DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR COBALT

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

September 2024

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Comments provided by Reviewer #1

ATSDR Charge Questions and Responses and Reviewer Comments

Acute-duration inhalation MRL

QUESTION: Do you agree that the study selected was the appropriate one.

COMMENT 1: I agree – my only concerns is that the outcome of interest is a less serious health outcome and of questionable severity.

RESPONSE: *The critical effect (elevated neutrophils in bronchoalveolar lavage fluid [BALF]; Viegas et al. 2022a) is considered a less-serious lowest-observed-adverse-effect level (LOAEL) by ATSDR. While not a serious effect, it is the most sensitive respiratory effect identified and is considered a key event in particle-induced lung inflammation and toxicity. Since respiratory effects progress to histopathological findings at higher concentrations, it is considered the first detectible event in a progressive chain of adverse respiratory effects on the respiratory system. Therefore, it is a health-protective endpoint on which to base the acute-duration inhalation MRL. Additional support was identified in a study by Weber et al. (2023) and added to Appendix A.*

Regarding the LOAEL endpoint of elevated neutrophils in BALF identified in the study by Viegas et al. (2022a, 2022b), neutrophil-mediated inflammation is considered a key event in particle-induced lung inflammation and toxicity (Lam et al. 2023). In support, Viegas et al. (2022a, 2022b) reported that inflammatory changes at the LOAEL (2.2 mg Co/m^3) progressed to histopathological changes at the next tested concentration (6.7 mg Co/m^3). Furthermore, data from several other inhalation studies with particulates support that increased neutrophils in BALF correlated well with histological changes in the alveoli in 90-day studies in rats (Weber et al. 2023). Weber et al. (2023) proposed that progressive inflammatory changes were associated with macrophage destruction, which decreased particle clearance from the lungs.

COMMENT 2: In addition, group sizes in this study are small.

RESPONSE: *The principal study by Viegas et al. (2022a) used five female rats/group. While for longer-duration studies (e.g., intermediate-duration), 10 animals per group is often the minimum required to be considered adequate, it is generally acceptable to utilize only 5 animals/group for acute-duration studies. Based on the study results, the animal number used had sufficient statistical power to detect intergroup differences. Therefore, ATSDR considers the number of animals/group used in the Viegas et al. (2022a) study to be sufficient.*

QUESTION: Do you agree with the health endpoint selected, the point of departure (NOAEL), the exposure time adjustments in calculating the $NOAEL_{ADJ}$, and the human equivalency adjustments in calculating the $NOAEL_{HEC}$ for the MRL? If you disagree with any of these, please specify what they should be.

COMMENT 3: As I mentioned above the changes seen are mild (elevated neutrophils in broncoalveolar lavage fluid) – this may not represent a clear adverse health effects absent other evidence of respiratory tract injury or pathology. I agree with subsequent steps.

RESPONSE: *Please see response to Comment 1 regarding adversity of elevated neutrophils in BALF, and revisions made to address concerns raised by Reviewer 1.*

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 4: I agree with both the MRLs that have been determined and the uncertainty factors that have been used.

RESPONSE: *No response needed.*

QUESTION: Do you agree that the approach used for deriving the MRL was appropriate.

COMMENT 5: I agree.

RESPONSE: *No response needed.*

QUESTION: Do you consider that rounding the $NOAEL_{HEC}$ at an intermediate step is or is not mathematically appropriate?

COMMENT 6: Appropriate – although some would carry the calculation and round the value at the end.

RESPONSE: *No change requested.*

QUESTION: Do you agree with the final MRL value? If not, please provide your rationale and recommendation for study selection and for either deriving or not deriving this MRL. If you agree with this MRL, then the acute- and chronic-duration MRLs will respectively be 0.0003 and 0.0001 mg Co/m^3.

COMMENT 7: A general concern that I have is that the physicochemical properties of different forms of cobalt can vary (e.g., solubility). As the ATSDR points out this can lead to marked difference in toxicokinetics and toxicity. The document shouls cealry iindicate that the MRLs developed for less toxic/less soluble forms should be health protective.

RESPONSE: *In the of the acute-duration MRL section of Appendix A, the text in Selection of the Principal Study section discusses recent data indicating two distinct classes of cobalt compounds based on high versus low toxicity (Danzeisen et al. 2022a, 2022b; Derr et al. 2022; van den Brule et al. 2022; Verougstraete et al. 2022; Viegas et al. 2022a). The Reviewer states that MRLs developed for less toxic/less soluble forms should be health protective; however, the inverse is true. The most health-protective MRL would be based on the most toxic compound. Since the acute-duration inhalation study is based on a compound in the "high toxicity" group, the principal study is considered health protective. Slight revisions to the text in Appendix A were made to specifically indicate this point.*

> Based on these findings, the study by Viegas et al. (2022a, 2022b), which identified an early key event for respiratory toxicity following exposure to a cobalt compound belonging to the "high acute toxicity" group (cobalt sulfate heptahydrate), appears to be an appropriate, health-protective study on which to base the acute-duration inhalation MRL.

Intermediate-duration oral MRL

QUESTION: Do you agree that the study selected was the appropriate one.

COMMENT 8: Yes – it is a more robust study where exposure assessment is well characterized.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the health endpoint, the benchmark dose model selected, and the point of departure ($BMDL_{1SD}$) for the MRL? If you disagree with any of these, please specify what they should be.

COMMENT 9: I agree with each of these steps.

RESPONSE: *No response needed.*

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 10: The document is a bit inconsistent as to whether effects in neonates would be expected to occur at levels lower than that seen in adults. The ATSDR should clearly indicate why an additional uncertainty factor was not considered (I am not advocated for this).

RESPONSE: *ATSDR does not have a specific uncertainty factor for neonates (see Draft Guidance for the Preparation of Toxicological Profiles.). Therefore, additional text regarding if an uncertainty factor was considered is unwarranted. The uncertainty regarding children's susceptibility is addressed in the 10-fold uncertainty factor for human variability (i.e., susceptibility).*

QUESTION: Do you agree that the approach used for deriving the MRL was appropriate.

COMMENT 11: Yes it was appropriate.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the final MRL value? If not, please provide your rationale and recommendation for study selection and for either deriving or not deriving this MRL.

COMMENT 12: I agree with the final MRL.

RESPONSE: *No response needed.*

GENERAL COMMENTS

COMMENT 13: The chronic oral MRL discussion could consider dietary intake of this essential metal – in essence this could provide a ground truthing exercise or may even be a. reasonable way to frame the chronic oral MRL.

RESPONSE: *Despite being part of vitamin B12, which is an essential vitamin, the United States does not have a recommended dietary intake or tolerable upper intake levels for cobalt that can be used as a starting point (e.g., NOAEL) value for which to start framing a potential chronic oral MRL.*

COMMENT 14: I was also surprised that certain cobalt-containing drugs went un mentioned (e.g., hydroxocobalamin used in humans for the treatment of cyanide poisoning).

RESPONSE: *As discussed in Section 2.1, the scope of the profile is to evaluate sources of environmental exposure above background levels. Medical uses of cobalt are beyond the scope of the profile. Text in Section 2.1 was revised to add hydroxocobalamin to the list of medical uses that are beyond the scope of the profile.*

> Additionally, studies evaluating kinetics and potential toxic effects associated with medical applications of cobalt (e.g., implantable medical devices, hydroxocobalamin for treatment of cyanide poisoning) are not included in the profile, as the profile is concerned with environmental exposures via inhalation, oral, or dermal routes.

