DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR COPPER

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 Copper 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

Peer Reviewer #1 did not provide responses to the charge questions. However, general comments on the profile were provided which have been pasted below the charge questions. Annotated comments on the profile have been incorporated.

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: No response was provided by the reviewer

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: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

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 was provided by the reviewer

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 was provided by the reviewer

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 response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

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: No response was provided by the reviewer

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 was provided by the reviewer

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: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

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: No response was provided by the reviewer

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 response was provided by the reviewer

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 response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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: No response was provided by the reviewer

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 was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

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: No response was provided by the reviewer

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 was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

Additional References from Reviewer*

***These are references cited within the reviewer's individual comments. Responses to the reviewer's comments specify the disposition of these references within the toxicological profile.*

Abbott SM, Malkani RG, Zee PC (2020). Circadian disruption and human health: A bidirectional relationship. *Eur J Neurosci.* 51: 567–583.

Cheng TF, Choudhuri S, Muldoon-Jacobs K (2012). Epigenetic targets of some toxicologically relevant metals: a review of the literature. *J. Appl. Toxicol*. 32: 643–653

Luojus MK, Lehto SM, Tolmunen T *et al.* (2015) Serum copper, zinc and high sensitivity C-reactive protein in short and long sleep duration in ageing men. *J Trace Elem Med Biol* 32, 177-182.

Naveed M, Nicolaisen M, Kawamoto K, Moldrup P et al (2014). Simultaneous loss of soil biodiversity and functions along a copper contamination gradient: When soil goes to sleep. *Soil Sci. Soc. Am. J.* 78: 1239–1250

Ognik K, Cholewińska E, Juśkiewicz J, Zduńczyk Z et al (2019). The effect of copper nanoparticles and copper (II) salt on redox reactions and epigenetic changes in a rat model. *J Anim Physiol Anim Nutr.* 103: 675–686

Rossner P, Jr, Vrbova K, Rossnerova A, Zavodna T, Milcova A et al (2020). Gene Expression and Epigenetic Changes in Mice Following Inhalation of Copper(II) Oxide Nanoparticles. *Nanomaterials* 10, 550; doi:10.3390/nano10

Song CH, Kim YH, Jung KI (2012) Associations of zinc and copper levels in serum and hair with sleep duration in adult women. *Biol Trace Elem Res* **149**, 16- 21.

Yoshioka H, Nonogaki T, Shinohara Y, Suzui M (2018). Lethal chronotoxicity induced by seven metal compounds in mice. *Journal of toxicological sciences* 43 (2): 129 - 134

Yukihiro Y, Prosser RA (2020). Copper in the suprachiasmatic circadian clock: A possible link between multiple circadian oscillators. *Eur J Neurosci.* 51: 47–70.

Appendices

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

COMMENT: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

Annotated Comments on the Profile

COMMENT: The draft report may be difficult to read - contains too many complicated sentences. There are many instances where a sentence contains a string of 4-8 nouns and it is not easy to know the one to which the verb belongs. It is recommended that some effort should be made to edit the text to simpler sentences.

RESPONSE*: The reviewer provided grammatical and editorial edits throughout the profile that were incorporated as appropriate. Additionally, a grammatical edit was made to simplify sentences throughout the profile.*

COMMENT: The V-shaped form in the transition from copper essentiality to copper toxicity needs to be kept in mind. Some of the reported doses for systemic toxicity are higher than the copper levels required for normal body metabolism. This raises the question: At what level of exposure does copper change from being essential to being toxic? This conundrum should be mentioned or briefly discussed in the report.

RESPONSE*: Additional text was added to Section 1.1 to address the issue of essential vs. toxic levels of copper. The following text was added:*

> *"However, excess intake of copper can result in toxicity and may adversely interact with certain heavy metals. While copper is essential there is uncertainty as to what levels copper becomes toxic. Excess copper exposure can result from external environmental sources such as copper contamination in drinking water, and endogenously from disorders that disturb copper regulation in the body."*

Additionally, in the MRL worksheets, it is noted that the MRLs represent copper intake above what is expected to be necessary for essential functions.

COMMENT: The term, "copper ions" is used liberally throughout the report. In fact, free copper ion is very toxic and induces not only oxidative stress but also DNA damage. A delicate homeostatic balance must therefore be maintained between the uptake and efflux of copper to control the amount of free copper ion concentration within the cell. In many instances, it would appear that the studies being described involved "dissolved copper" or "copper in solution" rather than free copper ions.

RESPONSE*: The reviewer provided multiple edits that address specifying the form of copper including "free copper ions" when discussing studies in the profile, which we have incorporated into the profile. Additionally, we did an additional check on all instances where copper ions are discussed to check that the statements are accurate and consistent.*

COMMENT: It looks like the material in many sections (Section 5.4 in particular) came from an earlier version of the Tox Profile for Copper. The references in such sections are outdated, the science is no longer current, and there is a need to update the information to include any newer insights from the last two to three decades or so.

RESPONSE*: An edit was made regarding the older data in Chapter 5. The reviewer's recommended line edits were incorporated and an additional review of the entire toxicological profile was performed to update data and delete outdated section content.*

COMMENT: Loss of Biodiversity. Because of the antibacterial and antiviral properties of copper, loss of biodiversity should be expected in environments contaminated with copper including the superfund sites. This issue has not been well documented but has been reported. A study by Naveed et al (2014) found a 10% loss in soil biodiversity along a copper contamination gradient in the range of 110 to 800 µg/g. Loss of biodiversity has been linked to human health and well-being and is of concern in the EcoHealth paradigm.

RESPONSE*: Information on the loss of biodiversity associated with copper contamination from Naveed et al. 2014 has been added to the Other Media Subsection of section 5.4.2 (Transformation and Degradation) where copper as an essential nutrient is discussed. The following sentence has been added to the first paragraph of this subsection after the first two sentences: "However, loss of biodiversity has been reported in environments contaminated with copper. Naveed et al. (2014) found that increasing copper pollution resulting from a former wood preservation plant had a negative impact on plant growth and species. Earthworms, bacteria, nematodes, and fungi showed a similar response to increasing copper*

concentrations. Results of this study showed that there was a 10% loss in soil biodiversity within a copper concentration range of 110 to 800 mg/kg (Naveed et al. 2014)."

COMMENT: Circadian rhythm disorders and sleep disturbance. Copper is critically involved in proper function of the suprachiasmatic circadian clock through its ability to modulate cell metabolism, redox state, transcription, and neuronal activity. Circadian rhythm disorders have classically been associated with disorders of abnormal timing of the sleep–wake cycle. Both circadian rhythms and sleep are fundamental biological imperatives, which can be influenced by exposure to copper in the environment (Luojus et al., 2015; Song et al., 2015; Yoshioka et al., 2018; Yukihiro et al., 2020). Dysfunction in the circadian rhythm or wake-sleep cycle can play a role in a wide range of pathologies, including increased risk for cardiometabolic diseases, cognitive and mood disorders. Sleep disorder is a major public health problem of our time (Abbott et al., 2020), and any likely contribution of copper pollution to this disease burden needs to be given some attention.

RESPONSE*: ATSDR agrees with the reviewer comments. The following text was added to Chapter 6, Section 6.2 Identification of data needs:*

> *"Recent evidence strongly indicates that copper is critically involved in optimal functioning of the circadian clock by modulating cell metabolism, redox state, transcription, and neuronal activity (Yamada and Prosser, 2020). Alterations in these circadian rhythms are implicated in increased risk for cardiometabolic diseases, cognitive and mood disorders, and sleep disorders (Luojus et al., 2015; Song et al., 2015; Yoshioka et al., 2018; Yukihiro et al., 2020; Abbott et al., 2020). Studies that investigate the effects of inhalation, oral, and dermal exposure to copper that examine its effects on circadian rhythms need to be designed and conducted in animal and human paradigms. These studies need to examine the alterations in circadian machinery and the potential downstream alterations in physiology in the organisms."*

COMMENT: Epigenetic changes. Copper overload can affect the epigenome. Both in vitro and in vivo research, along with epidemiological studies have linked exposure to copper with specific gene promoter methylation and epigenetic histone protein modification as well as the signaling transduction. An important pathway involves the generation of excess ROS by copper exposure which exerts a genotoxic effect by modifying the level of global DNA methylation. Results of some studies on this topic are summarized by Cheng et al (2012), Ognik et al (2019) and Rossner et al (2020).

RESPONSE*: : ATSDR agrees with the reviewer comments. The following text has been added to Chapter 2, Section 2.20 Genotoxicity of copper:*

"Recent evidence indicates that exposure to copper can influence gene expression by binding to metal response elements and also via epigenetic mechanisms (Cheng et al. 2012). Human and animal studies have been used to demonstrated alterations to the epigenome. Human hepatocyte Hep3B cells treated with Cu2+ at 100–200 mM showed significant decreases in global histone acetylation (Kang et al 2004). Hypoacetylation detected in histones demonstrate that copper is capable of altering the epigenome (Cheng et al. 2012). Study by Melino et al. (2009) suggested that Cu might also modulate histone deacetylase (HDAC) activity, a crucial enzyme in the epigenetic machinery. In an in vivo study, rats were exposed to 6.5 mg/kg copper in feed which increased DNA methylation (Ognik et al. 2019). No significant trends in global DNA methylation related to inhalation copper exposure in ICR mice were observed in

Rossner et al. (2020). Changes in DNA methylation and acetylation caused by exposure to copper can lead to modifications on the epigenome which could potentially have transgenerational effects."

COMMENT: The bulleted items pertain to risks rather than hazards

***RESPONSE**: The comment refers to the following bulleted items in section 1.2 (Summary of Health Effects): "The review resulted in the following hazard identification conclusions:*

- *Gastrointestinal effects are a presumed health effect of copper exposure.*
- *Hepatic effects are a suspected health effect of copper exposure."*

A footnote was added to refer the reader to Appendix C where these conclusions are further defined.

COMMENT: Do you mean intestinal microbiome homeostasis?

***RESPONSE**: The comment refers to the following sentence in section 1.2 (Summary of Health Effects – Gastrointestinal Effects): "Evidence in laboratory animals indicates that oral copper exposure results in histological changes, such as ulcerations throughout the gastrointestinal tract, and changes in microbiota homeostasis at doses ≥2.4 mg Cu/kg/day (Cheng et al. 2020; Kadammattil et al. 2018; Khushboo et al. 2018; NTP 1993; Yamamoto et al. 2004)."*

ATSDR agrees with the reviewer. The wording has been changed in accordance with the reviewer's suggestion. The sentence now reads as follows:

> *"Evidence in laboratory animals indicates that oral copper exposure results in histological changes, such as ulcerations throughout the gastrointestinal tract, and changes in intestinal microbiome homeostasis at doses ≥2.4 mg Cu/kg/day (Cheng et al. 2020; Kadammattil et al. 2018; Khushboo et al. 2018; NTP 1993; Yamamoto et al. 2004)."*

COMMENT: Is this the only source of copper that can worsen the symptoms?

***RESPONSE**: The comment refers to the following sentence in section 1.2 (Summary of Health Effects – Hepatic Effects): "These diseases are not likely to be caused by excess copper intake, however symptoms can be worsened by excess oral copper intake such as from consuming milk boiled or stored in brass vessels."*

The example referred to is not exclusive. Examples are most commonly provided in the context of discussing these diseases based on the characteristics of the population commonly studied with regard to ICC and ICT. The phrase was reworded as follows to infer that other sources of copper may also worsen symptoms:

> *"These diseases can be exacerbated by excess oral copper intake (e.g., consuming milk boiled or stored in brass vessels) relative to the ability of the liver to safely store copper."*

COMMENT: "health effect **may** start to appear."

***RESPONSE**: The comment refers to the following sentence in section 2.1(Introduction): "The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear."*

The text the reviewer is referring to is included in all ATSDR Toxicological profiles. It is not specific for copper. Additional guidance on categorizing the adversity to health effects is contained in ATSDR's Guidance for the Preparation of Toxicological Profiles. ATSDR will consider revising the format and related text during its periodic review of the profile format and update of ATSDR's Guidance for the Preparation of Toxicological Profiles.

COMMENT: This study is problematic and can be deleted. The only substance that contains 99.75% copper is purified metallic copper. It sounds somewhat unrealistic that the female rats were fed 4 grams of unadulterated metallic copper per kg/body wt. In what form was the copper administered?

***RESPONSE**: The comment refers to the following sentence in section 2.2 (Death – Oral): "No death was reported in a toxicity study of 3 female rats fed 4,988 mg Cu/kg-bw, as a substance containing 99.75% of copper by weight (Durando et al. 2005)."*

The Durando (et al. 2005) study was removed from the LSE table due to its poor quality, but was mistakenly kept in the related text. This has been corrected. Durando et al. (2005) has been removed from the text due to lack of certainty on the copper concentration in the test substance, and insufficient information on exposure conditions.

COMMENT: somewhat awkward -- too many nouns together; rework the sentence

***RESPONSE**: The comment refers to the following sentence in section 2.2 (Death – Oral): "Intermediate- and chronic-duration oral exposure animal studies examining death reported mixed results on copper lethality." The sentence was rephrased to read more clearly as follows:*

> *"Oral exposure animal studies examining copper lethality reported mixed results following intermediate- or chronic-durations."*

COMMENT: See previous comment on this compound; page 87, lines 17-19

***RESPONSE**: The comment refers to the following sentence in section 2.2 (Death – Dermal): "The substance was an industrial coating with metallic copper as the sole active ingredient at 99.75% by weight." This study by Durando (2005) was removed from the profile due to poor study quality as there was uncertainty in the concentration of copper in the test substance, and the study lacked adequate reporting on the exposure conditions.*

COMMENT: the implication here is that "copper air pollution" is different from "copper in PM2.5", which is not always true

RESPONSE*: The comment refers to the following sentence in section 2.5 (Cardiovascular – Inhalation): "These studies have reported associations between copper air pollution and in PM2.5 and cardiovascular mortality and heart disease. The details from these studies are presented in Section 2.1, Table 2 4." This sentence was edited for clarity to state: "These studies have reported associations between copper air pollution, cardiovascular mortality, and heart disease."*

COMMENT: Vomit of copper sulfate can be bluish but I am not sure what "bluish vomiting" means

RESPONSE*: The comment refers to the following sentence in section 2.5 (Cardiovascular – Oral): "Conversely, two case studies of patients who initially presented with bluish vomiting reported elevated blood pressure following accidental ingestion of copper sulfate, one in a 65-year-old man who accidentally ingested approximately 10 g copper sulfate diluted in water, and one in a 22-year-old man who accidentally ingested 1 cup of copper sulfate powder (Higny et al. 2014; Hassan et al. 2010)." The text was changed to blue-colored vomiting. The studies described the vomit as blue. The sentence now read as follows:*

> *"Conversely, two case studies of patients who initially presented with blue-colored vomitus reported elevated blood pressure following accidental ingestion of copper sulfate, one in a 65-year-old man who accidentally ingested approximately 10 g copper sulfate diluted in water, and one in a 22-year-old man who accidentally ingested 1 cup of copper sulfate powder (Higny et al. 2014; Hassan et al. 2010)."*

COMMENT: These reports would seem inconsistent with the fact that the known lethal dose for copper sulfate is much higher than 15-18 mg.

RESPONSE*: The comment refers to the following sentence in section 2.5 (Cardiovascular – Oral): "Ingestion of copper sulfate crystals resulted in fatal cardiac arrest in two cases, one in a 26-year-old man who intentionally ingested an unknown amount of crystals, and another in a 60-year-old man who accidentally ingested 15-18 mg of crystals (Gupta et al. 2018; Griswold et al. 2017)."*

The patient had ingested this amount of a copper sulfate containing colorant. There isn't any indication from the authors that the amount ingested is underestimated. No revisions were made based on this comment.

