Disposition of Peer Review Comments for Toxicological Profile for Mercury

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 comment draft of the Toxicological Profile for Mercury were:

Harvey Clewell, Ph.D.
Principal Consultant
Ramboll US Consulting, Inc.
3107 Armand Street
Monroe, LA 71201

William Pan, Ph.D.
The Elizabeth Brooks Reid and Whitelaw
Reid Associate Professor of Population Studies
310 Trent Drive, Room 227, Box 90519
Durham, NC 27708

Kristine Vejrup, Ph.D.
Associate Professor
2 Norwegian Institute of Public Health
Postbox 4404, Nydalen, NO 0403 Oslo, Norway
Division of Occupational and Environmental Medicine
Lund University, SE, 221 85 Lund, Sweden

Comments provided by Reviewer #1

GENERAL COMMENTS

COMMENT 1: Attached manuscripts
Section 2.15 page 273: Chatzi x2
Section 2.16.1 s. 295: Strain
Section 2.16.1 s. 330: Skogheim
Section 2.18 page 411: Ramon
Section 2.18 page 414: Papdopoulou
Section 2.20 page 431:
Lu
Gundacker
Cardenas x2
Weyde

RESPONSE: *The studies listed by the Reviewer in the comment above were reviewed and added to the profile as appropriate. Specific information on the addition of these studies is also noted in Responses to Comments shown below. Note that the correct author of the Chatzi publications is Stratakis.*
Section 2.15 page 273: Chatzi x2 [Stratakis] (Comment 12)
Section 2.16.1 s. 295: Strain (Comment 32)
Section 2.16.1 s. 330: Skogheim (Comment 33)
Section 2.18 page 411: Ramon (Comment 34)
Section 2.18 page 414: Papdopoulou (Comments 16, 29, 30, and 35)
Section 2.20 page 431:
Lu (Comment 17)
Gundacker (Comment 20)
Cardenas x2 (Comments 18 and 19)
Weyde (Comment 20)

COMMENT 2: Publications on Gut microbiota and mercury exposure that might be relevant:

The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study. Rothenberg SE, Keiser S, Ajami NJ, Wong MC, Gesell J, Petrosino JF, Johs A. Toxicol Lett. 2016 Feb 3;242:60-67. doi: 10.1016/j.toxlet.2015.11.022. Epub 2015 Nov 25.

Longitudinal changes during pregnancy in gut microbiota and methylmercury biomarkers, and reversal of microbe-exposure correlations. Rothenberg SE, Wagner CL, Hamidi B, Alekseyenko AV, Andrea Azcarate-Peril M. Environ Res. 2019 May;172:700-712. doi: 10.1016/j.envres.2019.01.014. Epub 2019 Jan 11.

Environmental Exposures and Autoimmune Diseases: Contribution of Gut Microbiome. Khan MF, Wang H. Front Immunol. 2020 Jan 10;10:3094. doi: 10.3389/fimmu.2019.03094. eCollection 2019

Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: A pilot study of Chinese children. Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W. Environ Res. 2019 Apr;171:501-509. doi: 10.1016/j.envres.2019.01.060. Epub 2019 Feb 5

Subchronic oral mercury caused intestinal injury and changed gut microbiota in mice. Zhao Y, Zhou C, Wu C, Guo X, Hu G, Wu Q, Xu Z, Li G, Cao H, Li L, Latigo V, Liu P, Cheng S, Liu P. Sci Total Environ. 2020 Jun 15;721:137639. doi: 10.1016/j.scitotenv.2020.137639. Epub 2020 Feb 29

Adverse effects of methylmercury on gut bacteria and accelerated accumulation of mercury in organs due to disruption of gut microbiota. Seki N, Akiyama M, Yamakawa H, Hase K, Kumagai Y, Kim YG. J Toxicol Sci. 2021;46(2):91-97. doi: 10.2131/jts.46.91

Nutrient-toxic element mixtures and the early postnatal gut microbiome in a United States longitudinal birth cohort. Laue HE, Moroishi Y, Jackson BP, Palys TJ, Madan JC, Karagas MR. Environ Int. 2020 May;138:105613. doi: 10.1016/j.envint.2020.105613. Epub 2020 Mar 3

Randomized open-label pilot study of the influence of probiotics and the gut microbiome on toxic metal levels in Tanzanian pregnant women and school children. Bisanz JE, Enos MK, Mwanga JR, Changalucha J, Burton JP, Gloor GB, Reid G. mBio. 2014 Oct 7;5(5):e01580-14. doi: 10.1128/mBio.01580-14