COMMENT 15: The ATSDR document equates ocular irritation (chromodacryorrhea) with direct ocular irritation. This clinical sign is also associated with general stress (e.g., seen with systemic toxicity) and may or may not be evidence of ocular irritation (e.g., lacrimation, corneal changes).

RESPONSE: *Attribution of ocular irritation to direct contact with metallic particulates in the air is consistent with the Draft Guidance for the Preparation of Toxicological Profiles. Additionally, attributing findings to point-of-contact irritation with the test substance at moist tissues (rather than systemic toxicity) is consistent with nasal, larynx, tracheal, and bronchiole irritation and damage found at the same (and lower) inhalation concentrations in rats and mice in the same study (NTP 1991).*

COMMENT 16: One other comment relates to neurologic signs. It is not clear which types of signs lead to a severe effect versus a less severe effect. It would be an improvement if the document could provide some guidance.

RESPONSE: *Due to the numerous organ systems included in the profile, it is not possible to provide examples of less serious and serious effects for every system. However, the introduction of Section 2.1 gives a broad overview of severe versus less severe effects:* "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear.

For full details, the Reviewer can refer to the Draft Guidance for the Preparation of Toxicological Profiles, which provides guidance for all organ systems. In the context of neurological endpoints, anything that is considered a significant dysfunction is considered a serious LOAEL (e.g., ataxia, brain congestion, reactive gliosis, memory impairments), while less severe effects are considered "less serious" LOAELs (e.g., lethargy, decreased activity, anxiety-like behaviors). Please note, if one "serious" effect is noted at a dose/concentration, that dose/concentration is considered a serious LOAEL (but other effects noted at that dose/concentration would be included in the LSE table even if they are less serious effects). Some effects may be less serious with a mild impairment (mild motor weakness) and serious with a marked impairment (dragging hind legs).

Comments provided by Reviewer #2

GENERAL COMMENTS

COMMENT 1: This has been my first experience as an evaluator of a ATSDR toxicological profile. Based on my academic expertise in general toxicology (including human health risk assessment) and, specifically, on a career-long research and continued interest in the toxicity of cobalt, I consider myself as having the qualifications needed for this evaluation. I went carefully through the entire document, reading thoroughly (and making annotations) the sections (including tables and figures) dealing with toxicology and epidemiology, and more superficially sections for which I have less expertise (e.g. environmental exposures). With few exceptions, I did not consult original publications (many of which I had ever read) to check their content against statements made in the draft report.

As I stated when accepting the invitation to review, I do not have any commercial interests in relation to cobalt (or any other metal). I do recognize that I was/am pleased that one of my early publications (Nemery et al. 1992) is considered the critical study for setting MRL for chronic inhalation, but I do not think that this has clouded my judgement. I have also suggested adding some other publications by my group.

Overall, I have been impressed by the overall quality and thoroughness of the draft document. I detected no major issues and generally agree with the conclusions proposed in the document.

In the following text, I made brief comments for each chapter, and I mentioned generally minor editorial issues and occasional typos, as they arise in the document.

RESPONSE: *ATSDR thanks the Reviewer for their comments. Responses to annotated comments are below.*

ANNOTATED COMMENTS

Ch. 1. Relevance to public health.

COMMENT 2: Consider mentioning here (or elsewhere) that because of the need to decarbonize the economy (and transport) the need and use of cobalt (and hence opportunities for exposures) are expected to increase considerably over the next decade [see, e.g., Sovacool et al. 2020 doi: 10.1126/science.aaz6003). Consider also mentioning/emphasizing that cobalt is a so-called "critical" or "strategic" commodity (in the USA and EU), on account of its economic importance and supply risks.

RESPONSE: *The potential for increased cobalt production and recycling to support electric vehicle battery production and recycling to meet the U.S. mandate to phase out fossil fueled activities from 2023 to 2050 to increase air emissions was discussed in Section 5.3.1. The potential impact on exposure (due to increased use) was added to the profile in Chapters 1 and 5.*

Section 1.1: Additionally, the usage of cobalt in rechargeable batteries is expected to increase considerably over the next few decades to support electric vehicle battery production and recycling in response the U.S. mandate to phase out fossil fueled activities from 2023 to 2050. Due to this, the U.S. Department of Energy (DOE) considers cobalt a "critical" commodity.

Section 5.1: Workers in the following industries can be exposed to higher levels of cobalt via airborne dust and direct contact: hard metal production or processing (tool production, grinding, etc.);

coal mining; metal mining, smelting, and refining; lithium-cobalt battery production or recycling (including electric vehicle batteries); cobalt dyes and paints; and cobalt chemical production. Populations living near these industrial sites might also be exposed to higher levels of cobalt.

Section 5.2.3: The leading use of cobalt globally is in rechargeable battery electrodes (USGS 2020). Cobalt is also an important component in energy storage for sustainable and alternative energies, which are on the rise world-wide, including solar photovoltaics, wind turbines, fuel cells, and nuclear reactors (Karduri 2023; Sovacool et al. 2020). In the United States, the need for cobalt to support production of electric vehicles is expected to increase considerably over the next few decades in order to support the U.S. mandate to phase out fossil fueled activities from 2023 to 2050 (WH 2021a, 2021b). The U.S. DOE (2023) classifies cobalt as a "critical" energy resource. However, since most cobalt mining processes occur outside of the United States, development of alternate technology (such as lithium iron phosphate or lithium iron-manganese-phosphate batteries) would decrease dependency on the D.R. Congo and China for cobalt supply (DOE 2023).

Section 5.7: Workers in the hard metal industry (tool production, grinding, etc.); coal mining; metal mining, smelting, and refining; lithium-cobalt battery production or recycling (including electric vehicle batteries); cobalt dye painting industry; and cobalt chemical production can be exposed to higher levels of cobalt via airborne dust and direct contact. Of these industries, the largest group is the hard metal industry.

However, with increases in electric vehicle battery production and recycling in the early 2020s to meet the U.S. mandate to phase out fossil fueled activities from 2023 to 2050 (WH 2021a, 2021b), the population of workers with greatest risk of exposure may shift in future years.

In a Japanese nickel-hydrogen battery plant, workers were exposed to were exposed to a mixture of metallic cobalt, cobalt oxyhydroxide and nickel hydroxide dust (Yokota et al. 2007). Measured breathing zone air levels of cobalt over two consecutive working days ranged from 4 to 330 µg/m^3 (mean of 67 µg/m^3). On day 1, urinary cobalt levels increased from 10.7 µg pre-shift to 38.6 µg post-shift. Day 2 levels were more stable, at 25.7 µg pre-shift and 28.2 µg post-shift. Urinary levels were not well correlated with air levels; study authors attributed this to use of respirators by workers (Yokota et al. 2007).

COMMENT 3: Page 1, lines 8-9: largest use is NOW in rechargeable batteries (including in electrical vehicles), followed by … and hard metal tools.

RESPONSE: *Section 1.1 was revised exactly as suggested by the Reviewer.*

Ch. 2. Health effects.

COMMENT 4: Overall excellent and comprehensive coverage.

RESPONSE: *ATSDR thanks the Reviewer for this feedback.*

COMMENT 5: The existence of different chemical species of cobalt (chapter 4), could be summarized early on in chapter 2, with emphasis on the main issues (solubility) with impact on health effects.