COMMENT: This sentence is confusing; the biokinetics of copper in the stomach should be a function of dose

RESPONSE*: The comment refers to the following sentence in section 2.6 (Gastrointestinal – Oral): "Symptoms of gastrointestinal upset following acute exposure to copper are suspected to be a direct contact effects associated with the concentration of copper in the stomach at a specific time rather than the daily dose (Donohue 1997)." Based on the feedback from another reviewer, significant edits were made to this statement to clarify that the Cmax is more likely to cause effects rather than the 24-hour intake. The sentence now reads as follows:*

> *"Symptoms of gastrointestinal upset following acute exposure to copper are suspected to be a direct contact effect, in that the symptoms result from the maximum serum concentration (C_{max}) of*

copper in the gastrointestinal system at a timepoint rather than the 24-hour intake (Donohue 1997)."

COMMENT: On what part of the body was the copper powder spilled?

***RESPONSE**: The comment refers to the following sentence in section 2.7 (Hematological – Dermal): "Hypoxemia and hemolytic anemia were observed in a 2-year-old female child who spilled a copper powder (Donoso et al. 2007)." This sentence was edited to provide further detail on the exposure as follows:*

"Hypoxemia and hemolytic anemia were observed in a 2-year-old female child who spilled a copper powder on her face and inhaled some of the powder (Donoso et al. 2007). "

COMMENT: Unclear

***RESPONSE**: The comment refers to the following sentence in section 2.7 (Hematological – Other Routes): "Hemolytic anemia was observed in a 41-year-old female who intentionally subcutaneous injected 2.5 g copper glycinate in solution (Oon et al. 2006)." Additional detail was added regarding the exposure as follows:*

"Hemolytic anemia was observed in a 41-year-old female who intentionally subcutaneously injected a total of 2.5 g copper glycinate in solution via syringe among 3 different sites on the forearm (Oon et al. 2006)."

COMMENT: How is the low copper level causally related to Wilson's disease?

***RESPONSE**: The comment refers to the following sentence in section 2.9 (Hepatic – Oral – Wilson's Disease): "The manifestations of Wilson's disease are not considered to be related to exposure to high levels of copper." There is no evidence to support that low copper levels are causally related to Wilson's disease but rather the impaired excretion of copper is implicated. The purpose of the noted statement is to inform that exposure to copper itself is not a cause for the development of Wilson's disease. The sentence was edited as follows:*

"The manifestations of Wilson's disease are not considered to be related to exposure to high levels of copper, but rather the individuals impaired excretion of copper."

COMMENT: Sentence should be reworked. Something like "Two out of six pigs fed a diet containing ……"

***RESPONSE**: The comment refers to the following sentence in section 2.9 (Hepatic – Oral – Idiopathic copper toxicosis (ICT)): "Pigs fed a diet containing 16.5 mg Cu/kg/day for 46 days displayed jaundice in 2/6 pigs, while pigs given 18.7 mg Cu/kg/day for 49 days displaced jaundice in 5/6 pigs and AST levels elevated by >100%, compared to controls (Suttle and Mills 1966)." The sentence was reworded as follows:*

"Two out of six pigs fed a diet containing 16.5 mg Cu/kg/day for 46 days displayed jaundice, while five out of six pigs given 18.7 mg Cu/kg/day for 49 days displaced jaundice and AST levels elevated by >100%, compared to controls (Suttle and Mills 1966)."

COMMENT: Elevated ammonium as a sign of adverse effect of exposure to copper powder does not seem to make biologic sense

***RESPONSE**: The comment refers to the following sentence in section 2.10 (Renal – Inhalation): "A two-year-old female child who inhaled an unknown amount of a copper powder developed renal failure accompanied by elevated ammonium and oliguria (Donoso et al. 2007)." The phrase "elevated ammonium" was deleted from the sentence in agreement with the reviewer. The sentence now states:*

"A two-year-old female child who inhaled an unknown amount of a copper powder and spilled some on her facial skin developed renal failure accompanied by oliguria (Donoso et al. 2007)."

COMMENT: Were copper ions actually measured in the studies?

***RESPONSE**: The comment refers to the following sentence in section 2.10 (Renal – Oral): "Increased BUN and serum creatinine correlated positively with free copper ions and hepatic MDA and inversely with GSH and total antioxidant capacity (TAC)." Change was made as requested and the sentence now reads:*

"Increased BUN and serum creatinine correlated positively with free copper levels and hepatic MDA, and inversely with GSH and total antioxidant capacity (TAC)."

COMMENT: This is a lot of copper. How does this compare with the lethal doses mentioned in Section 2.2?

***RESPONSE**: The comment refers to the following sentence in section 2.13 (Endocrine – Oral): "A 53-year-old man developed acute pancreatitis after intentionally ingesting 120g copper sulfate (Lubica et al. 2017)." Additional detail was added that the dose is well above recorded lethal doses in human but that in this case the patient survived due to medical intervention. The text was edited as follows:*

"A 53-year-old man developed acute pancreatitis after intentionally ingesting 120 g copper sulfate well above reported lethal doses, however medical intervention prevented death (Lubica et al. 2017)."

COMMENT: string of six nouns

***RESPONSE**: The comment refers to the following sentence in section 2.14 (Immunological – Oral): "In rats exposed to 199 mg Cu/kg/day for 21 days, serum tumor necrosis factor alpha levels were increased 1.55 times greater than in controls (Seven et al. 2018)." There isn't another way to state this but edits were made to clarify the sentence, as follows:*

"In rats exposed to 199 mg Cu/kg/day for 21 days, serum tumor necrosis factor-alpha (TNF) levels were increased 1.55 times greater than in controls (Seven et al. 2018)."

COMMENT: this long sentence should be simplified

RESPONSE*: The comment refers to the following sentence in section 2.15 (Neurological – Oral): "The lowest dose where neurological effects were seen was 4 mg Cu/kg/day in rats that showed neurobehavioral changes evidenced by decreased passive avoidance response (refraining from an act or response that would produce an aversive stimulus), increased immobility time in a forced swim test, decreased entries in open arm test, and decreased exploration time (Kumar et al. 2019)." This sentence was split into two and edited as follows:*

> *"The lowest LOAEL for neurological effects was 4 mg Cu/kg/day in rats that showed neurobehavioral changes (Kumar et al. 2019). Changes include decreased passive avoidance response (refraining from an act or response that would produce an aversive stimulus), increased immobility time in a forced swim test, decreased entries in open arm test, and decreased exploration time (Kumar et al. 2019)."*

COMMENT: this sentence should be reworked

RESPONSE*: The comment refers to the following sentence in section 2.15 (Neurological – Oral): "Changes in TAC, GSH, and MDA correlated with functional neurological impairment demonstrated by changes in grip strength, plus rotarod, and y-maze tests results in rats exposed to 39.8 mg Cu/kg/day for 30–90 days; neurotoxicity increased with dose (Kumar et al. 2016b)." The sentence was split into two sentences and reworded for clarity as follows:*

> *"In rats exposed to 39.8 mg Cu/kg/day for 30–90 days, changes in TAC, GSH, and MDA correlated with functional neurological impairment (Kumar et al. 2016b). This was based on changes in grip strength, plus rotarod, and y-maze test results in rats exposed to 39.8 mg Cu/kg/day for 30–90 days; neurotoxicity increased with dose (Kumar et al. 2016b)."*

COMMENT: What is the threshold for this?

RESPONSE*: The comment refers to the following sentence in section 2.16 (Reproductive – Oral): "Khushboo et al. (2018) suggests an association between copper overload and decreased synthesis of testosterone." This sentence was rephrased as using the term copper overload is misleading since this experimental rat study doesn't state a threshold where this effect would occur. The sentence was edited as follows:*

> *"Khushboo et al. (2018) suggests an association between excess oral copper exposure and decreased synthesis of testosterone."*

COMMENT: String of 8 nouns; sentence needs to be reworked

RESPONSE*: The comment refers to the following sentence in section 2.19 (Cancer – Inhalation): "In a carcinogenicity study in rats orally exposed to copper gluconate, 62 mg Cu/kg/day, for 6 weeks the number of glutathione S-transferase placental form (GST-P) positive single hepatocytes examined increased significantly compared to controls (Abe et al. 2008)." This sentence was rephrased but there*

was not another way to describe "glutathione S-transferase placental form positive single hepatocytes" and the sentence now reads:

> *"In a carcinogenicity study rats were orally exposed to copper gluconate, 62 mg Cu/kg/day, for 6 weeks, and a significant increase in the number of glutathione S-transferase placental form (GST-P) positive single hepatocytes was seen compared to controls (Abe et al. 2008)."*

COMMENT: convert 5 mM to mg/L for sake of consistency

RESPONSE*: The comment refers to the following sentence in section 2.20 (Genotoxicity): "Caicedo et al. (2008) found that copper at concentrations of 5 mM did not induce DNA damage in human CD4+ T lymphocytes, whereas Husain and Mahmood (2019) found DNA damage to human lymphocytes at copper concentrations of 2.5 mM." The conversion to mg/L was added in parentheses, as follows:*

> *"Caicedo et al. (2008) found that copper at concentrations of 5 mM (318 mg/L) did not induce DNA damage in human CD4+ T lymphocytes, whereas Husain and Mahmood (2019) found DNA damage to human lymphocytes at copper concentrations of 2.5 mM (159 mg/L)."*

COMMENT: change to mg/L

RESPONSE*: The comment refers to the following sentence in section 2.20 (Genotoxicity): "Caicedo et al. (2008) found that copper at concentrations of 5 mM did not induce DNA damage in human CD4+ T lymphocytes, whereas Husain and Mahmood (2019) found DNA damage to human lymphocytes at copper concentrations of 2.5 mM." The conversion to mg/L was added in parentheses, as follows:*

> *"Caicedo et al. (2008) found that copper at concentrations of 5 mM (318 mg/L) did not induce DNA damage in human CD4+ T lymphocytes, whereas Husain and Mahmood (2019) found DNA damage to human lymphocytes at copper concentrations of 2.5 mM (159 mg/L)."*

COMMENT: This sentence belongs in the Reproductive section

RESPONSE*: The comment refers to the following sentence in section 2.20 (Genotoxicity): "In vitro testing of rabbit spermatozoa exposed to copper sulfate found sperm abnormalities including altered anterior part of the sperm head and in the connection segment (Roychoudhury et al. 2010)." This statement was moved to the reproductive section and removed from the genotoxicity section per the reviewer's recommendation.*

COMMENT: Too many nouns together; sentence is unclear and should be reworked

RESPONSE*: The comment refers to the following sentence in section 2.21 (Copper Nanoparticles): "The CuNPs particle surface charge nano dimension aid diffusion through tissues and cells thus assisting with systemic distribution (Chang et al. 2012)." Upon further review, this sentence seems unnecessary and was removed from the profile.*

COMMENT: It would be nice to cite a few examples here

RESPONSE*: The comment refers to the following sentence in section 2.21 (Copper Nanoparticles): "Most of the copper nanoparticle toxicity studies use in vivo and in vitro approaches and most of the toxicity studies thus far focus on aquatic organisms and/or microorganisms." A citation was added to a review paper that highlights studies on these organisms, as follows:*

> *"Most of the copper nanoparticle toxicity studies use in vivo and in vitro approaches and most of the toxicity studies thus far focus on aquatic organisms and/or microorganisms (Chang et al. 2012)."*

COMMENT: This sentence is not clear scientifically. Gives the wrong impression that high dietary copper is okay because of homeostatic control

RESPONSE*: The comment refers to the following sentence in section 3.1 (Toxicokinetics): "High dietary copper results in lower copper absorption as part of copper homeostasis." This sentence was edited for clarity as follows:*

> *"Dietary copper intake and copper absorption are tightly regulated by copper homeostasis maintenance."*

COMMENT: It should be the exact opposite; zinc deficiency is sometimes associated with copper toxicity. Also contradicts the following statement from page 160, line 30: "…….a diet high in zinc can result in copper deficiency (Igic et al. 2002; Myint et al. 2018)"]

RESPONSE*: The comment refers to the following sentence in section 3.1.1 (Absorption): "Turnlund et al. (1988) found that diets low in zinc (below the dietary requirement) decreased copper absorption in humans; 48.1% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 16.5 mg Zn/day (zinc dietary requirement is 15 mg/day), and 37.2–38.5% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 5.5 mg Zn/day." After reviewing the study, this sentence was edited to be more consistent with what is concluded in the study, as follows:*

> *"Turnlund et al. (1988) found that diets with zinc intake slightly above the Recommended Dietary Allowance did not interfere with copper absorption nor increased fecal copper loss. While absorption significantly varied between study groups (48.1% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 16.5 mg Zn/day; 37.2–38.5% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 5.5 mg Zn/day), both groups had positive cooper balance at both levels."*

COMMENT: these results may be an anomaly compared to data from many other studies.

RESPONSE*: The comment refers to the following sentence in section 3.1.1 (Absorption): "Turnlund et al. (1988) found that diets low in zinc (below the dietary requirement) decreased copper absorption in humans; 48.1% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 16.5 mg Zn/day (zinc dietary requirement is 15 mg/day), and 37.2–38.5% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 5.5 mg Zn/day." This sentence was rewritten for clarity as follows:*

> *"While absorption significantly varied between study groups (48.1% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 16.5 mg Zn/day; 37.2–38.5% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 5.5 mg Zn/day), both groups had positive cooper balance at both levels."*

COMMENT: Populations at greater exposure risk to unusually high levels of copper are discussed in…

***RESPONSE**: The comment refers to the following sentence in section 3.2 (Children and Other Populations that are Unusually Susceptible): "Populations at greater exposure risk to unusually high exposure levels to copper are discussed in Section 5.7, Populations with Potentially High Exposures." The text the reviewer is referring to is included in all ATSDR Toxicological profiles. It is not specific for copper. ATSDR will consider the reviewers suggestion during periodic reviews of templated format and text of ATSDR's toxicological profiles in accordance with future updates to ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: this clause is not clear; should be reworked

***RESPONSE**: The comment refers to the following sentence in section 3.2 (Children and Other Populations that are Unusually Susceptible): "A cross-sectional study (see Table 2 7) in mother-infant pairs measured serum copper concentrations in the umbilical cord and did not find any association between copper in cord serum and low infant birth weight (Yang et al. 2020)." Additional detail was added to the sentence for clarity:*

> *"A cross-sectional study (see Table 2-7) in mother-infant pairs measured serum copper concentrations in the umbilical cord blood collected at birth, and no association between copper in cord serum and low infant birth weight was observed (Yang et al. 2020)."*

COMMENT: Stick with one decimal place throughout; it is unlikely that the measurement in this particular study was accurate to the second decimal place

***RESPONSE**: The comment refers to the following sentence in section 3.3.1 (Biomarkers of Exposure): "Fifteen days after admission, the patient's plasma copper level was 33.99 µmol/L (normal range in 6-month to 6-year-old children: 14-30 µg/dL)." This sentence was edited to include ug/dL measurement as follows:*

> *"Fifteen days after admission, the patient's plasma copper level was 216 µg/dL (33.9 µmol/L) (normal range in 6-month to 6-year-old children: 14-30 µg/dL)."*

COMMENT: Concentrations should not be compared in µmol/L and µg/dL in the same sentence.

***RESPONSE**: The comment refers to the following sentence in section 3.3.1 (Biomarkers of Exposure): "Fifteen days after admission, the patient's plasma copper level was 33.99 µmol/L (normal range in 6-month to 6-year-old children: 14-30 µg/dL)." This sentence was edited to include ug/dL measurement as follows:*

"Fifteen days after admission, the patient's plasma copper level was 216 µg/dL (33.9 µmol/L) (normal range in 6-month to 6-year-old children: 14-30 µg/dL)."

COMMENT: What is "plasma serum"?

***RESPONSE**: The comment refers to the following sentence in section 3.3.1 (Biomarkers of Exposure): "Following chronic inhalation exposure to 111-464 mg Cu/m3 copper in dust, plasma serum levels greater than 31.8 µmol/L were observed in 16% of exposed factory workers (Suciu et al. 1981)." A correction was made to state serum copper levels in accordance with the language used in the study, as follows:*

"Following chronic inhalation exposure to 111-464 mg Cu/m^3 copper in dust, serum copper levels greater than 31.8 µmol/L were observed in 16% of exposed factory workers (Suciu et al. 1981)."