RESPONSE: *Studies of associations between mercury biomarkers and intestinal microbiome have been included in the text of Sections 2.7, 2.14, and 2.15.*

Section 2.7: Mercury ingestion can destroy and/or modify the composition of intestinal flora (Rice et al. 2014; Seki et al. 2021; Zhao et al. 2020). Mercury exposure biomarkers have been associated with changes in intestinal microflora profiles (Laue et al. 2020; Rothenberg et al. 2016a, 2019). Mercury biomarkers have also been associated with changes in microbiome profiles observed in certain disease states including autism, gestational diabetes, and autoimmune disease (Khan and Wang 2020; Zhai et al. 2019; Zhang et al. 2021).

Section 2.14: Mercury biomarkers have also been associated with changes in microbiome profiles observed in gestational diabetes (Zhang et al. 2021).

*Section 2.*15: These include: (1) …. (7) inhibition of nitric oxide production; (8) increased formation of ROS and lipid peroxidation; and (9) alteration of the intestinal microbiome (Khan and Wang 2020).

COMMENT 3: Contribution of trace element exposure to gestational diabetes mellitus through disturbing the gut microbiome. Zhang Y, Chen T, Zhang Y, Hu Q, Wang X, Chang H, Mao JH, Snijders AM, Xia Y. Environ Int. 2021 Aug;153:106520. doi: 10.1016/j.envint.2021.106520. Epub 2021 Mar 25.

RESPONSE: *This study has been included in Sections 2.7 and 2.14.*

Section 2.7: Mercury ingestion can destroy and/or modify the composition of intestinal flora (Rice et al. 2014; Seki et al. 2021; Zhao et al. 2020). Mercury exposure biomarkers have been associated with changes in intestinal microflora profiles (Laue et al. 2020; Rothenberg et al. 2016a, 2019). Mercury biomarkers have also been associated with changes in microbiome profiles observed in certain disease states including autism, gestational diabetes/ and autoimmune disease (Khan and Wang 2020; Zhai et al. 2019; Zhang et al. 2021).

Section 2.14: Mercury biomarkers have also been associated with changes in microbiome profiles observed in gestational diabetes (Zhang et al. 2021).

COMMENT 4: Publications on dietary exposure assessments:

Methylmercury exposure assessment using dietary and biomarker data among frequent seafood consumers in France CALIPSO study. Sirot et al. 2008 Environmental research.

Risk assessment of methylmercury based on internal exposure and fish and seafood consumption estimates in Taiwanese children. Shu Han You et al. International journal of hygiene and environmental health. 2018.

Effects of Uncertainties on Exposure Estimates to Methylmercury: A Monte Carlo Analysis of Exposure Biomarkers versus Dietary Recall Estimation. Ravi N. Sanga Risk Analysis, Vol. 21, No. 5, 2001

An Exposure Assessment for Methylmercury from Seafood for Consumers in the United States. Clark D. Carrington Risk Analysis, Vol. 22, No. 4, 2002

Dietary mercury exposure in a population with a wide range of fish consumption--self-capture of fish and regional differences are important determinants of mercury in blood. Jenssen et. al. Sci Total Environ. 2012

RESPONSE: *Carrington and Bolger (2002; the Clark 2002 reference referred to in the comment is Carrington and Bolger 2002), Jenssen et al. (2012), and Sanga et al. (2001) are cited in Section 5.6 as part of the basis for the range in mercury intakes:*

> Total diet studies conducted in Asia, United States, and Europe suggest that intakes of total mercury ranging from 1 to 10 µg/day are typical (Carrington and Bolger 2002; EFSA 2014; EPA 1999b; Jenssen et al. 2012; Kim et al. 2016b; Sanga et al. 2001; WHO 1990).

Sirot et al. (2008) and You et al. (2018) have been included in Section 5.6:

> The dominant source of mercury intake and absorption from the diet derives from consumption of fish (Bloom 1992; Davis et al. 2014; De Winter-Sorkina et al. 2003; EFSA 2012; EPA 1999b; Kim et al. 2016b; Lescord et al. 2018; Mahaffey et al. 2004; Nielsen et al. 2015; Sirot et al. 2008; WHO 1990; You et al. 2014, 2018),…

COMMENT 5: Section 5.3.1 Air:

Mercury Contamination from Dental Amalgam. Anita Vazquez Tibau 1, Blanche D Grube 2 Review J Health Pollut. 2019 Jun

RESPONSE: *The Tibau and Grube (2019) publication was added to Section 5.3.1 as follows.*

> Mercury emissions from cremation, which contributed 0.17% of the total global anthropogenic mercury emissions in 2019 (UNEP 2018), are expected to increase as global cremations increase (Tibau and Grube 2019).