RESPONSE: *Section 2.1 was revised as shown below.*

In order to appropriately compare doses following exposures to various cobalt compounds, inhalation exposures are expressed throughout the profile as mg Co/m^3 and oral and dermal exposures are expressed as mg Co/kg/day, when possible. This allows for evaluation of relative toxicities and determination of whether certain chemical properties (e.g., solubility) impact toxicity. When available, information pertaining to relative toxicity of different cobalt compounds is discussed throughout Chapter 2.

COMMENT 6: page 11, line 24: "substance x" to be replaced by "cobalt"

RESPONSE: *Section 2.1 was revised exactly as suggested by the Reviewer.*

COMMENT 7: Tables 2.1: clear and comprehensive, but the rationale for the sequence of studies in the tables is not always obvious to me (human studies first, but no chronological sequence?); consider showing explanations for the main abbreviations and symbols on each separate page (also for other tables and figures).

RESPONSE: *The study order is sorted first by duration (acute, intermediate, chronic), second by species (human, monkey, rat, mouse, rabbit, guinea pig, hamster, dog, gerbil, cat, pig, ferret, mink, sheep, cow), third by author (alphabetically), and fourth by publication date (oldest to most recent). Regarding explanation of the abbreviations and symbols in the LSE tables and figures, please see the User's Guide provided in Appendix D.*

COMMENT 8: figures 2.1: clear and comprehensive, but the vertical interrupted lines (- - -) should also be explained, the horizontal line for MRL should be longer (matching the length shown in the legend) (also applicable for next figures)

RESPONSE: *It is assumed that the Reviewer meant Figure 2-2 (the LSE figure). The symbols in the figure key (circles, triangles, horizontal line) are not to scale for any of the point markers in the LSE figure; they are intended to inform the reader what the markers are and are auto-generated by the program that makes the figures. The size of these markers change depending upon how data rich (crowded) an LSE figure becomes. Therefore, the size of the horizontal lines was not altered. Regarding the vertical interrupted lines, they represent adjustments (duration, human equivalency calculations) and uncertainty factors applied to the point of departure during the calculation of the MRL. This is explained in the User's Guide provided in Appendix D (explanation associated with #15 on the sample LSE figure).*

COMMENT 9: tables 2.2: page 62: effects not mentioned for 77 (Umar et al. 2016)

RESPONSE: *No change. In the study by Umar et al. (2016), no exposure-related changes in pain threshold or responses were observed in Wistar rats following oral exposure to 23 mg Co/kg/day as cobalt chloride for 28 days. Therefore, 23 mg Co/kg/day is a NOAEL and the "Effects" column is blank intentionally.*

COMMENT 10: tables 2.3: consider including the publication by Mandervelt et al (1997) (doi: 10.1016/s0300-483x(97)03629-9) showing that CoCl2 tested positive (SI 2.8 at 0.5%) in a mouse local lymph node assay.

RESPONSE: *Mandervelt et al. (1997) was added to Section 2.14 (Immunological); doses were converted into mg Co/kg/day consistent with other mouse local lymph node assays (LLNAs) (e.g., Ikarashi et al. 1992a, 1992b). A study entry for Mandervelt et al (1997) was also added to Table 2-3.*

Additional LLNA studies in mice have confirmed skin sensitization at ≥12 mg Co/kg/day as cobalt chloride (Ikarashi et al. 1992b; Mandervelt et al. 1997).

COMMENT 11: Page 88, line 4: delete "like"

RESPONSE: *Section 2.4 was revised exactly as suggested by the Reviewer.*

COMMENT 12: Table 2.4: clear and comprehensive; page 93, legend: $FEF_{75/50/25}$ is "forced expiratory flow" (not "end respiratory flow")

RESPONSE: *The footnote in Table 2.4 (in Section 2.4) was revised exactly as suggested by the Reviewer.*

COMMENT 13: Page 94, line 5: delete "in" after "exposure", consider adding "excess" before "decline"; line 13: add "maximal" before "expiratory"

RESPONSE: *Section 2.4 was revised exactly as suggested by the Reviewer.*

COMMENT 14: Page 95, line 16: add "CO" (or carbon monoxide) after "uptake"

RESPONSE: *The sentence in Section 2.4 was revised for clarity.*

However, decreased alveolar capillary fractional uptake of carbon monoxide and altered diffusing capacity (i.e., decreased steady-state carbon monoxide uptake) were observed along with increased incidence of self-reported cough and sputum in exposed workers, compared to unexposed workers.

COMMENT 15: Page 97, line 26: consider replacing "reactivity" by "reaction"

RESPONSE: *No change. Inflammatory "reactivity" is the appropriate term when discussing adverse respiratory responses to adverse stimulants and reactive airway disease.*

COMMENT 16: 2.5. Cardiovascular: maybe start by explaining why this issue received a lot of attention: "cobalt cardiomyopathy in beer drinkers"(page 102, 2nd paragraph); maybe conclude that most studies turned out to be negative.

RESPONSE: *As stated in Section 2.5, due to co-exposure to high levels of ethanol, conclusions regarding cobalt's potential contribution to so-called "beer-cobalt cardiomyopathy" cannot be made. Due to this, further discussion or addition of more studies on this topic is not considered necessary.*

COMMENT 17: 2.7 Hematological: consider mentioning the issue of the (potential) use of cobalt in "blood doping" by athletes (see, e.g., Lippi et al. 2006; doi: 10.1186/1745-6673-1-18).

RESPONSE: *The scope of toxicological profiles is to address concerns regarding environmental exposures to cobalt. Therefore, the addition of a discussion regarding the use, and associated risks, of cobalt by athletes in an attempt to enhance the blood oxygen carrying capacity is beyond the scope of the profile.*

COMMENT 18: Page 114, line 7: bromsulph**T**alein

RESPONSE: *The typographical error of "bromsulphalein" in Section 2.9 was corrected to "bromsulphthalein."*

COMMENT 19: Page 116, line 20: consider adding "transient" before "increase"

RESPONSE: *Section 2.10 was revised exactly as suggested by the Reviewer.*

COMMENT 20: Page 123, line 8: consider capitalizing c (**C**-reactive), also elsewhere

RESPONSE: *Section 2.14 was revised exactly as suggested by the Reviewer (C-reactive protein was not discussed in other sections of the profile).*

COMMENT 21: Page 126, line 28: cobalt is **a** skin sensitizer

RESPONSE: *Section 2.14 was revised exactly as suggested by the Reviewer.*

COMMENT 22: Page 129, line 8: delete "following" (or add "administration of cobalt")

RESPONSE: *Section 2.15 was revised exactly as suggested by the Reviewer ("following" was deleted).*

COMMENT 23: Page 129, lines 31-33: unclear why this is reported here in the context of 2.15.

RESPONSE: *The sentence "*Singh and Junnarkar (1991) examined the effects of intraperitoneal and intravenous injections on both Wistar rats and Swiss-Webster mice and observed that it increased urine volume at various doses of cobalt chloride and cobalt sulfate (Singh and Junnarkar 1991)." *was moved to Section 2.10 (renal).*

COMMENT 24: Page 130, lines 2-5: similarly, "altered neurotransmission" ex vivo may be an effect at organ level rather than a truly neurological effect

RESPONSE: *The following clarifying statement was added to Section 2.15:*

> Additionally, these findings may represent changes in the examined organ tissue (e.g., respiratory, gastrointestinal, reproductive tissues) rather than (or in addition to) changes in the neurological system.

COMMENT 25: Page 135, line 13: "creatine" or "creatinine"?

