposure concentrations, where the highest BCF values were obtained at the lowest exposure concentrations."