Chapter 1

COMMENT 6: This chapter give an overall summary of the health effects of mercury exposure in humans and animals. I find this to be a sufficient introduction to the profile. The exposure conditions are described in a satisfactory way and are easy to access and understand.

RESPONSE: *No response needed.*

COMMENT 7: In general, exposure effects only observed in animal studies may not be of concern to humans. Some effects found in animal do not predict human reactions due to differences in physiology and metabolic pathways. An important factor is how the animal studies are designed, conducted and analysed, and if the methodology is comparable to results found in humans. Concerning effects of mercury exposure in animal studies where new endpoints and effects at lower levels of exposure are explored can reveal new knowledge that can be of concern for humans. I agree that effects observed in animal studies can be of concern to humans when studies are well conducted with good methodology.

RESPONSE: *No response needed.*

COMMENT 8: I agree with the MRLs that has been derived in the profile. Setting the MRLs for MeHg at 0.1 µg/kg bw day is contributing to showcase that dietary exposure to vulnerable groups are considered

in the derivation of MRLs. Several studies have reported that prenatal neurodevelopmental effects occur at maternal exposure levels lower that the current EFSA health-based guidance value 1.3 µg/kg bw week. I agree that there is not sufficient data to derive a MRL in many of the exposure scenarios. Some of the scenarios are not relevant as there is no significant exposure. I agree with the uncertainty factors set with each component.

RESPONSE: *No response needed.*

Chapter 2

COMMENT 9: Overall, this chapter give an adequately and exact summary of published findings on health effects of mercury exposure. The authors have done a thorough review of the studies and included human studies that are well designed and summed up short and easily read.

RESPONSE: *No response needed.*

COMMENT 10: As the profile is not aiming to include all published studies on mercury, I find that key publications on listed health effects are included. In the health outcomes where it is relevant to describe a dose-response relationship I find that this is mentioned. I think that the categorization cited in the LSE table are instructive and give a good overview of the results. The health effects discussed are extensive and I believe that most relevant possible mechanisms have been mentioned. I do not have a lot of experience in animal studies, but it seems that the best designed studies are included and described in the profile. Overall, I find the conclusions made in the chapter appropriate.

RESPONSE: *No response needed.*

COMMENT 11: I have made some suggestion on publications on findings that you might find relevant to include. A copy of each study are attaches and it is indicated where in the text each study should be included in the main document.

RESPONSE: *All publication provided by the Reviewer were reviewed and added to the profile as appropriate. Details on these publications are provided in comments below.*

COMMENT 12: Section 2.15 Immunological page 270 – I suggest including two studies from the Helix multicenter study;

1. In Utero Exposure to Mercury Is Associated With Increased Susceptibility to Liver Injury and Inflammation in Childhood. Chatzi 2021. investigated the effect of prenatal exposure to Hg on childhood liver injury by combining epidemiological results from a multicenter mother-child cohort with complementary in vitro experiments on monocyte cells that are known to play a key role in liver immune homeostasis and NAFLD. We used data from 872 mothers and their children (median age, 8.1 years; interquartile range [IQR], 6.5-8.7) from the European Human Early-Life Exposome (HELIX) cohort. We measured Hg concentration in maternal blood during pregnancy (median, 2.0 µg/L; IQR, 1.1-3.6). We also assessed serum levels of alanine aminotransferase (ALT), a common screening tool for pediatric NAFLD, and plasma concentrations of inflammation-related cytokines in children. We found that prenatal Hg exposure was associated with a phenotype in children that was characterized by elevated ALT (≥22.1 U/L for females and ≥25.8 U/L for males) and increased concentrations of circulating

interleukin (IL)- 1β, IL-6, IL-8, and tumor necrosis factor α (TNF-α). Consistently, inflammatory monocytes exposed in vitro to a physiologically relevant dose of Hg demonstrated significant up-regulation of genes encoding these four cytokines and increased concentrations of IL-8 and TNF-α in the supernatants. Conclusion: These findings suggest that developmental exposure to Hg can contribute to inflammation and increased NAFLD risk in early life.