RESPONSE: *The typographical error "creatine" in Section 2.17 was corrected to "creatinine."*

COMMENT 26: Page 137, line 10: reasonabl**Y**

RESPONSE: *The typographical error "reasonable" in Section 2.19 was corrected to "reasonably."*

COMMENT 27: Page 137, line 23: delete "the" before "just"

RESPONSE: *Section 2.19 was revised exactly as suggested by the Reviewer.*

COMMENT 28: Page 141, line 12: correct "evaluating" to "evaluat**ed**"

RESPONSE: *The typographical error "evaluating" in Section 2.19 was corrected to "evaluated."*

COMMENT 29: Page 143, line 12: "chloroform" ???

RESPONSE: *The incorrect compound name was corrected in Section 2.20.*
In vitro and *in vivo* studies of the genotoxic effects of cobalt are summarized in Tables 2-10 and 2-11, respectively.

COMMENT 30: Page 153, line 30: correct "thorough" to "through"

RESPONSE: *Section 2.21 was revised exactly as suggested by the Reviewer.*

COMMENT 31: Page 155, line 1: add "with" after "interfere"; replace HIF-α by HIF-**1**α (also elsewhere in paragraph)

RESPONSE: *Section 2.21 was revised exactly as suggested by the Reviewer.*

Ch. 3. Toxicokinetics etc.

COMMENT 32: 3.1. Toxicokinetics: seems fine to me; the absence of data for Co in the brain is conspicuous: does the blood-brain barrier prevent cobalt ions to enter the brain?

RESPONSE: *Cobalt appears to be able to cross the blood-brain barrier, as some animal studies described in Section 3.1.2 reported detectable cobalt in the brain following dietary exposure (Bourg et al. 1985; Danzeisen et al. 2020a). However, cobalt is (at least initially) preferentially distributed to the liver and kidney. Neurological effects reported in oral studies in animals support that there is a mechanism for cobalt to cross the blood-brain barrier.*

COMMENT 33: The following recent publication on transfer of cobalt across the placenta (human data) could be included: Kayembe-Kitenge et al. 2023 doi: 10.1016/j.jtemb.2023.127294. (admittedly published after end of search date)

RESPONSE: *The following information was added to Section 3.12:*

In a mother-infant study from the African Copperbelt mining region, in which populations have high environmental exposure to cobalt, a high degree of placental transfer of cobalt from mother to infant was observed, with higher concentrations in umbilical cord blood, compared to maternal cord blood (Kayembe-Kitenge et al. 2023). The levels in paired maternal-cord blood samples were highly correlated, as were maternal-placental and placental-cord samples.

COMMENT 34: Page 165, line 22: 3,000 mg/L should be checked because this seems extremely high

RESPONSE: *The units for Paustenbach et al. (2013) were corrected from mg/L to* µg/L *in Section 3.1.2.*

At serum cobalt concentrations up to 3,000 µg/L, it is estimated that 8.3–8.5% exists as free cobalt ions; the rest is bound to serum proteins, primarily albumin (Paustenbach et al. 2013).

COMMENT 35: Page 167, line 2: "rate of uptake" or "ratio of concentrations"?

RESPONSE: *Section 3.1.2 was revised for clarity.*

Section 3.1.2: Following oral administration of cobalt in volunteers for 31 days, Finley et al. (2013) reported that the rate of uptake for serum cobalt levels was 1.3 µg/L for every 1.0 µg/L of cobalt in whole blood.

COMMENT 36: Page 175, line 3: for consistency, consider expressing amounts as mg Co (rather than nmol)

RESPONSE: *The sentence in Section 3.1.4. was revised to include both the reported units (nmol) as well as converted units (µg Co).*

Section 3.1.4: Urinary excretion of cobalt increased from a pre-exposure level of 18.1 nmol (1.07 µg) to 38.5 nmol (2.27 µg) 24 hours after exposure in five subjects who were dermally exposed for 1 hour by keeping their hands in a solution containing 1,600 mg Co/L.

COMMENT 37: Page 178-9, table 3.8: please, add units

RESPONSE: *No change. Units were provided in the row headers. The units for the rate columns are* day^{-1}. *Fractions are unitless.*

COMMENT 38: Page 182, line 7: please add "after inhalation" before "varies";

RESPONSE: *Section 3.1.6 was revised exactly as suggested by the Reviewer.*

COMMENT 39: Page 182, line 15-16: unclear sentence

RESPONSE: *Section 3.1.6 was revised for clarity.*

While PBPK models are available (Section 3.1.5), they are either restricted to kinetic modeling in humans (ICRP models) or model assumptions for cobalt are based on human data (Unice et al. 2020a).

COMMENT 40: Page 183, figure 3.6: consider enlarging figure (landscape) to improve legibility

RESPONSE: *No change. Figure 3.6 is reproduced in its original format from Unice et al. (2020b), with permission from Elsevier. Due to figure size (with explanatory footnotes), it cannot be enlarged further in either profile or landscaped orientations.*

COMMENT 41: 3.2. Children: consider citing Banza Lubaba Nkulu et al, 2018 (doi: 10.1038/s41893-018-0139-4), showing that children have higher internal exposure to cobalt than adults, admittedly in a highly polluted environment; see also figure 4 showing a relationship, among children, between creatinine-corrected urinary 8-OHdG and urinary Co (even though this correlation does not mean that cobalt is causing the oxidative DNA damage)

RESPONSE: *Section 3.2 is intended to report populations that are unusually susceptible to toxic effects of exposure to cobalt, not populations with increased risk of exposure. Data from Banza Lubaba Nkulu (2018) were added to Section 2.20 (Genotoxicity) and Section 5.7 (Populations with Potentially High Exposure).*

Section 2.20: Increased levels of a urinary biomarker for oxidative damage (8-hydroxydeoxyguanosine) was also associated with increased urinary cobalt levels in children residing in mining regions of the Democratic Republic of the Congo (D.R. Congo) (Banza Lubaba Nkulu 2018). Similar associations were not observed in adults; however, median urinary cobalt levels in children were nearly 3 times the levels observed in adults. Increased exposure in children may be due to hand-to-mouth activities recognized to occur with greater frequency in children compared to adults; Banza Lubaba Nkulu (2018) did not address this confounding factor.

Section 5.7: Data from communities near mining operations in the D.R. Congo confirm increased risk of exposure in children. Median measured urinary cobalt levels in children were nearly 3 times higher than values measured in adults from the same community; approximately 150 and 50 µg/g creatinine, respectively, based on graphically presented data (Banza Lubaba Nkulu 2018). This is consistent with children ingesting up to 1,800 mg/day of soil from typical mouthing behavior as they play and lie on grass and soil (ATSDR 2001).

COMMENT 42: Page 189, line 23: delete “in” at end of line

RESPONSE: *Section 3.3.1 was revised exactly as suggested by the Reviewer.*

Ch. 4. Chemistry …

COMMENT 43: Useful chapter, which could have come upfront in the document.

RESPONSE: *ATSDR is not considering re-ordering chapter presentation in Toxicological profiles at this time.*

COMMENT 44: Table 4.1. What about the chemistry of "cobalt hydrocarbonyl" used in some (early) studies?

RESPONSE: *Cobalt hydrocarbonyl was not included in the list of selected cobalt compounds in Table 4-1 because it is not expected to be a primary source of human exposure to cobalt compounds due to instability when exposed to oxygen. A series of studies reported by Palmes et al. (1959) is included in the profile that evaluated potential effects of exposure to cobalt hydrocarbonyl; however, it is noted that exposure is to cobalt hydrocarbonyl plus oxide/carbonate decomposition products due to compound instability.*

Ch. 5. Potential for human exposure

COMMENT 45: Useful chapter, with focus on exposures, mainly in USA. I did not read/check this chapter (especially section 5.4) as thoroughly as the previous chapters. The information on occupational exposures is largely based on old literature. However, the growth in rechargeable lithium-cobalt batteries for electric vehicles will undoubtedly increase opportunities for exposure to cobalt in (new) mining & refining of cobalt, and in battery manufacture, vehicle industry and maintenance, disposal/recycling of batteries; this could be highlighted.