COMMENT: How generally true is this statement?

***RESPONSE**: The comment refers to the following sentence in section 3.3.1 (Biomarkers of Exposure): "Based on a hair growth rate of 10 mm per month, the copper levels in the first 2 cm proximal to the scalp would represent copper intake over 2 months (Hopps 1977)." This sentence was rephrased to state this calculation is specific to this study, as follows:*

"A study by Hopps (1977) calculated that for a hair growth rate of 10 mm per month, the copper levels in the first 2 cm proximal to the scalp would represent copper intake over 2 months."

COMMENT: Unusual definition of controls

***RESPONSE**: The comment refers to the following sentence in section 3.3.1 (Biomarkers of Exposure): "In an occupational study of workers exposed to unspecified levels of copper from fossil fuel combustion, oil distribution workers had a mean hair copper level of 69.6 ug/g, which was significantly higher than in controls ("non-exposed individuals living from the exposure") who had a mean hair copper level of 36.8 ug/g (Jaccob 2020)." Further information was added on how the study defined their controls. The revised sentence now reads:*

"In an occupational study of workers exposed to unspecified levels of copper from fossil fuel combustion, oil distribution workers had a mean hair copper level of 69.6 ug/g, which was significantly higher than in controls (defined in the study as non-exposed "healthy individuals living far from hazardous exposure with age and weight matching the test group") who had a mean hair copper level of 36.8 ug/g (Jaccob 2020)."

COMMENT: Also see the comment on page 147

***RESPONSE**: The comment refers to the following sentence in section 3.4 (Interactions with Other Chemicals): "Dietary zinc strongly affects copper absorption, and a diet high in zinc can result in copper*

deficiency (Igic et al. 2002; Myint et al. 2018).” The sentence on page 147 was edited for accuracy and the edit is provided in an earlier related comment.

COMMENT: Add interaction of copper with arsenic because of the common co-occurrence of these two elements in groundwater in many parts of the world

***RESPONSE**: The comment refers to the following sentence in 3.4 (Interactions with Other Chemicals): “Two mechanisms of action of tetrathiomolybdate have been proposed: (1) it reacts with copper-metallothionein to form a soluble complex that is excreted (Ogra et al. 1996), and (2) it can complex with non-ceruloplasmin-bound plasma copper, impeding its cellular absorption (Brewer et al. 2006).” Text was added in Section 3.4. Information from one somewhat recent study in rats was added, as follows:*

> *“A study in rats found that exposure to sodium arsenate resulted in increased copper concentration in the kidney (Cui and Okayasu 2008). Rats were orally exposed to varying doses of sodium arsenate daily for 4- and 16-weeks.”*

COMMENT: this is not a complete sentence

***RESPONSE**: The comment refers to the following sentence in section 3.4 (Interactions with Other Chemicals): “Suggesting a diet high in vitamin C can result in copper deficiency.” This sentence was edited for clarity as follows:*

> *“This suggests that a diet high in vitamin C can result in copper deficiency.”*

COMMENT: Free copper ions are very toxic and must be carefully controlled in biological systems.

***RESPONSE**: The comment refers to the following sentence in section 4.1 (Chemical Identity): “Copper ions are essential to human health and is found in many foods.” The reviewer’s edit was integrated as follows:*

> *“Copper is essential to human health and is found in many foods. Copper sulfate ($CuSO_4$) is an inorganic compound that can occur in nature.”*

COMMENT: Is there a “non-commercial” copper and what is it used for?

***RESPONSE**: The comment refers to the following sentence in section 4.1 (Chemical Identity): “It is the most common commercial copper.” This sentence was reworded to clarify that copper sulfate is the most common compound for commercial uses, as follows:*

> *“It is the most common compound used in commercial applications.”*

COMMENT: What is this?

***RESPONSE**: The comment refers to the following sentence in section 4.2 (Physical and Chemical Properties): “It is soluble in “ammonia of dilute acid” with the presence of an oxidizing agent.” The*

sentence has been rewritten as: "It is soluble in dilute acid and in ammonia with the presence of an oxidizing agent."

COMMENT: Add 'contamination by' here to the boilerplate

***RESPONSE**: The comment refers to the following sentence in section 5.1 (Overview): "However, the number of sites evaluated for copper and copper compounds is not known." The text the reviewer is referring to is included in all ATSDR Toxicological profiles. ATSDR will consider the reviewers suggestion during periodic reviews of templated format and text of ATSDR's toxicological profiles in accordance with future updates to ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: Edit boilerplate title: Number of NPL Sites where Copper and Copper Compounds have been Reported

***RESPONSE**: The comment refers to the title of Figure 5-1 in section 5.1 (Overview): "Number of NPL Sites with Copper and Copper Compound Contamination" The text the reviewer is referring to is included in all ATSDR Toxicological profiles. ATSDR will consider the reviewers suggestion during periodic reviews of templated format and text of ATSDR's toxicological profiles in accordance with future updates to ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT: Do you mean extraterrestrial source? Atmospheric deposition is strictly not a source but a pathway and does not belong with the specific sources mentioned in the sentence

***RESPONSE**: The comment refers to the following bullet in section 5.1 (Overview): "Industrial effluents, mining and production of copper and other metals, municipal solid waste management, and fossil fuel combustion account for a large portion of the total environmental releases of copper and copper compounds. Natural sources of copper releases include windblown dust, volcanoes, decaying vegetation, forest fires, sea spray, and space deposition." Changes were made as requested and space deposition was removed from the sentence:*

> *"Industrial effluents, mining and production of copper and other metals, municipal solid waste management, and fossil fuel combustion account for a large portion of the total environmental releases of copper and copper compounds. Natural sources of copper releases include windblown dust, volcanoes, decaying vegetation, forest fires, and sea spray."*

COMMENT: This sentence is out of place.

***RESPONSE**: The comment refers to the following sentence in section 5.1 (Overview): "Copper concentrations will be higher in soils that are close to sources of copper emissions. In anaerobic sediments, Cu(II) will be reduced to Cu(I), and insoluble cuprous salts will be formed." This sentence has been removed from the Overview but this information is stated in Chapter 5.*

COMMENT: This paragraph is all mixed between the sources of copper in air, water and soils; needs some re-arrangement

RESPONSE*: The comment refers to the first paragraph in section 5.1 (Overview). The paragraph has been rearranged so that it discusses air, then water, and then soil, as follows:*

> *"Copper and its compounds are naturally present in the earth's crust and can be discharged naturally to air and water during weathering. Mean copper concentrations in the atmosphere measured at multiple U.S. locations ranged between 0.013 to 0.0792 µg/m^3 from 2016 to 2019 (EPA 2020a). Airborne copper is associated with particulates that are derived from suspended soils, combustion sources, the manufacture or processing of copper-containing materials, or mine tailings. Copper associated with particulate matter is emitted into the air naturally from windblown dust, volcanoes, and anthropogenic sources, the largest of which are primary copper smelters and ore processing facilities. The major sources of releases to water are mining operations, agriculture, sludge from publicly owned treatment works (POTWs), and municipal and industrial solid waste. Mining and milling contribute the most waste. Copper is released to water as a result of natural weathering of soil and discharges from industries and sewage treatment plants. Copper compounds may also be intentionally applied to water to kill algae. Copper concentrations in groundwater vary widely from 0.2 to 98.4 µg/L (USGS 2020b). Copper is predominantly found in the Cu(II) state. Most of it is complexed or tightly bound to organic matter. Little is present in the free (hydrated) or readily exchangeable form. The combined processes of complexation, adsorption, and precipitation control the level of free Cu(II). The chemical conditions in most natural water is such that, even at relatively high copper concentrations, these processes will reduce the free Cu(II) concentration to extremely low values. The USGS reports the median level of copper in soil and sediment as 30 ppm (USGS 2016). Copper concentrations will be higher in soils that are close to sources of copper emissions."*

COMMENT: This statement is not exactly true; in many instances a worker is exposed to the copper compound that s/he is working with which is usually known or can be surmised].

RESPONSE*: The comment refers to the following sentence in section 5.1 (Overview): "Little information is available concerning the forms of copper to which workers are exposed." This sentence has been deleted from the overview and the profile entirely as there are updated statements throughout that discuss the forms of copper commonly found and used.*

COMMENT: The clause deleted does not belong in this paragraph on copper production].

RESPONSE*: The comment refers to the following sentence in section 5.2.1 (Production): "The copper content of ores ranges from 0.5 to 1 or 2% copper (Schlesinger et al. 2011a), whereas in igneous rock copper content ranges from 0.0005 to 0.011% (Duby 1980; Weant 1985)." Changes were made as requested and the sentence now reads:*

> *"The copper content of ores ranges from 0.5 to 1 or 2% copper (Schlesinger et al. 2011a)."*

COMMENT: Sentence needs to be reworked; meaning is not very clear. The antimicrobial property has little to do with the use of copper for heating, ventilation and air conditioning

RESPONSE*: The comment refers to the following sentence in section 5.2.3 (Use): "Since copper's antimicrobial properties make it useful for drinking water treatment; drinking water distribution; and heating, ventilation, and air conditioning it also has potential uses for reducing microbial contamination and health care-associated infections (Arendsen et al. 2019; Vincent et al. 2016)." According to Vincent et al. (2016) in section 3.4 of the paper, "the antimicrobial properties of copper are also used in air conditioning systems." Vincent et al. (2016) describes some studies testing copper to replace aluminum HVAC systems, so this sentence has been reworded to clarify that the use of copper for HVAC systems due to antimicrobial properties is also a potential use. The sentence now reads:*

> *"Since copper's antimicrobial properties make it useful for drinking water treatment and distribution, it also has potential uses for reducing microbial contamination and health care-associated infections by controlling microorganisms in heating ventilation and air-conditioning systems (Arendsen et al. 2019; Vincent et al. 2016)."*

COMMENT: It is not clear that the references to the SIC Codes in this paragraph is particularly helpful. Also, the last sentence is long and unwieldy

RESPONSE*: The comment refers to the following sentence in section 5.3 (Releases to the Environment): "Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005)."*

The text the reviewer is referring to is included in all ATSDR Toxicological profiles. ATSDR will consider the reviewers suggestion during periodic reviews of templated format and text of ATSDR's toxicological profiles in accordance with future updates to ATSDR's Guidance for the Preparation of Toxicological Profiles.

COMMENT: ?????

RESPONSE*: The comment refers to the following sentence in section 5.3 (Releases to the Environment): "Natural sources of copper releases include windblown dust, volcanoes, decaying vegetation, forest fires, sea spray, and space deposition (Georgopoulos et al. 2001; Rauch and Graedel 2007)." Changes were made as requested and space deposition was removed from the sentence:*

> *"Natural sources of copper releases include windblown dust, volcanoes, decaying vegetation, forest fires, and sea spray (Georgopoulos et al. 2001; Rauch and Graedel 2007)."*

COMMENT: Reviewer's note: The time/period for the estimated releases/emissions is critically important and should be included in all the inventory tables. Emissions have changed significantly over time in response to the various pollution control programs

RESPONSE*: The comment refers to Table 5-4 in section 5.3.1 (Air). The year is specified in the source footnote and the table title is specified in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles.. The text preceding this table points out that the data is from 2018. No changes were made to the table title.*

COMMENT: for what year/period

RESPONSE*: The comment refers to Table 5-7 in section 5.3.1 (Air). The period has been added to the table title, which is now*

"Table 5 7. Global Emissions of Copper from Anthropogenic Sources in the mid-1990s."

COMMENT: Deleted sentence is not particularly meaningful. Intuitively, the earth's crust is the source of all copper

RESPONSE*: The comment refers to the following sentence in section 5.3.1 (Air): "Since copper is a component of the earth's crust, the earth's crust is the primary natural source of copper." The suggested change was made and the sentence has been deleted from the toxicological profile.*

COMMENT: in what year/period

RESPONSE*: The comment refers to Table 5-8 in section 5.3.1 (Air). The year has been added to the table title, which now reads:*

"Table 5-8. Copper Emissions into the Atmosphere in 1984."

COMMENT: This is why the inventory tables need to have some indication of the date

RESPONSE*: The comment refers to the following sentence in 5.3.1 (Air): "No recent reports updating these estimates have been found but due to changes in these industries over time, emissions are likely different now." This suggestion has been noted and table titles were updated to indicate dates where needed.*

COMMENT: What was so special about the negative result from this old study? Is the aim to show that dump sites are not important sources of airborne copper?

RESPONSE*: The comment refers to the following sentence in section 5.3.1 (Air): "The mean copper deposition rates in the two areas were about the same: 0.55 mg/kg-month (range of 0.04–1.6 mg/kg-month) over the refuse dump and 0.51 mg/kg-month (range of 0.26–0.76 mg/kg-month) in the control*

area (Lodenius and Braunschweiler 1986)." The conclusions from the summary have been summarized in a sentence and added to the profile:

> *"Lodenius and Braunschweiler (1986) concluded that the refuse dump did not contribute to copper concentrations in urban air above normal values."*

COMMENT: Not always true; in many areas, long-range transported aerosols have smaller sizes than locally entrained soil particles

***RESPONSE**: The comment refers to the following sentence in section 5.3.1 (Air): "Copper associated with fine particles (10 µm) is likely to originate from windblown soil and dust (Schroeder et al. 1987)." Information has been added from a study discussing the differences in sizes based on origin. The previous sentence was changed to state:*

> *"Copper associated with particles (≤10 µm) has been suggested to originate from windblown soil and dust (Schroeder et al. 1987)." An additional sentence was added to follow: "Generally, aerosols from sea spray, dust, and volcanic mineral emissions tend to be larger than particles formed by condensation of gases in the troposphere (Buseck and Posfai 1999)."*

COMMENT: \ milligram or megagram?

***RESPONSE**: The comment refers to the following sentence in section 5.3.1 (Air): "Using an emission factor of 3,000 ppm developed by the European Copper Institute, Lifset et al. (2012) estimated that releases from fireworks in the United States increased from 40 Mg in 1975 to 220 Mg in 2000." The units used in the study are megagrams. The unit has been changed to the equivalent metric tons, which is also noted in the study, to avoid confusion. The sentence now states:*

> *"Using an emission factor of 3,000 ppm developed by the European Copper Institute, Lifset et al. (2012) estimated that releases from fireworks in the United States increased from 40 metric tons in 1975 to 220 metric tons in 2000."*

COMMENT: This statement can be confusing. Copper in soils can accumulate in plants and animals to levels that pose a health hazard to human beings

***RESPONSE**: The comment refers to the following sentence in section 5.3.3 (Soil): "However, even though the largest releases of copper are to land, uptake of copper in human populations through ingestion of copper in soil is expected to be minimal in comparison to the primary route of exposure through the ingestion of drinking water." The clarification made by the reviewer has been added:*

> *"Although the largest releases of copper are to land, uptake of copper in human populations through ingestion of copper in soil is expected to be minimal in comparison to the primary route of exposure through the ingestion of drinking water. However, copper in soil can accumulate in plants and animals to levels that may impact human health."*

COMMENT: long and convoluted; the sentence should be reworked

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Air): "Dry depositional fluxes of copper tend to decrease between highly urbanized area such as Chicago, Illinois with an average depositional rate of 0.06 mg/m2/day, to less urbanized areas such as South Haven, Michigan with rate of 0.007 mg/m2/day or areas with minimal anthropogenic activity such as Lake Michigan (between 6 and 10 km offshore) with a rate of 0.01 mg/m2/day (Paode et al. 1998)." The sentence has been split into two sentences so that it is clearer. It now reads:*

> *"Dry depositional fluxes of copper tend to be higher in highly urbanized areas and lower in less urbanized areas or areas with minimal anthropogenic activity. For example, average depositional rates were 0.06 mg/m2/day in Chicago, Illinois, 0.007 mg/m2/day in South Haven, Michigan, and 0.01 mg/m2/day 6 to 10 km offshore of Lake Michigan (Paode et al. 1998)."*

COMMENT: The decimal places for some of the numbers need be reconsidered. It is unlikely that the deposition rates were measured accurately to the fourth decimal place. 0.0049 mg/m2 is a very small number

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Air): "For rural areas, the range of bulk deposition reportedly is 0.018–0.5 kg/ha/year or 0.0049–0.14 mg/m2/day, and wet deposition is 0.033 kg/ha/year or 0.009 mg/m2/day." The studies report these data to the decimal places shown for the data in kg/ha/yr; the other data in mg/m2/day are converted. Some of the data has been changed for consistency among significant figures, but the studies report data to the decimal places seen. With some updated significant figures, the sentence now reads:*

> *"For rural areas, the range of bulk deposition reportedly is 0.018–0.5 kg/ha/year or 0.0049–0.1 mg/m^2/day, and wet deposition is 0.033 kg/ha/year or 0.0090 mg/m2/day."*

COMMENT: Very unusual units for the washout ratio; infact, the ratio should be unitless

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Air): "The washout ratio is 114,000–612,000 (µg/m3 rain)/(µg/m3 air) or, expressed on a mass basis, 140–751 (µg/kg rain)/(µg/kg air)." After checking the study, the sentence has been updated to state:*

> *"The washout ratio is 140–751 (Schroeder et al. 1987)."*

COMMENT: This is questionable. A number of studies have linked the recent changes in copper profiles in the polar snowfields and other remote regions to increasing atmospheric pollution with copper

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Air): "Soil was not the major source of copper in cities or nearby rural soils but was the predominant source for copper in the atmosphere over more remote areas (Fergusson and Stewart 1992)." Following this sentence, another one has been added with information from more recent studies which states:*

> *"However, high copper concentrations in snow and aerosols from polar snowfields and remote locations has been attributed to airborne pollution and long-range transport (Annibaldi et al. 2007; Dinu et al. 2020)."*

COMMENT: What is the reference for these estimates?