2. Association of Fish Consumption and Mercury Exposure During Pregnancy With Metabolic Health and Inflammatory Biomarkers in Children. Chatzi 2020. The study included 805 mothers and their singleton children. Among mothers, the mean (SD) age at cohort inclusion or delivery of their infant was 31.3 (4.6) years. A total of 400 women (49.7%) had a high educational level, and 432 women (53.7%) were multiparous. Among children, the mean (SD) age was 8.4 (1.5) years (age range, 6-12 years). A total of 453 children (56.3%) were boys, and 734 children (91.2%) were of white race/ethnicity. Fish intake consistent with health recommendations (1 to 3 times per week) during pregnancy was associated with a 1-U decrease in metabolic syndrome score in children (β = −0.96; 95% CI, −1.49 to −0.42) compared with low fish consumption (<1 time per week) after adjusting for maternal mercury levels and other covariates. No further benefit was observed with fish intake of more than 3 times per week. A higher maternal mercury concentration was independently associated with an increase in the metabolic syndrome score of their offspring (β per 2-fold increase in mercury concentration = 0.18; 95% CI, 0.01-0.34). Compared with low fish intake, moderate and high fish intake during pregnancy were associated with reduced levels of proinflammatory cytokines and adipokines in children. An integrated analysis identified a cluster of children with increased susceptibility to metabolic disease, which was characterized by low fish consumption during pregnancy, high maternal mercury levels, decreased levels of adiponectin in children, and increased levels of leptin, tumor necrosis factor α, and the cytokines interleukin 6 and interleukin 1β in children.

RESPONSE: *The correct citation for the first study noted in the Reviewer's comment is Stratakis et al. (2021). This study was added to the discussion of associations between BHg and plasma cytokine levels in children (Section 2.15), with additional results added to Table 2-37.*

Three studies examined the relationship between BHg and plasma cytokine levels in children (Hui et al. 2016; Monastero et al. 2017; Stratakis et al. 2021). A prospective study evaluated associations between maternal BHg and child plasma cytokine levels at age 8 years (Stratakis et al. 2021). Children were stratified into two groups: those at low risk and those at high risk for nonalcoholic fatty liver disease (NAFLD). This study found positive associations between maternal BHg and cytokine levels (IL-1β, IL-6, IL-8, and tumor necrosis factor-alpha [TNFα]) in children with high risk of NAFLD.

For the second publication listed in the Reviewer's comment, the correct citation is Stratakis et al. (2020). This paper was not added to the profile. Although a median maternal blood mercury concentration was reported for the study population, results for serum inflammatory mediators were stratified based on the number of fish meals per week. The blood mercury concentration of these groups was not reported. Therefore, results cannot be correlated to a mercury biomarker level.

COMMENT 13: Section 2.16.1 page 295. A follow up study in the SCDNS at 7 years were recently published. Associations of prenatal methylmercury exposure and maternal polyunsaturated fatty acid status with neurodevelopmental outcomes at 7 years of age: results from the Seychelles Child Development Study Nutrition Cohort 2. The American Journal of Clinical Nutrition, Volume 113, Issue 2, February 2021, Pages 304–313, https://doi.org/10.1093/ajcn/nqaa338 I do not have access to the publication through my institution so have not attached the manuscript of the study.

RESPONSE: *Strain et al. (2021) has been added to Table 2-40 and related text in Section 2.16.1.*

Neurobehavioral endpoints were re-examined at age 7 years in a second cohort from the SCDNS (Strain et al. 2021). The study did not find associations between maternal hair levels (mean 2.91 µg/g; range 0.01, 31.66) and scores of tests that evaluated executive function, cognition, and linguistic skills. The study found improved scores in association with maternal serum omega-3 levels and no interaction between serum omega-3 levels and maternal hair mercury.

COMMENT 14: Section 2.16.1 page 330. S: This study is based on the Norwegian Mother, Father and Child Cohort Study and included 705 ADHD cases, 397 ASD cases and 1034 controls. Cases were identified through linkage with the Norwegian Patient Registry. Maternal concentrations of 11 metals/elements were measured in blood at week 17 of gestation; cadmium; cesium; cobalt; copper; lead; magnesium; manganese; selenium; zinc; total arsenic; and total mercury. Multivariable adjusted logistic regression models were used to examine associations between quartile levels of individual metals/elements and outcomes. We also investigated non-linear associations using restricted cubic spline models. The joint effects of the metal/element mixture on ASD and ADHD diagnoses were estimated using a quantile-based g-computation approach. Results: For ASD, we identified positive associations (increased risks) in the second quartile of arsenic [OR = 1.77 (CI: 1.26, 2.49)] and the fourth quartiles of cadmium and manganese [OR = 1.57 (CI: 1.07 2.31); OR = 1.84 (CI:1.30, 2.59)], respectively. In addition, there were negative associations between cesium, copper, mercury, and zinc and ASD. For ADHD, we found increased risk in the fourth quartiles of cadmium and magnesium [OR = 1.59 (CI: 1.15, 2.18); [OR = 1.42 (CI: 1.06, 1.91)]. There were also some negative associations, among others with mercury. In addition, we identified non-linear associations between ASD and arsenic, mercury, magnesium, and lead, and between ADHD and arsenic, copper, manganese, and mercury. There were no significant findings in the mixture approach analyses. Conclusion: Results from the present study show several associations between levels of metals and elements during gestation and ASD and ADHD in children. The most notable ones involved arsenic, cadmium, copper, mercury, manganese, magnesium, and lead.