RESPONSE: *As per the Draft Guidance for the Preparation of Toxicological Profiles, Chapter 5 is focused on environmental exposure data in the United States, especially pertaining to areas of potential high exposures. For occupational exposure data, primary data is also focused on U.S. exposures. However, if occupational data are lacking from the United States, data from similar industries in other countries may be reported for reference.*

In response to the Reviewer's comment, a few recent references for hard metal occupational exposure were added, and some outdated non-U.S., non-quantitative data were deleted. One study with exposure information from a battery factory was located. Additionally, existing data in Section 5.7 were reorganized for better data presentation.

The majority of occupational exposure data is from the hard metal industry. Historical concentrations of cobalt in the air of hard metal manufacturing, welding, and grinding factories may range from 1 to 300 µg/m^3, compared to normal atmospheric levels of 0.4–2.0 ng/m^3 (Haddad and Zikovsky 1985; Koponen et al. 1982; Lichtenstein et al. 1975; Marsh et al. 2017a, 2017b; NIOSH 1989; Sauni et al. 2017; Svartengren et al. 2017). Data collected in hard metal factories since 2000 show a decrease in the uppermost reported concentrations, ranging from 0.9 to 190 µg/m^3 (Al-Abcha et al. 2021; Andersson et al. 2020, 2021; Hamzah et al. 2014; Hedbrant et al. 2022; Lantin et al. 2011, 2013). The maximum Occupational Safety and Health Administration (OSHA) permissible level is 100 µg/m^3.

In a Japanese nickel-hydrogen battery plant, workers were exposed to a mixture of metallic cobalt, cobalt oxyhydroxide, and nickel hydroxide dust (Yokota et al. 2007). Measured breathing zone air levels of cobalt over 2 consecutive working days ranged from 4 to 0.330 µg/m^3 (mean of 67 µg/m^3). On day 1, urinary cobalt levels increased from 10.7 µg pre-shift to 38.6 µg post-shift. Day 2 levels were more stable, at 25.7 µg pre-shift and 28.2 µg post-shift. Urinary levels were not well correlated with air levels; the study authors attributed this to use of respirators by workers (Yokota et al. 2007).

COMMENT 46: Page 203, line 26: "Cobalt is the third most abundant element in the earth's crust, …" is wrong! (correct on Page 229, line 23)

RESPONSE: *Section 5.2.1 was corrected as shown:*

Cobalt is the 33rd most abundant element in the earth's crust, averaging approximately 17.3 ppm (Dehaine et al. 2021).

COMMENT 47: Page 219, "Other media": consider using also findings published by Cheyns et al. 2014 (and references therein)

RESPONSE: *Section 5.5 is predominately focused on U.S.-relevant exposures. However, for comparison, exposure levels in food from Cheyns et al. (2014) from DR Congo mining communities were added to Section 5.5.4 as suggested by the Reviewer.*

Cobalt levels in food from the Copperbelt mining region of D.R. Conga are much higher than non-mining regions (Cheyns et al. 2014). Food items with the highest concentrations in the mining regions included leafy vegetables (46 µg/g dry weight), beans (22 µg/g dry weight), cassava leaves (12 µg/g dry weight), fruit vegetable (12 µg/g dry weight), and sweet potato leaves (6.5 µg/g dry weight). Concentrations for the same food items from non-mining regions were 1.2, 0.84, 1.5, 0.58, and 1.1 µg/g dry weight, respectively.

COMMENT 48: Page 230: very high values of cobalt have been demonstrated in soils/sediments in DR Congo's Copperbelt mining area (several publications available)

RESPONSE: *Information regarding high soil values in D.R. Congo's mining areas was added to Section 5.5.3.*

Cobalt levels in soils near mining sites in the D.R. Congo are much higher with concentrations of 116–311 mg/kg (Cheyns et al. 2014; Michée et al. 2023). Reported mean soil concentrations in regions distant from mining operations in the D.R. Congo were 20 mg/kg (Cheyns et al. 2014).

COMMENT 49: Page 237, line 33: "recent" Spanish study in a 1998 publication?

RESPONSE: *Section 5.6 was revised to remove the term "recent."*

However, according to Camean et al. (1998), the low levels of cobalt presently found in beer do not make a significant contribution to the total cobalt intake in heavy beer drinkers.

COMMENT 50: Page 238, lines 20-22: correct "creatine-" to "creatinine-" (twice);

RESPONSE: *Section 5.6 was revised exactly as suggested by the Reviewer.*

COMMENT 51: Page 248: insert space after "in"

RESPONSE: *Section 5.6 was revised exactly as suggested by the Reviewer.*

COMMENT 52: Page 252, line 1: $69.9x\mathbf{1}0^{-6}$

RESPONSE: *Section 5.7 was revised exactly as suggested by the Reviewer.*

COMMENT 53: Page 254, line 8: metal-to-metal (with hyphens); add "as" at end of line

RESPONSE: *Section 5.7 was revised exactly as suggested by the Reviewer.*

Ch. 6. Adequacy of database

COMMENT 54: I generally agree with the content and conclusions of this chapter.

RESPONSE*: ATSDR thanks the Reviewer for this feedback.*

COMMENT 55: Figure6.1; Suggest adding number of studies (n=…) next to each pie-diagram

RESPONSE: *ATSDR will consider this suggested change in future revisions of the Draft Guidance for the Preparation of Toxicological Profiles.*

COMMENT 56: Page 262, lines 17-19: consider the need for being alert for the physical and chemical properties of novel cobalt-containing applications

RESPONSE: *No change. While novel cobalt-containing products may have novel physical/chemical properties, that is beyond the scope of ATSDR profiles. Chapter 4 is focused on the physical and chemical properties of cobalt compounds only.*

Ch. 7 Regulations and guidelines

COMMENT 57: OK

RESPONSE: *No response required.*

Ch. 8 References

COMMENT 58: Not checked in detail, but looks carefully collated

RESPONSE: *ATSDR thanks the Reviewer for this feedback.*

COMMENT 59: Page 281, line 36: "Ventilatory" (one l)

RESPONSE: *The title for Gennart and Lauwerys (1999) was corrected in Chapter 8.*

Gennart JP, Lauwerys R. 1990. Ventilatory function of workers exposed to cobalt and diamond containing dust. Int Arch Occup Environ Health 62:333-336. https://doi.org/10.1007/BF00640843.

COMMENT 60: Page 293, line 20: "Bergamo"

RESPONSE: *This typographical error was in the title of the Mosconi et al. (1994b) reference. This reference is no longer cited in the profile; outdated non-U.S. occupational exposure data were removed from Section 5.7 (see response to Comment 45).*

COMMENT 61: Page 301, line 42: "superficial"

RESPONSE: *The suggested revision was not made. The term "surficial" is correct for the study by Szefer et al. (1996).*

Appendix A: MRL worksheets

COMMENT 62: Inhalation, acute: I agree with the reasoning and conclusion ***(set out in Memorandum of March 27, 2024)*** regarding an acute-duration inhalation MRL of 0.0003 mg Co/m^3. [I note that the rounding to lower values for $NOAEL_{Adj}$ (0.03 from 0.0333) and $NOAEL_{HEC}$ (0.01 from 0.0125) leads to a lower value (0.0003) than would have been obtained without rounding (0.00046), but this is acceptable to me].