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Water): "The mean residency times of copper in sediments are estimated to be 15 years in Lake Erie and 101 years in Lake Superior." The references have been added; the sentence now states:*

> *"The mean residency times of copper in sediments are estimated to be 15 years in Lake Erie and 101 years in Lake Superior (Georgopoulos et al. 2001; Nriagu et al. 1996)."*

COMMENT: Questionable or confusing statement. It is only the dissolved copper in POTW effluent and surface runoff that is mostly in complexed form. By and large, most of the copper in POTW effluent and surface runoff is bound to the solid phases

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Water): "In fact, most of the copper in POTW effluent and surface runoff is already in the form of complexes (Sedlak et al. 1997)." The sentence has been rewritten to state:*

> *"Most dissolved copper in POTW effluent and surface runoff is mostly already in complexed form (Sedlak et al. 1997)."*

COMMENT: This paragraph needs to be better organized; the old study in Chesapeake Bay can be replaced with more recent information

RESPONSE*: The comment refers to the following paragraph in section 5.4.1 (Transport and Partitioning – Water): "Much of the copper discharged into waterways is in particulate matter and settles out. Only 0.1% of copper applied to a commercial catfish pond remained in pond water (Liu et al. 2006). In the water column and in sediments, copper adsorbs to organic matter, hydrous iron and manganese oxides, and clay. In the water column, a significant fraction of the copper is adsorbed within the first hour of introduction, and in most cases, equilibrium is obtained within 24 hours (Harrison and Bishop 1984). In fact, most of the copper in POTW effluent and surface runoff is already in the form of complexes (Sedlak et al. 1997). Copper in wastewater discharged into Back River leading into Chesapeake Bay, Maryland contained 53 ppb of copper, of which 36 ppb (based on weight) were in the form of settleable solids (Helz et al. 1975). The concentration of copper rapidly decreased downstream of the outfall so that 2–3 km from the outfall, the copper concentration had fallen to 7 ppb. The concentration of copper in sediment 2–3 km downstream from the outfall was about a factor of 10 higher than in uncontaminated areas (e.g., Rappahannock River). Based on their data and the results from other studies, Helz et al. (1975) estimated that approximately 200 metric tons of copper entered the Chesapeake Bay from the effluent discharged from waste treatment plants annually."*

The sentence "Only 0.1% of copper applied to a commercial catfish pond remained in pond water (Liu et al. 2006)" was removed to better organize the paragraph. One recent study was located, although it does not address copper from effluent discharged from waste treatment plants. The following sentence was added at the end of the paragraph to summarize this newer study: "Whitall et al. (2010) concluded that copper released from antifouling paint on boats was a likely source of copper measured in the Choptank river estuary, a tributary of the Chesapeake Bay."

COMMENT: Convoluted sentence; should be reworked

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Water): "A study evaluated the importance of the absorption properties of different nonlithogenic components of aerobic estuarine sediment to copper binding by determining copper's absorptivity to model components (phases) in artificial seawater (Davies-Colley et al. 1984)." The sentence has been rewritten as follows:*

> *"Davies-Colley et al (1984) determined copper's absorptivity to model phases in artificial seawater in order to estimate copper distributions between estuarine sedimentary phases and water."*

COMMENT: Solubility is not the right term for the experiment described by the authors

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Sediment and Soil): "To determine the factors affecting copper solubility in soil, Hermann and Neumann-Mahlkau (1985) performed a study in the industrial Ruhr district of West Germany, which has a high groundwater table (10–80 cm from the surface) and a history of heavy metal pollution." Solubility has been changed to leachability in this sentence.*

COMMENT: ????

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Sediment and Soil): "The leaching of heavy metals by simulated acid rain (pH 2.8–4.2) was measured by applying this rainwater to columns containing humus layers obtained from sites in a Swedish spruce forest both near to and far from a brass mill (Strain et al. 1984)." After being unable to confirm the information in the cited study, it has been removed from the profile.*

COMMENT: What is the use of describing this 45-year old experiment in detail? Since then, many laboratory and field experiments have been reported on the release of copper and other trace metals from sludge (all types). It would make more sense to cite these newer references rather than dwell on the one outdated study

RESPONSE*: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Sediment and Soil): "Only small amounts (<0.01–0.87 ppm) of copper were found in the leachate (Ritter and Eastburn 1978)." The paragraph in which this sentence is found has been rewritten so that information from the older sources is condensed and newer information has been added. This paragraph was also combined with the one below it in order to consolidate the older information. The paragraph now states:*

> *"For concentrations up to 2mg of copper per liter of water, 25–75% of copper entering POTWs is removed in sludge, much of which is disposed of by spreading on land. Thus, it is useful to ascertain whether copper in sludge is apt to leach into soil. This did not appear to be the case: leachate collected from sludge-amended soil contained <12 ppb of copper (Perwak et al. 1980). Older studies found that small amounts of copper were found in leachate from soils treated with copper-containing sludge, and copper is typically confined to the upper 5-10 cm of soil (Breslin*

1999; Davis et al. 1988; Giusquiani et al. 1992; Ritter and Eastburn 1978). In soils receiving long-term, heavy applications of sludge, high copper concentrations (471 mg/kg in comparison to 19.1 mg/kg in unamended control soils) were reported to depths of up to 25 cm (Richards et al. 1998). Brown et al. (1983) found that copper remained in the upper 12.7 cm of soil treated with sewage sludge for a year. The mobility of copper into soil from sludge was found to be determined mainly by the amount of soil organic carbon and soil surface area (Domergue and Védy 1992; Gao et al. 1997). In addition, soils amended by sludge with low metal content were found to have increased sorption of copper due to the increased binding capacity provided by the "low metal" organics in the sludge (Petruzzelli et al. 1994). From the results of other work, the major portion of the copper (40–74%) is expected to be associated with the organic Fe-Mn-oxide and carbonate fractions of most soils (Ma and Rao 1997).

Recent studies on the long-term effects of soil treated with organic amendments such as sludge, manure, and compost, on copper availability have been published. Smolders et al. (2012) found that copper availability in soil treated long-term with organic amendments is lower than that in soil that has been spiked with Cu2+ salts because of its lower availability in the original matrix and due to aging reactions. Cagnarini et al. (2021) simulated long-term metal concentrations in soil treated with organic amendments in Switzerland. Copper concentrations have decreased over time and are projected to remain nearly constant or in decline through 2100 (Cagnarini et al. 2021). The model suggests that although concentrations of copper in soil treated with sewage sludge are expected to decrease, historic inputs of sewage sludge would result in exceedances of the threshold concentration that would persist through 2100. Copper availability in soil to which stabilized sewage sludge or biosolids has been applied has also been more recently studied; concentrations of copper in biosolid treated clay, calcareous, and sandy soil were significantly higher than in control samples (Mahdy et al. 2007)."

COMMENT: All the studies mentioned in this paragraph were published before the turn of this century. Does it mean that nothing has been published on the topic since then?

***RESPONSE**: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Sediment and Soil): "In addition, soils amended by sludge with low metal content were found to have increased sorption of copper due to the increased binding capacity provided by the "low metal" organics in the sludge (Petruzzelli et al. 1994)." Information from newer studies has been added (see comment and response above).*

COMMENT: The reasons for providing the details of this 35-year old experiment should be mentioned

***RESPONSE**: The comment refers to the following sentence in section 5.4.1 (Transport and Partitioning – Sediment and Soil): "Also, the availability of the copper in soil, as determined by its extractability with diethylenetriamine pentaacetic acid (DTPA) and nitrate, remained constant over a 4-year period at all depths." Much of the information in the paragraph in question is from the previous version of the ToxProfile. Upon further review, some of the information could not be confirmed based on the studies cited. Much of this paragraph has been deleted, and remaining info has been combined with the paragraph above (as shown in previous related response) discussing sewage treated soils.*

COMMENT: A fair amount of information has become available on the speciation of copper and other trace metals in airborne particulates

RESPONSE*: The comment refers to the following sentence in section 5.4.2 (Transformation and Degradation – Air): "Few data are available regarding the chemical forms of copper in the atmosphere and their transformations." This sentence has been deleted. Information from recent studies has been added to the paragraph:*

> *"Data is available on the speciation of copper in airborne particulates. It is generally assumed that metals of anthropogenic origin, especially those from combustion sources, exist in the atmosphere as oxides because metallic species are readily attacked by atmospheric oxidants. As these oxides age, sulfurization may occur, but only when SOX gases are present in the atmosphere in sufficient amount. For example, in Arizona, atmospheric copper oxide levels near copper smelters was strongly correlated with co-emitted sulfur (Schroeder et al. 1987). Copper was primarily bound to organics and sulfides in dry deposition near a smelter in China, and dust from the smelter and in deposition samples showed sulfides and oxides (Liu et al. 2021). Copper has been observed bound to fine aerosol particles as the sulfate and nitrate (Osan et al. 2010). The form of copper in the coarse fraction could be used to trace its source to soil resuspension or brake pad wear erosion (Osan et al. 2010)."*

COMMENT: several newer technologies (such as hyphenated ICP-MS procedures), etc have since become available and could be mentioned

RESPONSE*: The comment refers to the following sentence in section 5.4.2 (Transformation and Degradation – Water): "Various techniques may be used to classify the lability of different fractions of soluble copper; these techniques include solvent extraction, ion-specific electrodes, ion exchange, ultrafiltration, electrochemical methods such as anodic stripping voltammetry, and gel filtration chromatography (Harrison and Bishop 1984)." A source was located to support hyphenated ICP-MS as a technique for this. A sentence has been added following the one in question to mention this technique:*

> *"Newer technologies include hyphenated ICP-MS (Agilent Technologies 2012)."*

COMMENT: Cite pertinent references to support the statements in this paragraph

RESPONSE*: The comment refers to the following sentence in section 5.4.2 (Transformation and Degradation – Water): "The results suggested that the rates are controlled by Mg2+, Ca2+, Cl-, and HCO3 through their involvement in complex formation and ligand exchange." We do not have access to the study about Biscayne Bay, so those sentences have been deleted. Citations have been added for the statements in this paragraph. The paragraph in question now reads:*

> *"Cu(I) concentration is highest in the surface layer of seawater, and the hydrogen peroxide concentration increases in parallel to that of Cu(I) (Moffett and Zika 1987). In addition, the percentage of free Cu(I) is highest on the surface. Sharma and Millero (1988) measured the rate of Cu(I) oxidation in seawater as a function of pH, temperature, and salinity. The rate of reaction increased with pH and temperature and decreased with increasing ionic strength (or higher salinity) (Sharma and Millero 1988). The results suggested that the rates are controlled by*

Mg2+, Ca2+, Cl-, and HCO3 through their involvement in complex formation and ligand exchange (Sharma and Millero 1988)."

COMMENT: this is not always true; fairly soluble minerals can readily be mobilized and/or extracted

***RESPONSE**: The comment refers to the following sentence in section 5.4.2 (Transformation and Degradation – Sediment and Soil): "On the other hand, if a mineral form is deposited, it would be unavailable for binding." This sentence has been deleted from the profile.*

COMMENT: Please indicate the instrumentation used – specific ion electrode, graphite furnace, ICP-MS, other technologies

***RESPONSE**: The comment refers to Table 5-10 in section 5.5 (Levels in the Environment). Footnotes have been added to indicate the instrumentation used for the methods in the table.*

COMMENT: Most of the data reviewed in this section were collected 30-40 years ago. They may not be particularly relevant to the present-day environment considering the well documented reduction in ambient air pollution in the country. One suggestion would be to contrast the ambient airborne levels of copper 30-40 years ago with those of today

***RESPONSE**: The comment refers to section 5.5.1 (Air). All US-based data has been reviewed and all data found has been included. Only one recent study on ambient air concentrations of copper was identified and has been added to the second paragraph:*

"One recent study found that the mean concentration of copper in ambient air from 13 U.S. cities was 0.005 µg/m3, and concentrations ranged from 0.002 to 0.006 µg/m3 (Chen and Lippmann)." Most recent data appear to be from abroad. The older data has been condensed and a caveat was added to the second paragraph: "It should be noted that older data may not be representative of current concentrations, given the reduction of ambient air pollution in the United States."

COMMENT: Most of the data on copper in uncontaminated waters of over 1.0 ppb should be viewed suspicion. These older studies measured copper with atomic absorption spectrometry which had high detection limits. In addition, the samples were often compromised by contamination during collection, treatment and instrumental analysis. A cautionary note on this matter should be included in this report

***RESPONSE**: The comment refers to section 5.5.2 (Water). A sentence was added at the end of the first paragraph:*

"Data from older studies may have been analyzed with instrumentation with high detection limits, and samples were often contaminated during collection, treatment, and analysis."

COMMENT: It would be good to indicate whether the data shown pertains to filtered or total (unfiltered) concentration, especially for surface water

***RESPONSE**: The comment refers to the following tables in section 5.5.2 (Water): Tables 5-14, 5-15, and 5-16. Information on whether the samples were filtered or unfiltered has been added to the notes columns of these three tables when information was given in the study.*

COMMENT: decimal places??

***RESPONSE**: The comment refers to the following sentence in section 5.5.4 (Other Media): "Median copper concentrations ranged from 0 mg/kg wet weight in canned frigate to 6.865 mg/kg wet weight in frozen prawn (Olemdo et al. 2013)." The data are presented as reported in the study. No changes made.*

COMMENT: Not a good matric for comparing with sewage sludge

***RESPONSE**: The comment refers to the following sentence in section 5.5.4 (Other Media): "For comparison, the concentration of copper in cow's manure is ~5 ppm (Mumma et al. 1984)." The clause "for comparison" has been removed from this sentence.*

COMMENT: Is it possible/realistic that plasma TV consists of 70% copper and CRT TV contains 62.5% copper?