RESPONSE: *Skogheim et al. (2021) has been added to Table 2-48 and related text in Section 2.16.1.*

Several studies have examined association between mercury exposure biomarkers (blood or urinary mercury) and signs of autism spectrum disorder (Golding et al. 2016a, 2016b, 2017, 2018; Hertz-Piccioto et al. 2010; McKean et al. 2015; Ryu et al. 2017; Skogheim et al. 2021; Yau et al. 2014).

Skogheim et al. (2021) found a nonlinear relationship between maternal blood mercury concentrations and OR for autism spectrum diagnosis, with elevated ORs at maternal blood mercury levels (<1 µg/L) but not at levels from >1 to 5 µg/L. The OR for ADHD diagnosis was negative.

COMMENT 15: Section 2.18 Development Page 410 predominantly mercury form unknown (general population). I suggest including a study by Ramon from a Spanish cohort study 2009. Fish consumption during pregnancy, prenatal mercury exposure, and anthropometric measures at birth in a prospective mother-infant cohort study in Spain. Cord blood total mercury was measured in 554 newborns in a population-based cohort born from 2004 to 2006. Fish consumption was classified in 4 frequency categories (<1 portion/mo, 1-3 portions/mo, 1 portion/wk, and > or =2 portions/wk). When multivariate models were adjusted, newborns in the higher quartile of total mercury weighed 143.7 g less (95% CI: -251.8, -35.6; P for trend = 0.02) and had higher odds of being SGA for length (odds ratio: 5.3; 95% CI: 1.2, 23.9; P from likelihood ratio test = 0.03) without a linear relation (P for trend = 0.13) compared with those in the lowest quartile. Mothers consuming >/=2 portions/wk of canned tuna had newborns who weighed more than those who consumed <1 portion/mo (P for trend = 0.03) and a lower risk of

having infants who were SGA for weight (P for trend = 0.01). Consumption of > or =2 portions/wk of large oily fish was associated with a higher risk of being SGA for weight and consumption of lean fish with a lower risk of being SGA for length compared with the consumption of <1 portion/mo, but in neither case was there a linear relation (P for trend >0.05).

RESPONSE: *The Ramon et al. (2009) study noted in the Reviewer's comment was not added to the profile. Although a mean blood mercury concentration was reported for the study population, results were stratified into quartiles. However, the blood mercury concentrations for the quartiles were not reported. Therefore, results cannot be correlated to a mercury biomarker level.*

COMMENT 16: Section 2.18 Development Page 414 I suggest to include a study from the Norwegian Mother and child cohort study (MoBa) on prenatal mercury exposure and child growth - Association between exposure to mercury as a predominant unknown form and child body mass index -Maternal seafood intake during pregnancy, prenatal mercury exposure and child body mass index trajectories up to 8 years Eleni Papadopoulou et.al. This study found that Higher prenatal mercury exposure (top decile) was associated with a reduction in child's weight growth trajectory, with the estimates ranging from -130 g [95% Confidence Intervals (CI) ¼ -247, -12 g] at 18 months to -608 g (95% CI ¼ -1.102, -113 g) at 8 years. This study can be included at page 146 and 150. A copy of the study is attached.

RESPONSE: *A discussion of the Papadopoulou et al. (2021) study was added to Section 2.18, with additional details added to Table 2-71, as follows.*

> A prospective study of mother-child pairs assessed associations between maternal BHg and BMI in children from 1 month through 8 years of age (Papadopoulou et al. 2021). No associations were observed between maternal BHg in the top 10th percentile and BMI in girls ages 1 month through 3 years. However, inverse associations were observed between maternal BHg in the top 10th percentile and BMI in girls at ages 4, 5, 6, 7, and 8 years of age; no associations were observed for boys or for boys and girls combined at any assessment age.