RESPONSE: *No changes requested.*

COMMENT 63: Inhalation, intermediate: I agree with the reasoning and conclusion ***(set out in Memorandum of March 27, 2024)*** that no intermediate-duration inhalation MRL is proposed for cobalt.

RESPONSE: *No changes requested.*

COMMENT 64: Inhalation, chronic: I agree with the MRL of 0.0001 mg Co/m^3 (please, note, however, my potential CoI, as the lead author of the critical effect study)

RESPONSE: *No changes requested.*

COMMENT 65: Page A-14, line 40: correct "oar" to "ore"

RESPONSE: *The MRL section was revised exactly as suggested by the Reviewer.*

COMMENT 66: Oral, acute: I agree with the MRL of 0.03 mg Co/kg/day, even though this value is based on old, limited data

RESPONSE: *No changes requested.*

COMMENT 67: Oral, intermediate: I agree with the reasoning and conclusion ***(set out in Memorandum of March 27, 2024)*** regarding a provisional intermediate-duration oral MRL of 0.02 mg Co/kg/day.

RESPONSE: *No changes requested.*

COMMENT 68: Page A-30, line 9: correct “personnel” to “personal”

RESPONSE: *The MRL section was revised exactly as suggested by the Reviewer.*

COMMENT 69: Oral, chronic: I agree that no chronic-duration oral MRL can be derived

RESPONSE: *No changes requested.*

Appendix B.

COMMENT 70: Not checked in detail, seems OK.

RESPONSE: *No changes requested.*

Appendix C

COMMENT 71: I generally agree with the confidence ratings for the various publications (tables not all read in detail)

RESPONSE: *No changes requested.*

Appendix D, E, F and G

COMMENT 72: not evaluated.

RESPONSE: *No changes requested.*

Comments provided by Reviewer #3

ATSDR Charge Questions and Responses and Reviewer Comments

Acute-duration inhalation MRL

QUESTION: Do you agree that the study selected was the appropriate one.

COMMENT 1: Generally yes, however a more balanced weighing of the studies listed in Table A-1 would increase the overall confidence in the study and POD selection. The selection of the key study only on the basis of having the lowest NOAEC might be re-considered in the light of a quality assessment of the available data.

RESPONSE: *ATSDR does consider study quality assessment during selection of the critical effect and principal study. The criteria is not the lowest NOAEL, rather the lowest LOAEL. At first glance, in Table A-1, that would be the human study by Kusaka et al. (1986a). However, due to study limitations discussed in the Selection of the Principal Study section of the acute-duration inhalation MRL section of Appendix A, Kusaka et al. (1986a) was considered an inadequate study. The next two lowest LOAELs are both for BALF changes in rats in studies with moderate confidence (as per systematic review); a LOAEL of 2.2 mg Co/kg/day as cobalt sulfate by Viegas et al. (2022a) and 11.29 mg Co/kg/day as cobalt tetraoxide by Burzlaff et al. (2022a). The difference in LOAEL values is explainable based on cobalt bioaccessibility of the compounds. Appendix A was revised for clarity on this issue.*

Of these, the most sensitive is the study exposing rats to cobalt sulfate heptahydrate, which reported elevated neutrophils in BALF at ≥2.2 mg Co/m^3 (Viegas et al. 2022a, 2022b). The study by Burzlaff et al. (2022a) also reported BALF alterations following exposure to cobalt tetraoxide at a higher concentration. The differences in adverse effect level between Viegas et al. (2022a) and Burzlaff et al. (2022a) is attributable to differences in bioaccessibility of cobalt in the administered compound. A series of studies by this group of researchers suggests two groupings of compounds based on high acute toxicity (cobalt metal power, cobalt dihydroxide, cobalt monoxide, and cobalt sulfate heptahydrate) and low acute toxicity (cobalt tetraoxide, cobalt sulfide) (Danzeisen et al. 2022a, 2022b; Derr et al. 2022; van den Brule et al. 2022; Verougstraete et al. 2022; Viegas et al. 2022a). Toxicity findings in these studies are correlated with bioaccessibility of cobalt in the various compounds.

COMMENT 2: It may be debated on whether the sole increase in %PMN should be considered as adverse and as key event for the MRL derivation. The work by Weber et al. 2023[1] considered only the activation of granulocytes adverse, in case this occurs in combination with destroyed macrophages (visible as amorphous material intermingled with non-structured eosinophilic fine coarse to clumpy material that likely represents cell remnants). Otherwise, such effect is considered a non-adverse adaptive response.

RESPONSE: *The Reviewer is referring to statements in the introduction of Weber et al (2023), which include "*In this situation, neutrophilic granulocytes (polymorphonuclear neutrophils, PMN) often enter the alveolar space. The combination of destroyed macrophages and the activation of granulocytes might be considered an adverse effect. However, PMN are also part of the initial immune response and might

[1] Weber, K. et al. (2023) Regenerative and progressing lesions in lungs and lung-associated lymph nodes from fourteen 90-day inhalation studies with chemically different particulate materials. Toxicol. Lett. DOI:10.1016/j.toxlet.2023.12.011

support the clearance process. In many situations, an initial inflammation provides beneficial aspects, i.e., repair without further sequelae such as fibrosis (Weber et al., 2018)*." However, the introduction goes on to say: "*The distinction between an initial beneficial inflammation and a progressive inflammatory/adverse reaction needs to be made carefully*." The study conducted by Weber et al. (2023) goes on to conclude that "*The BALF analysis data used as an early indicator of inflammatory processes in the alveoli, correlated well with the histopathologic changes seen in the alveolar area." *Additional findings supported that the inflammatory process resulted in destruction of macrophages, which impaired clearance processes. Thus, in their overall conclusions, Weber et al. (2023) stated that adverse effects were "*confirmed by the increase in polymorphonuclear neutrophils in BALF*." The only response that Weber et al. (2023) concluded was non-adverse in their discussion was uptake of particles by macrophages (e.g., particle-laden macrophages). Therefore, the study by Weber et al. (2013) supports the classification of elevated neutrophils in BALF reported by Viegas et al. (2022a) as a less serious LOAEL. Weber et al. (2023) was added to Appendix A in support of the selection of the principal study for the acute-duration inhalation MRL.*

Regarding the LOAEL endpoint of elevated neutrophils in BALF identified in the study by Viegas et al. (2022a, 2022b), neutrophil-mediated inflammation is considered a key event in particle-induced lung inflammation and toxicity (Lam et al. 2023). In support, Viegas et al. (2022a, 2022b) reported that inflammatory changes at the LOAEL (2.2 mg Co/m^3) progressed to histopathological changes at the next tested concentration (6.7 mg Co/m^3). Furthermore, data from several other inhalation studies with particulates support that increased neutrophils in BALF correlated well with histological changes in the alveoli in 90-day studies in rats (Weber et al. 2023). Weber et al. (2023) proposed that progressive inflammatory changes were associated with macrophage destruction, which decreased particle clearance from the lungs.

QUESTION: Do you agree with the health endpoint selected, the point of departure (NOAEL), the exposure time adjustments in calculating the $NOAEL_{ADJ}$, and the human equivalency adjustments in calculating the $NOAEL_{HEC}$ for the MRL? If you disagree with any of these, please specify what they should be.