***RESPONSE**: The comment refers to the following sentence in section 5.5.4 (Other Media): "The average weight (mg/device) of copper in different electronic devices are: plasma TV (700,300), color CRT TV (625,600), LCD TV (206,000), laptop computer (102,800), LCD monitor (59,500), and cell phone (18,800) (Woo et al. 2016)." The units for these data are mg/device. For example, plasma TVs, on average, have 700,300 mg of copper. The sentence was rephrased to make this clearer:*

> *"The average weight of copper in different electronic devices is: 700,300 mg in plasma TVs, 625,600 mg in color CRT TVs, 206,000 mg in LCD TVs, 102,800 mg in laptop computers, 59,500 mg in LCD monitors, and 18,800 mg in cell phones (Woo et al. 2016)."*

COMMENT: Any idea where the copper in swimsuits came from or why it was added to such clothing?

***RESPONSE**: The comment refers to the following sentence in section 5.5.4 (Other Media): "Copper was detected in 64% of the samples at an average concentration of 27.9 mg/kg, with concentrations ranging from less than 0.15 to 328 mg/kg." Herrero et al. (2020) did not discuss why copper specifically may be found in swimsuits, but they did mention some reasons for additives such as trace metals in fabrics. That reasoning has been added after the sentence in question:*

> *"Although Herrero et al. (2020) does not specifically discuss the origins of copper in swimsuits, the authors do note that many swimsuits are made of artificial fibers so that they may be water repellant or fast drying. Metals may be used in the textile industry as dyes, antimicrobials, and water repellants (Herrero et al. 2020)."*

COMMENT: This is an interesting use for this compound from the exposure perspective and needs further discussion in the report

RESPONSE*: The comment refers to the following sentence in section 5.6 (General Population Exposure): "Since copper sulfate is used as a preservative in fresh fish and as a water treatment in ponds and other freshwater surfaces, children who eat fresh fish more often may be exposed to it." Further information on the use of copper sulfate as a preservative in fish was not located. No changes were made.*

COMMENT: Any known instances for this? If so, they need to be cited here

RESPONSE*: The comment refers to the following sentence in section 5.7 (Populations with Potentially High Exposures): "Exposure to high levels of free Cu(II) can occur, for example, from swimming in water that was recently treated with a copper-containing algicide." No studies/literature were identified describing known instances of exposure from swimming in copper-treated water. No changes were made.*

COMMENT: Any known instances for this? If so, they need to be cited here

RESPONSE*: The comment refers to the following sentence in section 5.7 (Populations with Potentially High Exposures): "Consumers who use skin-whitening agents could be at risk of high exposure to copper." Aside from the cited study on a correlation between skin whitening agent use and elevated serum copper (Ivanda et al. 2011), no studies were located that describe known instances of high copper exposure from using these products. More studies were located quantifying the concentration of copper in products, and those have been summarized before the sentence in question:*

> *"Copper concentrations ranged from 2.27 to 8.48 mg/kg in skin lightening creams sold in Nigeria (Sani et al. 2016; Theresa et al. 2011)."*

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 with the effects identified in the ATSDR document.

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: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

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

COMMENT: No response was provided by the reviewer

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 that MRLs for inhalation of any duration could not be determined from the reviewed lieterature.

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: No response was provided by the reviewer

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: An UF of 3 for a contact effect is appropriate; however, the scientific basis for this conclusion (copper is acting as a direct contact irritant accounting for gastroinstestinal signs including nausea, abdominal pain, and vomiting) needs additional support. The authors of the ATSDR document should consider reviewing what is known about vagal inputs and vomiting (e.g., see: Babic T, Browning KN. The role of vagal neurocircuits in the regulation of nausea and vomiting. Eur J Pharmacol. 2014;722:38-47. doi:10.1016/j.ejphar.2013.08.047) in evaluating the literature they cite. It would also be appropriate to better define what the authors mean by a direct contact effect – does this imply gastritis? irritation of the gastric mucosa? activation of the CRTZ? Most emetics that I am aware of (e.g., hydrogen peroxide, ipecac syrup) that are thought to work through direct gastric irritation have a short latency between administration and emesis (e.g., 15 minutes or less) – this time frame is also similar to that reported for copper sulfate and dogs (see below). However, the cited lietarture from the human studies does not seem to indicate that emesis occurred "immediately" after ingestion of drinking water containing elevated concentrations of copper.

RESPONSE: *In the MRL Summary, text was added that the direct contact effect is on the gastric mucosa. In the last paragraph of section, "Selection of the Critical Effect," information is provided supporting the direct contact effect that is stated throughout the document. In this paragraph, studies in dogs, shrews, and ferrets are cited where emesis was induced by copper sulfate contact in the stomach mediated by the vagus nerve. The cited studies in humans where vomiting is reported along with other gastrointestinal symptoms do not specifically report the timing of the symptoms in relation to the timing of the exposure. In these studies (Pizarro et al. 1999, 2001), participants only reported all symptoms experienced by the end of the day, and the study authors do not report whether these symptoms occurred immediately after ingestion of the drinking water containing copper sulfate. The authors from Pizarro et al. (2001) do attribute the vomiting effects to direct stimulation of the vomiting center in the central nervous system. The current justification for the direct contact effect was kept as is. No changes were made.*

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

COMMENT: A4, Line 23: The acute oral MRL may be a more conservative estimate as some evidence indicates that gastrointestinal symptoms may be due in part to a direct contact effect determined by the amount of copper in the stomach at a given time, especially at high exposure concentrations. Comment: It is not clear to me that the effects seen are indeed a "direct contact effect". The Pizarro study does not indicate when episodes of vomiting, nausea, and abdominal pain occurred relative to when the copper in drinking water was ingested. The Wang and Borison (1951) study conducted in mongrel dogs used a much higher dose rate (i.e., > 4 mg/kg) in animals that underwent partial or total vagotomy. As these authors point out (page 521) copper sulfate has both central and peripheral effects with respect to emesis. Emesis in the normal (presurgical) dogs had a latency of approximately 7-10 minutes – which could be consistent with a direct irritant effect. However, the human data does not provide this additional information. The ATSDR authors also state that: Several other studies in dogs and ferrets demonstrate that copper sulfate induced emesis results from contact in the stomach mediated by the vagus nerve (Makale and King 1992), and also show that 5-HT4 receptors and abdominal vagal afferents are closely associated and play a role inducing vomiting (Bhandari and Andrews 1991; Fukui et al. 1994).

In addition, the effectiveness of copper sulfate as an emetic in dogs shows a dose-response relationship. For example, Wang and Borinson also reprted that 91/107 dogs given 40 mg $CuSO_4$ in 50 ml water vomited whereas only 20/107 responded to 20 mg $CuSO_4$. The women on the Pizarro study were given a solution containing 5 mg Cu/L (equating to a much lower dose). The data remains uncertain as to whether

or not at the doses given to the women the effect is a contact effect. Whether copper is acting directly has implications for the use of uncertainty factors and it also impacts the rationale of using the acute level for the intermediate level (A12, Line 14).

RESPONSE: *The authors of Pizarro et al. (2001) support the notion that the gastrointestinal effects, particularly emesis results from "direct stimulation of the vomiting center in the central nervous system by absorbed copper ions." The drinking water studies are limited in that they do not report the timing of the effect relative to the timing of the exposures as study participants only reported symptoms that occurred throughout the day. Based on the effects observed in animal studies, and reports on the mechanism of gastrointestinal toxicity, ATSDR reviewers determined that a direct contact effect on the stomach is the most likely cause of the reported effects. Several observational and human case studies have reported gastrointestinal effects in humans exposed orally but are limited in reporting the timing of the symptoms. Knobeloch et al. (1994) did report two cases in infants who vomited immediately after ingesting formulas suspected of copper contamination. No changes were made in direct response to the reviewer's comment.*

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: The conclusions drawn in Chapter 2 are supported by the published literature.

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: Human studies were described adequately and significant limitations were also reported.

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: Animal studies were described adequately and significant limitations were also reported.

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: It was unclear from Appendix B why "laboratory mammals" did not include other species that have fairly rich data on copper effects and pharmacokinetics. For example, sheep are sensitive to the effects of copper and studies performed in cattle (e.g., excretion into milk) could also be relevant for this review. Eventhough these species are ruminants research conducted in these species could help improve our understanding of relative toxicokinetics (a data gap identified by ATSDR) and species differences in response.

RESPONSE: *For the ToxProfile health effects and toxicokinetics data, only experimental studies in lab animals are considered in addition to all available human studies. Studies were located regarding the excretion of copper into breastmilk, and observational studies in cattle or other non-laboratory animals are not considered for this discussion. No edits were made as a result of the reviewer's comment.*

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

COMMENT: Adequate attention has been 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: As I mentioned above copper has been widely studied in other mammalian species (e.g., sheep and cattle). For example, a PubMed search for sheep OR cattle AND copper AND toxicity yields nearly 500 items.

RESPONSE: *For the ToxProfile health effects and toxicokinetics data, only experimental studies in lab animals are considered in addition to all available human studies. All toxicology studies in mammals were captured in the literature search for the Copper ToxProfiles and screened through the title and abstract screening. Data from observational studies in farm animals such as cattle or sheep are not considered relevant in determining levels of toxicity in humans due to lack of controls and exposure conditions. No edits were made as a result of the reviewer's comment.*

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 any relevant studies.

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: NOAELs and LOAELs were identified for each study.

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:

- **a.** Table 2.1. Drummond et al. 1986. Changes in pulmonary macrophage has been identified as a less serious effect – while changes in clearance of K. pneumoniae is deemed a serious effect. This is somewhat arbitrary since changes in pulmonary macrophage number of function can alter bactericidal activity in the lung (i.e., these are mechanistically linked).
- **b.** Table 2.2 COPPER NTP 1993 - Cytoplasmic alteration in kidney has been classified as both a less serious effect and a more serious effect seemingly based on incidence rates. This needs to be reconsidered. Likewise, weight loss (even when weight loss is > 25%) is identified as a less serious effect – yet severe cachexia would be classified as a serious effect if the same degree of weight loss occurred in humans over the same (relative) time frame (similarly Kumar et al. 2015 has a chronic weight loss of ~21% considered as a serious effect). For example, see also Page 97 Line 15 ("Reduced food intake, possibly the result of taste aversion, contributed to the deaths") which suggests severe weight loss could be considered a serious effect. It's also unclear when a relative weight change in an organ (e.g., testis) would be considered a serious effect. It's also unclear how inflammatory changes are handled with respect to less serious versus more serious classification (e.g., gliosis versus inflammation of clitoral gland).
- **c.** Kumar et al. 2019 – the decreased muscle strength (was this assessed using measurement of fore- or hindlimb grip strength) could account for the effects seen in the neurobehavioral battery (i.e., the neurotoxicology batteries used in rodent students evaluate multiple domains – not all of which by themselves would be considered to be evidence of neurotoxicity. For example, muscle weakness could be of a non-neurologic origin and if severe could decrease grip strength, alter spontaneous motot activity, etc). This may require additional evaluation of this study. Note that Kumar et al. 2016b reports similar changes using a different neurobehavioral battery – and in this case similar effects were considered a less sensitive effect.
- **d.** Table 2.2 Khushboo et al. 2018 – it's not clear why changes in spleen size is considered a less sensitive effect when I appears that multiple organs were similarly affected in this study (and classified as a severe effect)
- **e.** Table 2.2 COPPER NTP 1993 (mice) not clear why similar relative changes in organ weights (brains and testes) have been assigned different severity ratings.
- **f.** Table 2.2 Munley 2003b Dark discoloration or mottling of lung tissue in 3/21 rabbits has been classified as a severe effect – absent any histologic evaluation this could also represent a postmortem artifact.

RESPONSE:

a. *Decrease pulmonary macrophage was kept as a LOAEL based on ATSDR's guidance on classifying immune effects. The SLOAEL of 3.3 was deleted in consideration of the reviewer's comments.*
b. *In regard to the cytoplasmic alterations, both were classified as LOAELs, therefore the renal SLOAEL for females was taken out. This is supported by the study as it states that cytoplasmic alterations were less severe in females than males. For males, this effect was kept as a LOAEL.*
   In regard to body weight, several bodyweight LOAELs were corrected to SLOAELs. A few values representing 22-28% body weight changes were incorrectly classified as LOAELs. Per ATSDR guidance, >20% body weight change is classified as a SLOAEL.
   In regard to relative organ weight change, per ATSDR's guidance: "Organ weight change is considered an adverse effect if observed in a known target organ. Organ weight change in a known target tissue is considered a minimal LOAEL if the response is associated with no other alterations (morphologic, biochemical); organ weight change in this case may be representative of early-stage adverse effects." Inflammatory changes severity are determined on the incidence and presence of other health effects in the system.
   All organ weight changes were checked and edited as needed to ensure they are consistent with ATSDR's posted guidance regarding assessing organ weight change.
c. *Kumar et al. (2019) measured noticeably impaired muscle strength and coordination following the rotarod test. Together, this indicates a reduction in locomotor activity. The musculoskeletal effect of reduced muscle strength was kept as a LOAEL based on these indications. Neurological effects were classified as a SLOAEL since the study supports multiple other effect in addition to the changes in locomotor activity, including impairment recognition, passive avoidance retention in addition to biochemical changes. The study reports that "neurobehavioral results unveil significant copper neurotoxicity." The neurological effects in Kumar et al. (2016b) were changed to a SLOAEL upon further review of the reported effects).*
d. *For the other endpoints, accompanying effects that were observed would classify as a SLOAEL. For the spleen, the indication of the enlarged spleen on its own would be a LOAEL.*
e. *The SLOAEL for testis weight was changed to LOAEL to remain consistent with ATSDR's guidance on evaluating organ weight, which states the following: "Organ weight change is considered an adverse effect if observed in a known target organ. Organ weight change in a known target tissue is considered a minimal LOAEL if the response is associated with no other alterations (morphologic, biochemical); organ weight change in this case may be representative of early-stage adverse effects. Increased liver weight following exposure to known hepatotoxins is a good example of such an effect. Changes in the organ weight of nontarget organ tissues that are not associated with morphologic or biochemical alterations are not considered to be adverse effects."*
f. *The study authors attributed these observations and the mortality to be substance related. The effect was kept as is.*

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: The discussion of mechanism of action was completed.

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: I agree with the overall conclusions. I have some concerns that the classification of certain symptoms/signs may need to be reconsidered. Examples cited below extend beyond Chapter 2 but relate to Health Effects.

a. Table 2.2. Pizarro et al. 1999 – it is not clear whether headaches should be treated as a neurologic endpoint per se since the headaches seen in these subjects also occur with additional signs (possible secondary headaches) – these can have a musculoskeletal component to them rather than a neurologic origin per se.
b. Page 111, Line 25: Impaired muscle strength was observed in rats exposed to 4 mg Cu/kg/day for 16 weeks, measured by the rotarod test (Kumar et al. 2019). The rotarod does not evaluate muscle strength directly (versus grip strength measures [e.g., see: Takeshita, H., Yamamoto, K., Nozato, S. et al. Modified forelimb grip strength test detects aging-associated physiological decline in skeletal muscle function in male mice. Sci Rep 7, 42323 (2017). https://doi.org/10.1038/srep42323] which are cited later in the document as evidence of neurologic effects). Impaired rotarod performance can occur due to a variety of effects.
c. Page 122, Line 26: A 2-year-old female developed cyanosis around her mouth after spilling an unknown amount of a copper powder on her face. It was believed that she also inhaled some of the substance (Donoso et al. 2007). Cyanosis in this case where the child demonstrated lung effects severe enough to require mechanical ventilation is not indicative of a dermal effect per se versus decreased oxygenation (and possible contribution from methemoglobinemia). Similar comment applies to discussion of cyanotic changes on Page 123 (i.e., cyanosis is more indicative of a cardiovascular/respiratory effect versus a dermal response since the color change relates to subdermal capillaries).
d. Page 136, Line 17: These studies were limited in scope, thus cannot be determined whether the maximum threshold dose (MTD) for tumor development was achieved. – it should also be noted that these are shorter term studies and not 2-year cancer bioassays. Same location: In a carcinogenicity study in rats orally exposed to copper gluconate, 62 mg Cu/kg/day, for 6 weeks the number of glutathione S-transferase placental form (GST-P) positive single hepatocytes examined increased significantly compared to controls (Abe et al. 2008). It would be more appropriate to discuss this as a mechanistic study versus a study designed to evaluate the carcinogenicity of copper
e. Page 142, Line 23: avoid stating mental dysfunction when describing these studies. Changes in motor activity (exploratory behavior) can arise from multiple causes including musculoskeletal disorders. If a specific test of cognition was included in the studies this should be explicitly mentioned.
f. Page 239, Line 2: Clinical symptoms of neurotoxicity are observed in a drinking water study of 60 females, with 6 subjects reporting increased salivation (Pizarro et al. 1999). Increased salivation by itself is not indication of neurotoxicity – this statement should be updated if the Pizarro study does report neurologic effects. Although salivation can be a sign of neurotoxicity – however increased salivation can also occur as a result of other processes (e.g., inflammation of the oral cavity).