COMMENT 17: Section 2.20 Page 431. Genotoxicity in epidemiology studies. Urine mercury levels correlate with DNA methylation of imprinting gene H19 in the sperm of reproductive-aged men. Lu 2018 Plos One.
Methods: A total of 616 men, aged from 22 to 59, were recruited from Reproductive Medicine Clinic of Maternal and Child Care Service Center and the Urologic Surgery Clinic of Shanxi Academy of Medical Sciences during April 2015 and March 2016. Demographic information was collected through questionnaires. Urine was collected and urinary Hg concentrations were measured using a fully-automatic double-channel hydride generation atomic fluorescence spectrometer. Methylation of imprinting genes H19, Meg3 and Peg3 of sperm DNA from 242 participants were examined by bisulfite pyrosequencing. Spearman's rank and multivariate regression analysis were used for correlation analysis between sperm DNA methylation status of imprinting genes and urinary Hg levels. Results: The median concentration of Hg for 616 participants was 9.14μg/l (IQR: 5.56-12.52 μg/l; ranging 0.16-71.35μg/l). A total of 42.7% of the participants are beyond normal level for non-occupational exposure according to the criterion of Hg poisoning (≥10 μg/L). Spearman's rank analysis indicated a negative correlation between urinary Hg concentrations and average DNA methylation levels of imprinted genes H19 (r_s = -0.346, p <0.05), but there was no such a correlation for Peg3 and Meg3. Further, we analyzed the correlation between methylation level at individual CpG site of H19 and urinary Hg level. The results showed a negative correlation between urinary Hg concentrations and three out of seven CpG sites on H19 DMR, namely CpG2 (r_s = -0.137, p <0.05), CpG4 (r_s = -0.380, p <0.05) and CpG6 (r_s = -0.228, p <0.05). After adjusting age, smoking, drinking, intake of aquatic products and education by multivariate regression analysis, the results have confirmed the correlation as mentioned above.

RESPONSE: *The Lu et al. (2018) publication evaluates DNA methylation in sperm. In this profile, DNA methylation is considered as an epigenetic mechanism, not a genotoxicity endpoint. This study was added to the mechanisms section of Section 2.17.*

> Several mechanisms may be involved in the toxicity of mercury compounds to the reproductive system (Ferguson and Chin 2017; Lu et al. 2018; Schuurs 1999; Tan et al. 2009; Wirth and Mijal 2010). Proposed mechanisms include the following: (1) altered hormonal regulation of the hypothalamic-pituitary-gonadal axis; (2) disruption of steroidogenesis; (3) enzyme inhibition; (4) inhibition of DNA, RNA, and protein synthesis; (5) decreased mitochondrial energy production and alterations of microtubule assembly in sperm tails; (6) altered estrogen production resulting in decreased numbers, size, and quality of ova; (7) agonist activity at estrogen receptors; (8) genetic polymorphisms; and (9) DNA methylation in sperm.

COMMENT 18: Section 2.20 page 431. Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. Cardenas 2017 Sci Rep. Prenatal exposure to mercury, a known neurotoxic metal, is associated with lower cognitive performance during childhood. Disruption of fetal epigenetic programming could explain mercury's neurodevelopmental effects. We screened for epigenome-wide methylation differences associated with maternal prenatal blood mercury levels in 321 cord blood DNA samples and examined the persistence of these alterations during early (n = 75; 2.9-4.9 years) and mid-childhood (n = 291; 6.7-10.5 years). Among males, prenatal mercury levels were associated with lower regional cord blood DNA methylation at the Paraoxonase 1 gene (PON1) that persisted in early childhood and was attenuated in mid-childhood blood. Cord blood methylation at the PON1 locus predicted lower cognitive test scores measured during early childhood. Methylation at the PON1 locus was associated with PON1 expression in an independent set of cord blood samples. The observed persistent epigenetic disruption of the PON1 gene may modulate mercury toxicity in humans and might serve as a biomarker of exposure and disease susceptibility.

RESPONSE: *The Cardenas et al. (2017) study evaluates associations between DNA methylation and altered cognitive performance. In this profile, DNA methylation is considered as an epigenetic mechanism, not a genotoxicity endpoint. This study was added to the mechanisms section of Section 2.16, cited as Cardenas et al. (2017a).*

> A variety of toxicodynamic mechanisms contributing to neurological effects of methylmercury have been proposed. These include alteration or disruption of regulation of intracellular calcium homeostasis, the cytoskeleton, mitochondrial function, oxidative stress, neurotransmitter release, and DNA methylation (Aaseth et al. 2020; Cardenas et al. 2016, 2017a; Culbreth and Aschner 2016; Johansson et al. 2007; Patel and Reynolds 2013; dos Santos et al. 2016).