COMMENT 3: <u>health endpoint selected</u>
The selection of lung function impairment/inflammatory response as leading health effect is supported for the derivation of the acute-duration MRL.

RESPONSE: *No response required.*

COMMENT 4: <u>point of departure</u>
It is recognized that the BMD calculation with all six %PMN values of the 24 hrs timepoints did not lead to an adequate fit. Generally, the %PMN values for Viegas et al. (2022b) show (i) a high variability between timepoints (control values for the different timepoints range form 0,7% to 4.6%) (ii) for the 24 hrs timepoint no dose-dependency especially in the low-concentration range. Especially the last point might have led to the rejection during the BMD modelling.

It might therefore be considered to re-run the BMD to assess whether a suitable model output could be achieved (i) using the values at a different timepoint, such as 8 or 16 hrs and/or (ii) excluding the 0.02 and 0.07mg Co/m³ concentrations at the 24 hrs timepoint to achieve an acceptable model output.

Due to the high variability of the %PMN values and the lack of a concentration-response relationship, a BMDL10 is preferred over a NOAEC even if the number of data points is reduced.

RESPONSE: *Regarding modeling different timepoints, the 24-hour timepoint was selected as it is the only timepoint that shows dose-response with a LOAEL 2.2 mg Co/m^3. The 8-hour timepoint was not selected for modeling because the LOAEL for that timepoint is 6.7 mg Co/m^3. The 16-hours timepoint was not selected for modeling because statistically significant effects are observed at 2.2 mg Co/m^3 but not 6.7 mg Co/m^3 (lacking dose-response).*

Regarding dropping datapoints from the modelling, ATSDR does not drop low concentration data in attempts to gain model fit for BMD modeling, as what occurs in the low-concentration range is critical to inform the shape of the concentration-response curve. Sometimes it is appropriate to drop high-concentration data (if exposure-related effects remain at lower concentrations) since ATSDR is most interested in what is occurring at the low end of the curve.

COMMENT 5: calculating the NOAEL(ADJ)
The calculation of the NOAEL(ADJ) is generally supported, however the rounding of the value 0.03 mg/m³ vs. 1/30 mg/m³ introduces an error of approx. 33%. Instead, the formula for the NOAEC(ADJ) might directly be included in the NOAEC(HEC) formula and thereby eliminating the rounding error

RESPONSE: *No change. For dosimetric calculations (e.g., conversion of compounds into mg metal, adjusted values, HEC calculations), ATSDR considers each calculated dose is considered an independent calculation. Thus, each converted dose/concentration value should be expressed in the same number of significant figures as the original dose/concentration. This will ensure that converted doses/concentrations are not more precise than the reported values provided by study authors. For the principal study, the reported concentration at the NOAEL in terms of cobalt sulfate heptahydrate was 1 mg/m^3. Since there is only one significant figure provided in the original dose, the converted dose, in terms of cobalt content, is 0.2 mg Co/m^3 and the duration adjusted NOAEL is 0.03 mg Co/m^3. ATSDR is in the process of updating the Draft Guidance for the Preparation of Toxicological Profiles to provide clarity on the issue of maintaining the correct number of significant figures in dosimetric adjustments.*

COMMENT 6: MPPD modelling and human equivalency adjustments

The use of the NOAEC(HEC) for the MRL derivation is supported. However, some of the input parameters require adjustment/verification as detailed below.

The relative density of 1.95 as reported in Viegas et al. (2022b) was used for the MPPD deposition modelling. However, taking into account the handbook data on the relative density of solid cobalt sulfate (see table below), the Vigas et al. (2022b) value appears to originate from the aqueous solution used in the in vivo inhalation experiment. Since the atmosphere was generated using a collision nebulizer with subsequent drying of the aqueous solution, one may safely assume that the animals were exposed towards solid cobalt sulfate hexahydrate (as also confirmed in the ATSDR's Toxicological Profile – Draft, page A-5, lines 8-11). It therefore appears prudent to use the density of the solid sobalt sulfate for the MPPD deposition modelling, as follows:

relative density	source
3.71	O'Neil, M.J. et al. (Eds.) (2006): The Merck Index: An encyclopedia of chemicals, drugs, and biologicals, 14th Ed. Merck & Co., Inc, Whitehouse Station, NJ, USA, 411.
3.71	Lide, D.R. (ed.) (2008) CRC Handbook of chemistry and physics. 88th Edition, CRC press, New York, 4.1-4.163.

RESPONSE: *No change. It is not appropriate to use the density of the anhydrous salt for MPPD deposition modeling. Density of the solid compound decreases as hydration increases. For example, the density for cobalt sulfate heptahydrate as sold by Sigma Aldrich (in powder form) is 2.03. Density as reported by the study authors was retained in MPPD calculations.*

COMMENT 7: MPPD modelling and human equivalency adjustments

The MPPD modelling for the human deposition pattern is unclear. On the one hand the footnotes b, c and d suggest that three different deposition calculations were performed:

1. Human at sleep
2. Human at rest
3. Human at light activity,

using different input parameters for (i) breathing frequency, (ii) tidal volume and (iii) breathing scenario. However, this is not clear from the table A-3 itself, as only one value for the alveolar region deposition fraction is reported. Is this the mean of the three different deposition calculations?

It is recommended, for transparency reasons, to report the individual results for the three deposition calculations and use the mean of these three calculations for the NOAEL(HEC) calculation.

RESPONSE: *Three deposition calculations were not calculated. Time-weighted-averages (TWAs) for breathing frequency and tidal volume were calculated based on different activity levels. The TWA value reported in Table A-3 was the input used in the MPPD model to calculate a single deposition fraction representative of deposition over a 24-hour period. The following statement was added to Appendix A for clarification just before Table A-3:*

> The TWA values were then used in the calculation of the deposition fraction (to represent TWA deposition over a 24-hour period).

COMMENT 8: MPPD modelling and human equivalency adjustments

The input parameters for the MPPD modelling should be harmonized. In table A-3 reports a breathing frequency of 14.7 and a tidal volume of 875mL for humans, which equates to a ventilation rate of 18.5 m^3/day. However, this value is not used in table A-4, instead the value as given in EPA(1994) is used. This discrepancy should be adjusted by the 18.5 m^3/day also for the NOAEC(HEC) calculation – subject to verification of the values, see next comment.

RESPONSE: *No change. The default U.S. EPA (1994) human and animal ventilation rates used by ATSDR in dosimetry calculations are allometric values that also account for body weight (they are not just a simple breathing frequency × tidal volume calculation).*

COMMENT 9: MPPD modelling and human equivalency adjustments

Some values presented in Table A-3 and A-4 and used in the BMD/NOAEC(HEC) calculations deviate from values given in EPA 2019[2]:

[2] U.S. EPA. Integrated Science Assessment (ISA) for Particulate Matter (Final Report, Dec 2019). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-19/188, 2019.

ATSDR's Toxicological Profile – Draft, Table A-4	EPA(2019), Table 4-1
SA human= 54m²	SA human= 140 m² male, 100 m² female
SA rat= 0.34m²	SA rat= 0.4m²
ATSDR's Toxicological Profile – Draft, Table A-3	EPA(2019), Table 4-2 (males)
footnote c: 625mL at sleep 750 mL at rest 1250mL at light activity	footnote c: mL at sleep not provided 625 mL at awake rest 1000mL at light exertion

It is proposed that the values given in Tables A-3 and A-4 are checked for validity, given that the newer publication EPA (2019) reports different values.