RESPONSE:

a. ATSDR agrees and "headaches" was deleted from this effect text which now reads as follows: "Increased salivation in six females"
b. The study authors in Kumar et al. (2019) reported in the methods that the rota-rod test was used to evaluate muscle strength and coordination, and then reported that muscle strength was

impaired. Along with other tests performed, authors determined signs of neurotoxicity attributed to the copper exposure.

c. *Statements regarding cyanosis as a dermal effect were taken out of this paragraph and included elsewhere as appropriate. Including:*

> *Section 2.4: "A 2-year-old female child developed an acute respiratory distress syndrome with dyspnea, bilateral hyperinflation, and interstitial infiltrates of the lungs following inhalation of copper dust (Donoso et al. 2007). These effects were further indicated by cyanosis on the patient."*
>
> *Section 2.7: "Cyanosis, a blueish discoloration of the skin usually associated with methemoglobin accumulation, has also been reported in several case studies (Du and Mou 2019; Hassan et al. 2010; Malik and Mansur 2011; Sinkovic et al. 2008; Yang et al. 2004)."*

d. *This study discussion was kept as a carcinogenicity study based on the discussion provided by the study authors. The additional detail was added per the reviewer's recommendation, as follows:*

> *"These studies were limited in scope, thus cannot be determined whether the maximum threshold dose (MTD) for tumor development was achieved. Additionally, the studies were shorter term and not multi-year cancer bioassays. In a carcinogenicity study, rats were orally exposed to copper gluconate, 62 mg Cu/kg/day, for 6 weeks, and a significant increase in the number of glutathione S-transferase placental form (GST-P) positive single hepatocytes was seen compared to controls (Abe et al. 2008)."*

e. *The language was changed to state instead state changes in motor activity, as follows:*

> *"Neurotoxic findings following oral or intravenous CuNP injection in rodents include changes in motor activity, oxidative stress in numerous brain regions (thalamus, hypothalamus, and medulla) in addition to increasing the levels of AChE in the hippocampus and striatum along with decreased exploratory behavior (Fahmy et al. 2020; Luo et al. 2020)."*

f. *Increased salivation in this case was classified as a neurological LOAEL based on ATSDR's guidance.*

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 text is adequate – as noted earlier pharmacokinetic studies performed in other mammalian species could be informative (especially excretion of copper in milk).

RESPONSE: *No revisions were suggested.*

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

COMMENT: I am not aware that models exist for mammals (a fish PBPK model has been published - Wang and Wang, 2016).

RESPONSE: *PBPK models in fish are not considered relevant regarding human exposure. No revisions were made.*

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: This section was adequate and interspecies differences were considered and discussed.

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: Children's health issues were considered and discussed adquately.

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: ATSDR has identified several at risk populations including humans with inborn errors of copper metabolism. It does not appear that the derived acute MRL considers these susceptible subpopulations when applying uncertainty factors (I am not advocating for this – it appears the document is largely silent on this concern).

RESPONSE: *A caveat was added in the MRL Worksheets to state that the MRL is not applicable to individuals with copper disease such as Wilson's disease. The following statement is in the oral acute MRL worksheet:*

> *"The MRL is based off of concentrations in excess of those that a person would normally be exposed from diet. For further information on dietary copper intake, see Section 5.6. Since Pizarro et al. (1999) uses individuals from the general population, the MRL is not protective of individuals with Wilson's disease, which causes excess accumulation of copper in the body. This condition is further is discussed in Section 2.9."*

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

COMMENT: As noted measurement of copper in blood, urine, and tissues is a specific biomarker of exposure.

RESPONSE: *No revisions were suggested.*

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

COMMENT: The biomarkers of effect (e.g., measurement of serum enzymes) are not specific for copper. I am unaware of any specific biomarker of effect for copper.

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: Copper interactions with other trace minerals is complex. For example, copper – iron – and manganese can interact; however, the draft document does not consider this interaction. In some cases the interactions could be considered primary (e.g., copper and molybdenum) and in others secondary (or even tertiary as may be the case with manganese). Incoproprating a 'Mulders chart' might be illustrative for the reader.

RESPONSE: *Additional text was added regarding copper interactions with other trace minerals, including manganese. Molybdenum is discussed in this section but additional detail was added. A Mulders chart was not added as it is typically used to describe the interaction of trace minerals in plants and does appear relevant in the discussion of interactions within the human body. The following text was added to Section 3.4:*

> *"A study in rats found that exposure to sodium arsenate resulted in increased copper concentration in the kidney (Cui and Okayasu 2008). Rats were orally exposed to varying doses of sodium arsenate daily for 4- and 16-weeks. Exposure to manganese in rats resulted in a similar effect as 7-day exposure to manganese in diet, water, or gavage increased copper levels in the liver (Mercadante et al. 2016). Exposure by diet and gavage resulted in decreased copper levels in bile; both effects suggest a relationship between manganese and copper hepatobiliary excretion."*
>
> *"Interactions with copper sulfate may differ as molybdenum may lower the activity of sulfide oxidase resulting in the accumulation of copper sulfide (Vyskocil and Viau 1999)"*

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: The text could be strengthened by discussing what is known about copper interactions with metal transporters (e.g., DMT-1 and others) and this interaction).

RESPONSE: *Where relevant Section 3.4 discusses interactions with substances and describes the mechanism of this interaction including if there is an effect on a copper-containing protein. The interaction of copper with metal transporters themselves are not discussed in this section.*

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: I spot checked certain values and they appeared correct based on independent sources (e.g., PubChem)

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, data for different chemical forms is 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: This discussion appears 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: This discussion appears complete. I am not aware of any additional relevant 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: This discussion appears complete. I am not aware of any additional relevant 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: This discussion appears complete. Proper units for each medium are used. Quality discussions are adequate for this document. I am not aware of any additional relevant 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 populations discussed in this section. I also agree that the text has adequately described sources and pathways for exposure.

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: As I mentioned before – studies in other mammals may help fill some of the datagaps (e.g., Ward JD, Spears JW, Kegley EB. Bioavailability of copper proteinate and copper carbonate relative to copper sulfate in cattle. J Dairy Sci. 1996 Jan;79(1):127-32; Bengoumi M, Essamadi AK, Tressol JC, Faye B. Comparative study of copper and zinc metabolism in cattle and camel. Biol Trace Elem Res. 1998 Aug;63(2):81-94; Du Z, Hemken RW, Harmon RJ. Copper metabolism of holstein and jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. J Dairy Sci. 1996 Oct;79(10):1873-80. – multiple others).

RESPONSE: *Non-experimental studies in non-laboratory mammals are not considered for inclusion in the health effects discussion per ATSDR guidance on evaluating studies relevant to human exposure. No revisions were made.*

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

COMMENT: The data needs are relatively "cookbook" – i.e., we need more studies on ____. This section could be strengthened by considering the essentiality of copper and how additional data may influence derivation of an MRL or providing advice to the public or health professionals.

RESPONSE: *The current data needs identify gaps in the data that currently affect the ability to derive oral and inhalation MRLs. The current needs emphasize gaps for endpoints where further evidence is needed to better understand effects and mechanisms in humans. Additional detail was added to the neurological section based on recent literature. This section does not necessarily aim to provide advice to the public or health professionals but rather provides justification on how a data need would be beneficial.*
The following text was added to the data needs:

> *"Recent evidence strongly indicates that copper is critically involved in optimal functioning of the circadian clock by modulating cell metabolism, redox state, transcription, and neuronal activity (Yamada and Prosser, 2020). Alterations in these circadian rhythms are implicated in increased risk for cardiometabolic diseases, cognitive and mood disorders, and sleep disorders (Luojus et al., 2015; Song et al., 2015; Yoshioka et al., 2018; Yukihiro et al., 2020; Abbott et al., 2020). Studies that investigate the effects of inhalation, oral, and dermal exposure to copper that examine its effects on circadian rhythms need to be designed and conducted in animal and human*

paradigms. These studies need to examine the alterations in circadian machinery and the potential downstream alterations in physiology in the organisms."

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

COMMENT: I did not detect bias in this presentation.

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: This discussion appears complete.

RESPONSE: *No revisions were suggested.*

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

COMMENT: I don't see a need to remove any of these items.

RESPONSE: *No revisions were suggested.*

Additional References from Reviewer*

***These are references cited within the reviewer's individual comments. Responses to the reviewer's comments specify the disposition of these references within the toxicological profile.*

Appendices

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

COMMENT: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

Annotated Comments on the Profile

COMMENT: Page 1, Line 12: However, excess intake of copper can result in toxicity and heavy metal poisoning. Comment: the use of the term heavy metal could be misleading here. Although there is no formal definition of what a heavy metal is (or isn't) is often restricyed in toxicology to metals such as

mercury and lead. These metals produce distinctly different toxicological effects when compared with copper (and their modes of action also differ).

RESPONSE*: The comment refers to the following sentence in section 1.1 (Overview and U.S. Exposures): "However, excess intake of copper can result in toxicity and adversely interact with other heavy metals." Referring to databases, such as ScienceDirect, heavy metals are comprised of essential and nonessential metals. The text here was modified slightly to make the statement less encompassing of all heavy metals by stating "certain heavy metals" since this is discussed in detail elsewhere in the profile.*

COMMENT: Page 3, Line 29: Liver effects in humans are observed in individuals with Wilson's disease, Indian childhood cirrhosis, and Idiopathic copper toxicosis which all alter copper homeostasis and primarily result in liver toxicity. These diseases are not likely to be caused by excess copper intake, however symptoms can be worsened by excess oral copper intake possibly originating from consuming milk boiled or stored in brass vessels. Comment: This statement isn't entirely accurate – for example Wilson's disease is due to excess copper intake (for the affected individuals) relative to the ability of the liver to safely store copper (e.g., in early stages storing capacity has not been exceeded).

RESPONSE*: The comment refers to the following sentences in section 1.2 (Summary of Health Effects – Hepatic Effects): "Liver effects in humans are observed in individuals with Wilson's disease, Indian childhood cirrhosis, and Idiopathic copper toxicosis which all alter copper homeostasis and primarily result in liver toxicity. These diseases are not likely to be caused by excess copper intake, however symptoms can be worsened by excess oral copper intake such as from consuming milk boiled or stored in brass vessels."*

The wording was changed to be more accurate in references to these diseases, and the sentences now read as follows:

> *"Individuals with Wilson's disease, Indian childhood cirrhosis, and Idiopathic copper toxicosis are particularly susceptible to liver toxicity caused by altered copper homeostasis. These diseases can be exacerbated by excess oral copper intake (i.e. consuming milk boiled or stored in brass vessels) relative to the ability of the liver to safely store copper."*

COMMENT: Page 4, Line 1: Change to: Liver effects which included elevated hepatic marker enzymes, lipid damage, and extensive histopathological observations in the liver including acute swelling of hepatocytes, coagulative necrosis represented by karyolysis of nuclei, and hyperplasia of the epithelium lining of bile ducts were seen in doses as low as 1.6 mg Cu/kg/day in experimental animals exposed daily for 30 days (Hashish and Elgaml 2016).

RESPONSE*: The comment refers to the following sentence in section 1.2 (Summary of Health Effects – Hepatic Effects): "Liver effects which included elevated hepatic marker enzymes, lipid damage, and extensive histopathological observations in the liver including acute swelling of hepatocytes, coagulative necrosis represented by karyolysis of nuclei, and hyperplasia of the epithelium lining of bile ducts were seen in doses as low as 1.6 mg Cu/kg/day in experimental animals exposed daily for 30 days (Hashish and Elgaml 2016)."*

The wording provided by the reviewer appears to be the same as what was written in the text. The text was edited for clarity:

> *"Liver effects were seen in doses as low as 1.6 mg Cu/kg/day in experimental animals exposed daily for 30 days, effects included elevated hepatic marker enzymes, lipid damage, and extensive histopathological observations in the liver such as acute swelling of hepatocytes, coagulative necrosis represented by karyolysis of nuclei, and hyperplasia of the epithelium lining of bile ducts (Hashish and Elgaml 2016)."*

COMMENT: The document could be strengthened by clearly identifying animal species when discussing findings (especially for critical studies).

RESPONSE*: The document was reviewed to ensure clarity in the animal species being discussed for a given study. Minor edits were made throughout Chapter 2 to add in the specific species being discussed, as follows:*

> *Section 2.2 (Death): "Lifetime exposure of mice to 42 mg Cu/kg/day as copper gluconate in drinking water resulted in a 12.8% reduction of the maximum lifespan (from 986 to 874 days) and a 14.4% decrease in their mean survival time (Massie and Aiello 1984)."*
>
> *Section 2.4 (Respiratory): "Combined, the mice, hamster, and rabbit studies indicate that there may be species differences in response to the inhalation of particulate copper."*
>
> *Section 2.5 (Cardiovascular): "The hearts were weighed and no exposure-related differences in relative heart weight were noted for exposed and non-exposed rats."*
>
> *Section 2.9 (Hepatic): "However, Abe et al. (2008) found no significant difference in liver weight in Fischer 344 rats receiving 62 mg Cu/kg/day for 6 weeks compared to controls. Pregnant Wistar rats exposed to 130 mg Cu/kg/day prior to mating and during gestation (up to 73 days total) showed histopathological changes in the liver including hypertrophy and degeneration of hepatocytes and areas of focal necrosis (Haddad et al. 1991). Seven et al. (2018) reported hepatocellular degeneration and necrosis, and karyolysis and karyomegaly in some hepatocytes with a dose of 199 mg Cu/kg/day in male Sprague-Dawley rats."*
>
> *Section 2.16 (Reproductive): "At a dose of 15 mg Cu/kg/day no reproductive differences were observed in the rabbits(Munley 2003a)."*
>
> *Section 2.18 (Other Noncancer): "In the same study, copper sulfate induced emesis (vomiting) in shrews but not pica behavior."*

COMMENT: Table 2.2 Rana and Kumar 1980 – correct spelling for pyknosis (similar correction needed for pyknotic nuclei (Kumar et al. 2016a).

RESPONSE*: The comment refers to Table 2-2 in section 2.1 (Introduction). This spelling was corrected.*

COMMENT: Table 2.2 Sakhaee et al. 2012 - correct spelling for apoptotic bodies.

RESPONSE*: The comment refers to Table 2-2 in section 2.1 (Introduction). The spelling was corrected to state "…and presence of apoptotic bodies"*

COMMENT: Page 96, Line 15 – Capitalize Streptococcus

RESPONSE*: The comment refers to the following sentence in section 2.2 (Death – Inhalation): "In mice exposed to copper sulfate with a of streptococcus aerosols challenge, mortality ranged from 54–70% higher compared to controls, and the mean survival time decreased by 4.2–5.9 days following a single 3-hour exposure to 0.56 mg/m^3 (Drummond et al. 1986)." Changes were made as requested and the sentence now reads: "In mice exposed to copper sulfate with a Streptococcus aerosols challenge, mortality ranged from 54–70% higher compared to controls, and the mean survival time decreased by 4.2–5.9 days following a single 3-hour exposure to 0.56 mg/m^3 (Drummond et al. 1986)."*

COMMENT: Page 101: Line 16: Reword the following sentence: However, such a difference could also be influences by the study (i.e. particular matter, particle size, and composition). It's also unclear how PM would be a factor (do you mean chemical form of the copper?).