COMMENT 19: Section 2.20 page 431. Prenatal Exposure to Mercury: Associations with Global DNA Methylation and Hydroxymethylation in Cord Blood and in Childhood. Cardenas 2017 Environ Health Perspect. Methods: Within Project Viva, a U.S. prebirth cohort, we examined associations of maternal second trimester red blood cell mercury (RBC-Hg) concentrations with global 5-hydroxymethylcytosine (%-5hmC) and 5-methylcytosine (%-5mC) DNA content in blood collected at birth (n=306), early childhood (n=68; 2.9 to 4.9 y), and midchildhood (n=260; 6.7 to 10.5 y).
Results: Median prenatal RBC-Hg concentration was 3.23µg/g [interquartile range (IQR)=3.29]. At birth, median cord blood %-5mC, %-5hmC, and their ratio were 4.95%, 0.22%, and 24.37, respectively. The mean adjusted difference [95% confidence interval (CI)] of blood %-5hmC for a doubling in prenatal RBC-Hg concentration was -0.013% (-0.029, 0.002), -0.031% (-0.056, -0.006), and 0.005% (-0.007, 0.018) at birth, early, and midchildhood, respectively. The corresponding relative adjusted change in the genomic ratio of %-5mC to %-5hmC for a doubling in prenatal RBC-Hg concentration was 4.70% (0.04,

9.58), 22.42% (7.73, 39.11), and 0.73% (-4.18, 5.88) at birth, early, and midchildhood, respectively. No associations were present between prenatal maternal RBC-Hg and %-5mC at any time point. Conclusions: Prenatal mercury exposure was associated with lower %-5hmC genomic content and a corresponding increase in the ratio of %-5mC to %-5hmC in cord blood. This association was persistent in early but not midchildhood blood. Our results demonstrate the potential malleability of epigenetic modifications associated with mercury exposure in utero. https://doi.org/10.1289/EHP1467.

RESPONSE: *The Cardenas et al. (2017) study evaluates associations between cord blood Hg and global DNA methylation. In this profile, DNA methylation is as an epigenetic mechanism, not a genotoxicity endpoint. This study was added to the mechanisms section of Section 2.18, cited as Cardenas et al. (2017b).*

Prenatal exposure to mercury has been shown to alter DNA methylation in pregnant women and infants (Cardenas et al. 2017b; Weyde et al. 2021).

COMMENT 20: Section 2.20 page 431.

1. Functional gene polymorphisms related to mercury assosiated disease phenotypes. The relevance of the individual genetic background for the toxicokinetics of two significant neurodevelopmental toxicants: mercury and lead. Gundacer 2010 Mutat Res. This review summarizes the genetic factors that modify their toxicokinetics. Understanding toxicokinetics (uptake, biotransformation, distribution, and elimination processes) is a key precondition to understanding the individual health risks associated with exposure. We selected candidate susceptibility genes when evidence was available for (1) genes/proteins playing a significant role in mercury and lead toxicokinetics, (2) gene expression/protein activity being induced by these metals, and (3) mercury and lead toxicokinetics being affected by gene knockout/knockdown or (4) by functional gene polymorphisms. The genetic background is far better known for mercury than for lead toxicokinetics. Involved are genes encoding L-type amino acid transporters, organic anion transporters, glutathione (GSH)-related enzymes, metallothioneins, and transporters of the ABC family. Certain gene variants can influence mercury toxicokinetics, potentially explaining part of the variable susceptibility to mercury toxicity. Delta-aminolevulinic acid dehydratase (ALAD), vitamin D receptor (VDR) and hemochromatosis (HFE) gene variants are the only well-established susceptibility markers of lead toxicity in humans. Many gaps remain in our knowledge about the functional genomics of this issue. This calls for studies to detect functional gene polymorphisms related to mercury- and lead-associated disease phenotypes, to demonstrate the impact of functional polymorphisms and gene knockout/knockdown in relation to toxicity, to confirm the in vivo relevance of genetic variation, and to examine gene-gene interactions on the respective toxicokinetics. Another crucial aspect is knowledge on the maternal-fetal genetic background, which modulates fetal exposure to these neurotoxicants. To completely define the genetically susceptible risk groups, research is also needed on the genes/proteins involved in the toxicodynamics, i.e., in the mechanisms causing adverse effects in the brain. Studies relating the toxicogenetics to neurodevelopmental disorders are lacking (mercury) or very scarce (lead). Thus, the extent of variability in susceptibility to heavy metal-associated neurological outcomes is poorly characterized.
2. Gestational blood levels of toxic metal and essential element mixtures and associations with global DNA methylation in pregnant women and their infant. Kjell Vegard F. Weyde 2021 Science of total environment. Methods: Using 631 mother-child pairs from a prospective birth cohort (The Norwegian Mother, Father and Child Cohort Study), we measured maternal blood concentration (gestation week ~18) of five toxic metals and seven and 5-hydroxymethylcytosine (5hmC) in mothers during pregnancy and their newborn children (cord blood). Multiple testing was adjusted for using the Benjamini and Hochberg false discovery rate (FDR) approach.