RESPONSE: *Regarding surface area values, the "typical" values reported in Table 4-1 of EPA (2019) did not replace the EPA (1994) guidelines. As evidence for this, EPA (2020) used default surface area values (along with other parameters) from the EPA (1994) guidelines to calculate pulmonary HEC values (HEC_{PU}) for Table 3 in its Provisional Peer-Reviewed Toxicity Value (PPRTV) document for trans-1,2-dichloroethylene. Until EPA updates its guidance to recommend revised default parameters for dosimetry calculations, ATSDR will continue to use the surface area values reported by EPA (1994).*

Regarding tidal volumes, ATSDR has elected to retain values from ICRP (1994) because it provides a singular source with values for all three activity levels required to calculate TWA values. To ensure this approach would not impact the overall HEC calculation, ATSDR ran the MPPD model using the sleeping value from ICRP (1994) plus awake rest and light exertion values from EPA (2019), which results in a TWA value of 750 m: (625 mL × 16 hours) + (1,000 mL × 8 hours)]/24 hours. While this 14% decrease in tidal volume results in a 7% decrease in the predicted total pulmonary deposition in humans, this parameter change did not alter the overall HEC value of 0.01 mg Co/m^3.

COMMENT 10: MPPD modelling and human equivalency adjustments

The NOAEC(HEC)=0.0125 mg Co/m³ is rounded to 0.01 mg Co/m³. This rounding introduces an error of 20%, which is neither justified nor required at this stage. It is therefore recommended to maintain the mathematically correct value of 0.0125 mg Co/m³.

RESPONSE: *As discussed in response to Comment 5, the $NOAEL_{HEC}$ was rounded to retain the correct number of significant figures based upon the original concentration of 1 mg/m^3 (in terms of cobalt sulfate heptahydrate). It is not appropriate for a dosimetrically adjusted value to have more significant figures than the originally reported value.*

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 11: The uncertainty factor of 3 for the extrapolation from animals to humans should be set to 1, as any uncertainty is intrinsically covered by the HEC calculation, which directly compares the deposited doses between rats and humans.

RESPONSE: *The uncertainty factor of 3 for the extrapolation from animals to humans is accounting for the toxicodynamic component of the uncertainty factor ($10^{0.5}$), as dosimetric calculations only account for the toxicokinetic component of the uncertainty factor (reduced the toxicokinetic component to 1).*

QUESTION: Do you agree that the approach used for deriving the MRL was appropriate.

COMMENT 12: In case this question is aimed at whether the implementation of an acute MRL was at all justified, this is agreed.

RESPONSE: *No response needed.*

QUESTION: Do you consider that rounding the $NOAEL_{HEC}$ at an intermediate step is or is not mathematically appropriate?

COMMENT 13: The derivation of the MRL uses the rounded NOAEL(HEC), which is an inappropriate inaccuracy and significantly increases the conservatism, since the rounded NOAEL(HEC)=0,01mg/m³ is 20% lower than the non-rounded value of NOAEL(HEC)=0,0125mg/m³.

RESPONSE: *ATSDR has determined dosimetric calculations (e.g., conversion of compounds into mg metal, adjusted values, HEC calculations) should be considered an independent calculation, rather than intermediate steps during the MRL calculation. Thus, each converted dose/concentration value should be expressed in the same number of significant figures as the original dose/concentration when the number of significant figures are known. This decision was made because it is mathematically inappropriate to have converted doses/concentrations that are more precise than the original doses/concentrations reported by study authors. ATSDR is in the process of updating the Draft Guidance for the Preparation of Toxicological Profiles to provide clarity on this issue. As discussed in response to Comments 5 and 10, the original concentration was reported as only one significant figure by Viegas et al. (2022a); therefore, results of all dosimetric conversion calculations are also reported as one significant figure.*

QUESTION: Do you agree with the final MRL value? If not, please provide your rationale and recommendation for study selection and for either deriving or not deriving this MRL. If you agree with this MRL, then the acute- and chronic-duration MRLs will respectively be 0.0003 and 0.0001 mg Co/m³.

COMMENT 14: The final MRL is not agreed, rationale provided above.

RESPONSE: *No changes were made to the acute-duration inhalation MRL. Please see responses to Comments 1 through 13 for rationale.*

COMMENT 15: Rounding of values should be avoided, since this introduces an unnecessary additional conservatism in the MRL derivation.

RESPONSE: *Please see previous responses to Comments 5 and 9 regarding maintenance of correct significant figures in dosimetric conversions.*

Intermediate-duration oral MRL

QUESTION: Do you agree that the study selected was the appropriate one.

COMMENT 16: The study Danzeisen et al. 2020a is a GLP, guideline-compliant sub-chronic oral study in rats, which is reliable without restriction. The further studies listed in table A-16 either lack quality or relevance for the MRL assessment. Agree with the study selection.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the health endpoint, the benchmark dose model selected, and the point of departure ($BMDL_{1SD}$) for the MRL? If you disagree with any of these, please specify what they should be.

COMMENT 17: Agree with the BMD calculation. The selection of the linear model suggests a non-threshold mechanism for the haematological effect, depending on the curve fit one may consider selecting the polynomial or power model which would be more appropriate for the observed thresholded effect.

RESPONSE: *As noted in the footnote in Table A-18, both the 2-degree and power models converged on the linear model (provided the exact estimates and curve fits). Therefore, ATSDR did not make any changes in model selection for the red blood cell count in male rats (Danzeisen et al. 2020a).*

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 18: The uncertainty factor for the inter-species variability (extrapolation from animals to humans) consists of (i) a factor 4 correction for differences in metabolic rate for rats to humans and (ii) a factor of 2.5 for remaining differences. The metabolism of cobalt as an inorganic substance can be excluded. There are no reasons to assume that this behaviour which is based on the physico-chemical properties of the substance will be different between rats and humans. Therefore, it may be considered justified to apply a substance-specific assessment factors accounting for a correction for differences in metabolic rate of 1 instead of using the respective factor of 4.
The overall uncertainty factor should therefore be 25.

RESPONSE: *The suggestion that the toxicokinetic component of the uncertainty factor can be excluded based on the lack of metabolism of metals ignores other key elements of toxicokinetics that can differ between animals and humans, most notably absorption. As discussed in Section 3.1.6, available data indicate that interspecies differences have been observed for absorption of soluble cobalt compounds (Ayala-Fierro et al. 1999; Bailey et al. 1989; Barnaby et al. 1968; Hollins and McCullough 1971; Kirchgessner et al. 1994; Naylor and Harrison 1995; Schade et al. 1970; Taylor 1962; Van Bruwaene et al. 1984). As noted in Appendix A, available toxicokinetic data and PBPK models are inadequate for cobalt-specific dosimetric extrapolations for the oral route. Therefore, the default uncertainty factor for 10 is retained for extrapolation for animals to humans.*

QUESTION: Do you agree that the approach used for deriving the MRL was appropriate.

COMMENT 19: In case this question is aimed at whether the implementation of an intermediate MRL was at all justified, this is agreed.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the final MRL value? If not, please provide your rationale and recommendation for study selection and for either deriving or not deriving this MRL.

COMMENT 20: The final MRL is not agreed, rationale provided above.

RESPONSE: *The Reviewer did not agree on the final rational based on: (1) the potential for selection of a BMD model providing a non-threshold response, and (2) use of a full uncertainty factor of 10 for animal to human extrapolation. Based on responses provided to the comments on these issues above, ATSDR has not changed the model selection for the BMD modeling or the uncertainty factor for animal to human extrapolation. Therefore, ATSDR has not changed the intermediate-duration oral MRL value.*