RESPONSE*: The comment refers to the following sentence in section 2.4 (Respiratory – Inhalation): "However, such a difference could also be influenced by the characteristics of the material used in the study design (e.g., particle size, and composition)." The reviewer's recommended was considered in editing the sentence, as follows:*

> *"However, such a difference could also be influenced by the study (i.e. particle size, and composition)."*

COMMENT: Also line 30: Reword: In pregnant rabbits, post-mortem evaluation of the lungs no exposure-related changes… (add revealed?)

RESPONSE*: The comment refers to the following sentence in section 2.4 (Respiratory – Oral): "In pregnant rabbits, post-mortem evaluation of the lungs found no exposure-related changes following daily exposure to 7.5–30 mg Cu/kg/day, as copper hydroxide, from gestation days 7 to 28 (Munley 2003a). In the sentence, "found" was changed to "revealed."*

COMMENT: Page 103, Line 27 ("Male Wistar rats exposed to 50.9 mg Cu/kg/day of copper sulfate for 30 days showed flabby, enlarged, congested hearts upon histological examination (Khushboo et al. 2018") – it's not clear from this study that they relied on histologic evaluations to assess the heart (they did for the testes) – the pathology described is more consistent with a gross pathology finding.

RESPONSE*: The comment refers to the following sentence in section 2.5 (Cardiovascular – Oral): "Male Wistar rats exposed to 50.9 mg Cu/kg/day of copper sulfate for 30 days showed flabby, enlarged, congested hearts upon histological examination (Khushboo et al. 2018)." The sentence was change to state that this finding was following gross pathology. The sentence now read as follows:*

> *"Male Wistar rats exposed to 50.9 mg Cu/kg/day of copper sulfate for 30 days showed flabby, enlarged, congested hearts upon gross pathology (Khushboo et al. 2018)."*

COMMENT: Page 104, Line 23: Change likely to possibly (in: While initial exposure was via the inhalation route, it is likely that the observed gastrointestinal effects were due to oral exposure to copper).

RESPONSE*: The comment refers to the following sentence in section 2.6 (Gastrointestinal – Inhalation): "While initial exposure was via the inhalation route, it is likely that the observed gastrointestinal effects were due to oral exposure to copper." Changes were made as requested and the sentence now reads: "While initial exposure was via the inhalation route, it is possibly that the observed gastrointestinal effects were due to oral exposure to copper."*

COMMENT: Page 106, Line 1: I found the following sentence confusing: Symptoms of gastrointestinal upset following acute exposure to copper are suspected to be a direct contact effects associated with the concentration of copper in the stomach at a specific time rather than the daily dose (Donohue 1997). It may be more clear to state that effects are driven by Cmax rather than total 24-hr intake. Line 4 same page: I am also confused by: Copper sulfate has a direct contact effect on the peripheral nervous system followed by a systemic effect on the central nervous system associated with absorbed copper intake (Horn et al. 2014; Wang and Borison 1951). How would this "direct contact effect" (portal of entry?) occur. This statement requires additional explanation.

RESPONSE*: The comment refers to the following sentence in section 2.6 (Gastrointestinal – Oral): "Symptoms of gastrointestinal upset following acute exposure to copper are suspected to be a direct contact effects associated with the concentration of copper in the stomach at a specific time rather than the daily dose (Donohue 1997)." The sentence was edited to explain that the Cmax is what relates to the health effects rather than daily intake. The other sentenced noted by the reviewer was also edited to clarify what is meant by the statement "direct contact effect." The sentences now read:*

> *"Symptoms of gastrointestinal upset following acute exposure to copper are suspected to be a direct contact effect, in that the symptoms result from the maximum serum concentration (C_{max}) of copper in the gastrointestinal system at a timepoint rather than the 24-hour intake (Donohue 1997). A study by Wang and Borison (1951), hypothesized a biphasic mechanism of copper sulfate induced emesis. The effect of copper sulfate on the peripheral nervous system followed by a systemic effect on the central nervous system was associated with the absorbed copper intake (Horn et al. 2014; Wang and Borison 1951)."*

COMMENT: Page 108, Line 10: Change to: A study by Gotteland et al. (2001) was designed to evaluate whether there was a change in the permeability of the gastric and intestinal mucosa in following exposure to a bolus intake of a 10 mg/L copper sulfate solution. Twenty percent of subjects experienced a nausea response and 5 % reported vomiting response.

RESPONSE*: The comment refers to the following sentences in section 2.6 (Gastrointestinal – Oral): "A study by Gotteland et al. (2001) was designed to evaluate whether there was a change in the permeability of the gastric and intestinal mucosa in following exposure to a bolus intake of a 10 mg/L copper sulfate solution. Twenty percent of subjects experienced a nausea response and 5 % a vomiting response." Suggested edits were accepted, and the sentence now reads: "A study by Gotteland et al. (2001) was designed to evaluate whether there was a change in the permeability of the gastric and intestinal mucosa following exposure to a bolus intake of a 10 mg/L copper sulfate solution. Twenty percent of subjects experienced nausea, and 5 % reported vomiting."*

COMMENT: Page 110, Line 21 – change monophils to monocytes.

RESPONSE*: The comment refers to the following sentence in section 2.7 (Hematological – Oral): "One study in rabbits observed changes in blood composition including unspecified decreases in neutrophils, eosinophils, platelets, monophils, and basophils at 16 mg Cu/kg/day for 50 days (Shen et al. 2005)." Changes were made as requested and the sentence now reads: "One study in rabbits observed changes in blood composition including unspecified decreases in neutrophils, eosinophils, platelets, monocytes, and basophils at 16 mg Cu/kg/day for 50 days (Shen et al. 2005)."*

COMMENT: Page 110, Line 29: Hypoxemia and hemolytic anemia were observed in a 2-year-old female child who spilled a copper powder (Donoso et al. 2007). This case report has been cited multiple times and represents a mixed exposure (per authors: "A previously healthy 2-year-old child unintentionally spilled a bottle containing a powdered golden pigment on her face, inhaling part of the contents." It would be more accurate to include that the dermal exposure (here and elsewhere) may also have an inhalation component since there was evidence that inhalation occurred based on copper stains of BALF.

RESPONSE*: The comment refers to the following sentence in section 2.7 (Hematological – Dermal): "Hypoxemia and hemolytic anemia were observed in a 2-year-old female child who spilled a copper powder (Donoso et al. 2007)." Relevant text was checked to ensure that all locations where the Donoso et al. (2007) study is discussed, that it is stated that both inhalation and dermal exposures occurred. This sentence was edited as follows:*

> *"Hypoxemia and hemolytic anemia were observed in a 2-year-old female child who spilled a copper powder on her face and inhaled some of the powder (Donoso et al. 2007)."*

Other related edits include:

> *In Section 2.9: "One case study reported elevated aspartate aminotransferase (AST) and bilirubin in a 2-year-old female child who accidentally inhaled a copper powder and got some on her face (Donoso et al. 2007)."*
>
> *In Section 2.10: "A two-year-old female child who inhaled an unknown amount of a copper powder and spilled some on her facial skin developed renal failure accompanied by oliguria (Donoso et al. 2007)."*

COMMENT: Page 116, Line 3: Change Bennington terrier dogs to Bedlington terrier dogs

RESPONSE*: The comment refers to the following sentence in section 2.9 (Hepatic – Idiopathic copper toxicosis (ICT).): "Additionally, a number of studies examined animals with genetic defects similar to Wilson's disease, primarily in Long Evans Cinnamon (LEC) rats and Bennington terrier dogs." Changes were made as requested and the sentence now reads:*

> *"Additionally, a number of studies examined animals with genetic defects similar to Wilson's disease, primarily in Long Evans Cinnamon (LEC) rats and Bedlington terrier dogs."*

COMMENT: Page 134, Line 18: This hypothesis is supported by the small number of metal fume fever reports from the many industries that use copper under conditions where particulate matter might not be present in air (Borak et al. 2000). I found this confusing – is the issue a lack of PM in the air (which at best would be relative) or other metal fumes?

RESPONSE*: The comment refers to the following sentence in section 2.18 (Other Noncancer – Inhalation): "This hypothesis is supported by the small number of metal fume fever reports from the many industries that use copper under conditions where particulate matter might not be present in air (Borak et al. 2000)." The evidence from this study was rewritten for clarity, as follows:*

> *"A review by Borak et al. (2000) supports this hypothesis as it reviewed occupational reports of metal fume fever and concluded that there is insufficient evidence to suggests these were caused by copper fumes or dusts as other agents appeared to contribute to reported symptoms."*

COMMENT: Page 134, Line 22: Change to: Male rats ingested kaolin indicating pica behavior following exposure once to 10 mg Cu/kg, as copper sulfate pentahydrate but they did not exhibit vomiting (Yamamoto et al. 2004). Rats are incapable of vomiting ths this is not an unexpected finding (see: Horn CC, Kimball BA, Wang H, Kaus J, Dienel S, Nagy A, et al. (2013) Why Can't Rodents Vomit? A Comparative Behavioral, Anatomical, and Physiological Study. PLoS ONE 8(4): e60537).

RESPONSE*: The comment refers to the following sentence in section 2.18 (Other Noncancer – Oral): "Male rats ingested kaolin indicating pica behavior following exposure once to 10 mg Cu/kg, as copper sulfate pentahydrate but they did not exhibit vomiting (Yamamoto et al. 2004)." The last phrase regarding the vomiting was deleted per the reviewer's comment:*

> *"Several experimental studies reported various noncancerous effects in rats or monkeys. Male rats ingested kaolin indicating pica behavior following exposure once to 10 mg Cu/kg as copper sulfate pentahydrate (Yamamoto et al. 2004). "*

COMMENT: Page 142, Line 25: Symptoms of gastrointestinal distress were observed in pregnant rats following oral administrated of CuNP for 15 days (Luo et al. 2020). Avoid using symptoms to describe effects seen in animal studies. Same location: There is some evidence that CuNP cause pulmonary inflammation and may reduce lung clearance, thus increasing the risks of pulmonary infections (Kim et al. 2011) – indicate the route of exposure.

RESPONSE*: The comment refers to the following sentence in section 2.21 (Copper Nanoparticles): "Symptoms of gastrointestinal distress were observed in pregnant rats following oral administrated of CuNP for 15 days (Luo et al. 2020)." The sentence was deleted as the information does not appear to be a finding of the cited study. The sentences where this study is elsewhere cited were reviewed for accuracy as well.*

The second part of the reviewer's comment refers to the following sentence in section 2.21 (Copper Nanoparticles): "There is some evidence that CuNP cause pulmonary inflammation and may reduce lung clearance, thus increasing the risks of pulmonary infections (Kim et al. 2011)." This sentence was edited based on the reviewer's suggestions as follows:

"A murine pulmonary infection model indicates some evidence that CuNP causes pulmonary inflammation and may reduce lung clearance, thus increasing the risks of pulmonary infections (Kim et al. 2011)."

COMMENT: Page 143, Line 7: CuNPs caused developmental effects in rats, including a dose-dependent change in fetal weight, induction of oxidative stress in fetal liver, and increased expression of pro-inflammatory cytokines (Luo et al. 2020). These changes would not be considered to be developmental effects per se)they could indicate fetal toxicity was present).

RESPONSE*: The comment refers to the following sentence in section 2.21 (Copper Nanoparticles): "CuNPs caused developmental effects in rats, including a dose-dependent change in fetal weight, induction of oxidative stress in fetal liver, and increased expression of pro-inflammatory cytokines (Luo et al. 2020)." The wording was changed based on the reviewer's suggestion, as follows:*

"CuNPs resulted in fetal toxicity in rats, including a dose-dependent change in fetal weight, induction of oxidative stress in fetal liver, and increased expression of pro-inflammatory cytokines (Luo et al. 2020)."

COMMENT: Page 143, Line 11: Absorption of CuNPs occurs predominantly through inhalation and ingestion, which may potentially explain its higher rate of aggregation in the brain (translocation through the olfactory bulb) than in the gastrointestinal system (as seen with copper) (Naz et al. 2020). I assume translocation through the olfactory bulb refers to the possibility of direct translocation via the olfactory epithelium ("nose-to-brain" transport). If this is correct, then this should be clarified.

RESPONSE*: The comment refers to the following sentence in section 2.21 (Copper Nanoparticles): "Absorption of CuNPs occurs predominantly through inhalation and ingestion, which may potentially explain its higher rate of aggregation in the brain (translocation through the olfactory bulb) than in the gastrointestinal system (as seen with copper) (Naz et al. 2020)." The Naz et al. 2020 was reviewed and edits were made for clarity, as follows:*

"Absorption of CuNPs occurs predominantly through inhalation and ingestion, which may potentially explain its higher rate of aggregation in the brain (direct translocation via the olfactory bulb) than in the gastrointestinal system (as seen with copper) (Naz et al. 2020)."

COMMENT: Page 143, Line 23. However, copper nanoparticles are expected to be more toxic than copper. This sentence needs additional clarification – e.g., discuss relative to copper salts. As written it implies that CuNP are not copper (which they are) – also CuNP seems to be inconsistently used as an abbreviation in this section.

RESPONSE*: The comment refers to the following sentence in section 2.21 (Copper Nanoparticles): "However, copper nanoparticles are expected to be more toxic than copper." The sentence was edited based on the reviewer's comment as follows:*

"However, CuNPs are expected to be more toxic than copper oxide ions."

COMMENT: Page 144, Line 7: The first phase distributes copper by the portal vein via active transport, ultimately about 75% of this copper is taken up by the liver. This sentence is confusing. The active transport mechanisms occur at cell membranes which then results in copper being transported into the liver and other organs or out of other tissues (e.g., GIT). Transport within the portal vein itself does not constitute active transport per se.

RESPONSE*: The comment refers to the following sentence in section 3.1 (Toxicokinetics): "The first phase distributes copper by the portal vein via active transport, ultimately about 75% of this copper is taken up by the liver." These phrases were edited for clarity based on the reviewer's comment, as follows:*

> *"Distribution: Following absorption, copper is distributed by a 2-phase process. The first phase distributes copper by transport to portal venous circulation where copper is bound to serum protein and ultimately about 75% of this copper is taken up by the liver. In the second phase, copper is bound primarily to ceruloplasmin in the liver, is released to systemic blood circulation, and is redistributed to other organ tissues including the brain, kidneys, muscles, and connective tissues."*

COMMENT: Page 145, Line 12: Peak copper absorption occurred 1-2 hours after ingestion of a single oral dose of copper gluconate in a controlled study of obese males (Boullata et al. 2017). This is what the abstract states; however, was absorption itself measured in this study or were peak blood concentrations seen at this time. Please clarify.

RESPONSE*: The comment refers to the following sentence in section 3.1.1 (Absorption): "Peak copper absorption occurred 1-2 hours after ingestion of a single oral dose of copper gluconate in a controlled study of obese males (Boullata et al. 2017)." This detail from the study was added, as follows:*

> *"Peak copper absorption, measured through a one-compartment model based on serum concentrations, occurred 1-2 hours after ingestion of a single oral dose of copper gluconate in a controlled study of obese males (Boullata et al. 2017). "*

COMMENT: Page 147, Line 12: Change to: a ceruloplasmin

RESPONSE*: The comment refers to the following sentence in section 3.1.2 (Distribution): "ATP7B has two primary functions: the transfer of copper to a ceruloplasmin that is secreted into the blood and then other organs, and excretion of copper from the body through bile (Guttmann et al. 2018)." Changes were made as requested and the sentence now states:*

> *"ATP7B has two primary functions: the transfer of copper to a ceruloplasmin that is secreted into the blood and then other organs, and excretion of copper from the body through bile (Guttmann et al. 2018)."*

COMMENT: Page 237, Line 8: The intermediate-duration oral animal database was not adequate for the derivation of an intermediate-oral MRL. This statement is inconsistent with Page 7, Line 6 that states: The oral database was adequate for the derivation of acute- and intermediate-duration oral MRLs for copper.

RESPONSE*: The comment refers to the following sentence in section 6.2 (Identification of Data Needs – Intermediate-Duration MRLs): "The intermediate-duration oral animal database was not adequate for the derivation of an intermediate-oral MRL." The text in Section 1.3 Minimal Risk Levels (MRLs) was edited to be consistent with Section 6.2, as follows:*

> *"The oral database was adequate for the derivation of acute- duration oral MRL for copper. The acute-duration oral MRL was applied to the intermediate-duration oral MRL."*

COMMENT: A4, Line 11: Change to: There are insufficient data for derivation of an intermediate-duration inhalation MRL as the studies for this route and duration are limited to two studies in rabbits.

RESPONSE*: The comment refers to the following sentence in Appendix A: "There are insufficient data for derivation of an intermediate-duration inhalation MRL as the studies for this route and duration are limited two studies in rabbits." The written text appears to be the same as the reviewer's suggestion. No changes were made.*

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 am not sure that these studies would change any of the outcomes derived here, but I think it would be important to cite recent studies of copper in air pollution exposures and negative impacts in children [1, 2].

RESPONSE: *The Lopuszanska and Samardakiewicz (2020) systematic review referenced by the reviewer was not included as the discussion focused on the broader impacts of $PM_{2.5}$. The Pujol et al. (2016) study cited by the reviewer was added to Section 2.15 Neurological, as follows:*

> *"An epidemiological study on children aged 8 to 12 years found that airborne copper exposure was significantly associated with poorer motor performance and detectable signs of brain damage (Pujol et al. 2016). Copper levels, primarily attributed to traffic pollution, were measured inside and outside of participant schools; 2,827 children participated in behavioral testing and a subset of 263 children participated in brain imaging. The reaction time in children with higher exposure was reduced, while imaging showed copper associated with higher gray matter concentration in the striatum and copper appeared related to changes in the architecture of neural tissue diffusion (Pujol et al. 2016). Additional details on this study are in Table 2-4."*

The study details were also added to Table 2.4 as follows:

Pujol et al. 2016 **Study Type:** Cross-section study of 2,836 children aged 8-12 years from schools in Barcelona, Spain who completed behavioral testing to test motor function. A subgroup of 236 children had a 3D MRI, functional MRI, and diffusion tensor imaging (DTI) to test brain repercussions.	**Exposure:** Air samples were collected from all schools that participants attended to calculate years air pollution levels. Samples were collected twice during 1-week periods separated by 6 months in warm and cold weather months through 2012 and 2013. Indoor air in the classrooms and outdoor air in the school courtyards was measured. The primary source of copper was road traffic, followed by industrial activity. **Inclusion/Exclusion Criteria:** Children with dental braces. DTI images were excluded when image degradation was detectable.	**Outcomes:** Higher copper exposure was associated with poorer motor performance, which was significant for reaction time ($p=0.006$; $\beta=2.2$). This relationship was observed among the main study group and subgroup. Among the subgroup, copper exposure was associated with a higher proportion of gray matter in the brain tissue (striatum). No other significant alterations were seen on the MRI. In the DTI, copper was associated with increased neural tissue fractional anisotropy. **Limitations:** Potential head movement in children during imaging may affect the quality. Self-selection bias as parents opted into the study.

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: Yes, effects observed only in animals are likely to be of concern for humans as they do not represent currently known animal-specific phenotypic characteristics are noted.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, exposure conditions are adequately described. However, it would be nice, particularly given the intent of the document to try to use the same units for studies where possible.

RESPONSE: *Units were standardized where relevant and as recommended by other internal reviewers and peer-reviewers.*

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: The basis for not deriving MRLs is quite clear given the paucity of data for some of the categories, and I agree with these omissions.

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: No response was provided by the reviewer

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: Yes, I agree with the derivation of the Intermediate Oral MRL in lieu of the Acute Oral MRL actually being greater in value.

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: One concern throughout the document is the limited inhalation data that is available, and the potential for Cu to be taken up via the olfactory nerve and bypass the blood brain barrier; such effects would not be reflected in levels of Cu in serum.

RESPONSE: *The available inhalation data on human and laboratory mammals are limited and this data need is expressed in the profiles. 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, in general they adequately reflect the published literature.

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, in general adequately designed human studies are identified and limitations are addressed.

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, in general adequately designed animal studies are cited with adequate sample sizes and dosing.

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: Despite the limitations of the data base in general, there is good overlap in terms of the effects in animal and human studies where that information is available, i.e., the animal species were appropriate for the analyses.

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, adequate attention has been paid to dose-response relationships in both human and animal studies.

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 other animal studies that should be included. I mentioned above two studies of Cu in air pollution and its impact on children, that should be mentioned in the profile, but I'm not sure that they would be useful for dose-response or derivation of MRLs.

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 mentioned above two studies of Cu in air pollution and its impact on children, that should be mentioned in the profile, but I'm not sure that they would be useful for dose-response or derivation of MRLs. I have attached copies of both.

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, appropriate NOAELs and LOAELs were identified as appropriate to conditions of the studies.

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: While this use of less serious vs more serious has become the norm in these types of analyses, overall, I do not agree with its usage as it provides no context for the effect. As a neuroscientist, I could think about slower movement, for example, which if just considered alone doesn't seem particularly serious. However, if I am working putting parts on an assembly line, being too slow could result in my arm being caught in the moving machinery.

RESPONSE: *ATSDR notes the reviewer's concern however in discussing health effects, assumptions are not made on the implications that certain health effects may have on activities beyond the reported health effect. 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: This section could certainly be expanded somewhat to talk more about the potential interactions of metals and how that relates to toxicity. It is a larger problem than just excess copper, it actually leads to metal dyshomeostasis.

RESPONSE: *Interactions with other substances are discussed in Section 3.4 Interactions with Other Chemicals. Per comments provided by other reviewers, information was added to Section 3.4 and are reflected in specific responses and in the profile.*

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: Based on what data is available and its significant limitations in terms of focus, I do think that the conclusions reached are appropriate. Note p. 134 states that rats did not vomit after exposure to copper; rats are not able to vomit so this should be stated.

RESPONSE: *This statement was removed based on the reviewer's suggestion, and it now states:*

> *"Male rats ingested kaolin indicating pica behavior following exposure once to 10 mg Cu/kg as copper sulfate pentahydrate (Yamamoto et al. 2004). "*

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: There is very little discussion with respect to inhalation exposure of Cu relative to particle size, particularly potential problems with ultrafine particulate matter which is likely directly bypassing macrophages and moving into the blood stream where Cu can then move to various organs. In addition, it would seem critical to discuss the potential for nasal olfactory uptake of Cu on ultrafine particle air pollution. Such absorption means Cu can enter brain bypassing blood brain barrier, and elevated levels are not reflected in serum Cu levels. In addition, it would seem important to discuss whether or not for Cu can be excreted from brain back into the blood stream, which is critical to brain metal dyshomeostasis and its potential role in neurodegenerative diseases.

RESPONSE: *This ToxProfile for copper focuses predominantly on the toxicokinetics of copper metal and salts which are elaborately discussed in Chapter 3. Since health effects and toxicokinetics of ultrafine and*

nano- particles are an emerging matter of concern, these are briefly discussed in Chapter 2, Section 2.21. The following language is included in the text "Absorption of CuNPs occurs predominantly through inhalation and ingestion, which may potentially explain its higher rate of aggregation in the brain (direct translocation via the olfactory bulb) than in the gastrointestinal system (as seen with copper) (Naz et al. 2020). Copper homeostasis in the brain is maintained by a coordinated system of copper transporters and chaperones which transport copper across the membranes as required (Haywood 2019). CuNPs can be distributed throughout the body. The primary target organs in animals tend to be the brain, liver, kidney, and spleen where the CuNPs induce pathological changes and organ injuries.". No changes were made in response to the reviewer's comment.

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

COMMENT: Yes, supporting pharmacokinetic/pharmacodynamic information has been presented. Note: p. 144 Toxicokinetcs: please indicate in each bullet what species you are referring to.

RESPONSE: *These bullets summarize the data in the preceding chapters relative to humans. Therefore the bullets only discuss toxicokinetics as it is expected to occur in humans. No changes were made in response to the reviewer's comments.*

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, again, considering the limitations of the database, this has been adequately addressed.

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: Again, I think it would be important to cite the studies of Cu in air pollution and children that have been published cited above and attached.

RESPONSE: *This comment is responded to in an earlier comment by this reviewer which recommended epidemiological studies to add into the profile. Our evaluation of these studies and the inclusion of Pujol et al. (2016) are described in the first comment response for this reviewer.*

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: In addition, the aging brain may also be more vulnerable to such exposures. There is a significant literature relating Cu exposures to various neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease [3-10].

RESPONSE: *Recent evidence (such as the references provided by the reviewer) indicate that copper levels may play a role in the development of Alzheimer's disease. One epidemiological study that examined this relationship was included in the profile but additional studies could not be located to support that environmental exposures to copper contributed to the development of the disease. In order to better address the growing research on the topic, additional text was added to Chapter 6 under neurological data needs, as follows:*

> *"There is a growing body of literature that indicates copper may play role in the development of Alzheimer's disease and other similar neurodegenerative diseases (Pohanka 2019). Medical studies have found evidence that the metabolic balance and distribution of copper is disrupted in individuals with Alzheimer's disease (Coelho et al. 2020). However, the current literature is unclear on how environmental exposures to copper affect the development of Alzheimer's disease. Since the current neurological literature in animals and humans indicate copper can lead to neurological impairment and given that copper can distribute to the brain, epidemiological studies examining the relationship between environmental exposures to copper and Alzheimer's disease would be useful in understanding long term risks of exposure."*

Biomarkers of Exposure and Effect:

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

COMMENT: Yes, but it needs to be recognized in the text that there may be differences between serum levels and actual brain levels of Cu. Note, p. 158, while hair and nail levels are mentioned, are these validated? And to what other organ/system?

RESPONSE: *The reviewer is referring to the following text in Section 3.3.1 (Biomarkers of Exposure): "Copper levels in hair and nails can also be used to assess copper exposure." Additional text was added to this sentence to clarify that these biomarkers are not proven reliable, as follows:*

> *"Copper levels in hair and nails can also be used to assess copper exposure however the reliability of these biomarkers has not been established."*

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

COMMENT: Yes, they seem adequately specific.

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: It would be useful, as noted above, to include more information about Cu interactions with other metals, as this text is currently limited; a consequence of this is metal dyshomeostasis which has adverse effects in the periphery as well as in the brain.

RESPONSE: *Additional information was added on copper interactions with other substances. The following additions were made:*

> *"A study in rats found that exposure to sodium arsenate resulted in increased copper concentration in the kidney (Cui and Okayasu 2008). Rats were orally exposed to varying doses of sodium arsenate daily for 4- and 16-weeks. Exposure to manganese in rats resulted in a similar effect as 7-day exposure to manganese in diet, water, or gavage increased copper levels in the liver (Mercadante et al. 2016). Exposure by diet and gavage resulted in decreased copper levels in bile; both effects suggest a relationship between manganese and copper hepatobiliary excretion."*

The discussion of molybdenum was expanded with the following statement:

> *"Interactions with copper sulfate may differ as molybdenum may lower the activity of sulfide oxidase resulting in the accumulation of copper sulfide (Vyskocil and Viau 1999)."*

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: The text includes some discussion of oxidative stress as a mechanism of some such effects, but in a limited capacity.

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: Outside my expertise.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, information is provided as to the various forms of the substance.

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, to the best of my knowledge.

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, the text traces substances from point of release to receptor population and also known occurrences at NPL sites; I am not aware of any additional information on this topic.

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, it is covered, and I am not aware of any additional information on this topic.

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: In general this is covered. However, it is difficult to make judgements about exposure levels given the use of different metrics throughout this session. It would be helpful to readers to utilize the same metrics if possible within discussions, or to provide some comparison that allows readers to better gauge exposure levels across different metrics. Furthermore, much of the data on monitored levels is quite old; it would be useful, although maybe not possible to find some way to ascertain the extent to which these data are still relevant.

RESPONSE: *The data on exposure levels provided in the profile are the most updated data that were located, including data from US-based databases. Much of the recent data appears to be from outside of the U.S. Units in accordance with ATSDR guidance; for example, units used for concentrations in air are those used for the inhalation LSE table. Not all concentrations in the profile were converted to* mg/m^3 *because some concentrations are quite small and use of ng or µg were more appropriate. No additional edits were made in response to the reviewer's comment.*

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: In general, I agree, but as noted above, I believe that inhalation exposure to Cu, especially the potential with ultrafine particles is not really discussed. Clearly, information is limited but some studies are cited above.

RESPONSE: *A search was conducted on the uses and sources of ultrafine copper particles to identify studies to include in Chapter 5. Some general discussion of ultrafine particles and discussion specific to copper was added to section 5.7 (Populations with Potentially High Exposures):*

> *"Exposure to ultrafine particles of copper poses a risk to human health due to their smaller size, larger surface area, surface material, and physical characteristics (Schraufnagel 2020). Traffic exhaust is a common source of exposure, although homes near a trash burning site, bedrooms with burning coils for mosquito abatement, homes with adult smokers, and kitchens during domestic cooking are also sources of exposure to ultrafine particles (Schraufnagel 2020). Particles created by brake wear, including copper particles, are in the range of 2.8 µm (Wahlin et al. 2006). Copper has been identified in ultrafine particles leading to metal fume fever among welders (Schraufnagel 2020)."*

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 included some references which I think could be included in terms of potential exposure but I don't believe that they will change any of the derivations in the document.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Yes, I agree with the data needs.

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: I did not detect any bias in the text.

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 not aware of any additional regulations or guidelines.

RESPONSE: *No revisions were suggested.*

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

COMMENT: Not that I am aware of.

RESPONSE: *No revisions were suggested.*

Additional References from Reviewer*

**These are references cited within the reviewer's individual comments. Responses to the reviewer's comments specify the disposition of these references within the toxicological profile.*

Appendices

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

COMMENT: No response was provided by the reviewer

RESPONSE: *No revisions were suggested.*

Unpublished Studies (If Applicable to Review)

Lopuszanska, U. and M. Samardakiewicz, *The Relationship Between Air Pollution and Cognitive Functions in Children and Adolescents: A Systematic Review.* Cogn Behav Neurol, 2020. **33**(3): p. 157-178.

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Acevedo, K., et al., *Redox active metals in neurodegenerative diseases.* J Biol Inorg Chem, 2019. **24**(8): p. 1141-1157.

Bisaglia, M. and L. Bubacco, *Copper Ions and Parkinson's Disease: Why Is Homeostasis So Relevant?* Biomolecules, 2020. **10**(2).

Coelho, F.C., et al., *Agricultural Use of Copper and Its Link to Alzheimer's Disease.* Biomolecules, 2020. **10**(6).

Ejaz, H.W., W. Wang, and M. Lang, *Copper Toxicity Links to Pathogenesis of Alzheimer's Disease and Therapeutics Approaches.* Int J Mol Sci, 2020. **21**(20).

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Pal, I., M. Roy, and S.G. Dey, *Active-site environment of Cu bound amyloid β and amylin peptides.* J Biol Inorg Chem, 2019. **24**(8): p. 1245-1259.

Pohanka, M., *Copper and copper nanoparticles toxicity and their impact on basic functions in the body.* Bratisl Lek Listy, 2019. **120**(6): p. 397-409.

Annotated Comments on the Profile

COMMENT: Dark discoloration or mottling of lung tissue in 3/21 rabbits has been classified as a severe effect – absent any histologic evaluation this could also represent a postmortem artifact.

RESPONSE*: This comment refers to Table 2-2 in Section 2.1 (Introduction): "Dark discoloration or mottling of lung tissue in 3/21 rabbits." The study authors attributed these observations and the mortality to be substance related. No changes made.*