Results: The most sensitive marker of DNA methylation appeared to be 5mC levels. In pregnant mothers, elastic net regression indicated associations between 5mC and selenium and lead (non-linear), while in newborns results indicated relationships between maternal selenium, cobalt (non-linear) and mercury and 5mC, as well as copper (non-linear) and 5hmC levels. Several possible two-way interactions were identified (e.g. arsenic and mercury, and selenium and maternal smoking in newborns). None of these findings met the FDR threshold for multiple testing. No net effect was observed in the joint (mixture) exposure-approach using quantile gcomputation essential elements. We investigated associations as individual exposures and two-way interactions, using elastic net regression, and total mixture, using quantile g-computation, with blood levels of 5-methylcytocine (5mC)

RESPONSE: *The first study in the Reviewer's comment (Gundacker et al. 2010) evaluates the role of genetic polymorphisms in susceptibility to mercury-induced toxicity. This study was already included in Section 3.2 of the profile. For the second publication noted in the Reviewer's comment (Weyde et al. 2021), the study evaluates associations between cord blood Hg and global DNA methylation. In this profile, DNA methylation is considered as an epigenetic mechanism, not a genotoxicity endpoint. This study was added to the mechanisms section of Section 2.18.*

Prenatal exposure to mercury has been shown to alter DNA methylation in pregnant women and infants (Cardenas et al. 2017b; Weyde et al. 2021).

Chapter 3

COMMENT 21: I find the discussion in this section adequate. To my knowledge all relevant models and supporting data have been presented in this section. There is adequate discussion of differences in toxicokinetics between humans and animals in the section. The section covers relevant information on susceptible populations.

RESPONSE: *No response needed.*

COMMENT 22: Section 3.2.3 page 465 Here I suggest including results from Seki 2021 Journal of toxicological sciences. Adverse effects of methylmercury and gut bacteria and accelerated accumulation of mercury in organs due to disruption of gut microbiota.

RESPONSE: *Seki et al. (2021) has been added to the text of Section 3.1.3.*

Methylmercury can react with hydrogen sulfide and hydrogen persulfide produced by gastrointestinal tract bacteria to form thiol complexes of methylmercury (Seki et al. 2021).

COMMENT 23: Exposure and effect specific for the substance are well described in the section. I would suggest to also include a section with information about dietary exposure assessment for methylmercury and the possibilities/disadvantages of using food frequency questionnaire data and food mercury databases to estimate MeHg exposure in population. Just as biomarkers of blood and hair total mercury contentrations reflect methylmerury intake, assessment of methylmercury dietary exposure will reflect the magnitude of mecury exposure given accurate methodology. Publications relevant to describe dietary exposure assessment in population studies are attached.

1. Methylmercury exposure assessment using dietary and biomarker data among frequent seafood consumers in France CALIPSO study. Sirot et al. 2008 Environmental research.

2. Risk assessment of methylmercury based on internal exposure and fish and seafood consumption estimates in Taiwanese children. Shu Han You et al. International journal of hygiene and environmental health. 2018.
3. Effects of Uncertainties on Exposure Estimates to Methylmercury: A Monte Carlo Analysis of Exposure Biomarkers versus Dietary Recall Estimation. Ravi N. Sanga Risk Analysis, Vol. 21, No. 5, 2001
4. An Exposure Assessment for Methylmercury from Seafood for Consumers in the United States. Clark D. Carrington Risk Analysis, Vol. 22, No. 4, 2002
5. Dietary mercury exposure in a population with a wide range of fish consumption--self-capture of fish and regional differences are important determinants of mercury in blood. Jenssen et. al. Sci Total Environ. 2012

RESPONSE: *Carrington and Bolger (2002; the Clark 2002 reference referred to in the comment is Carrington and Bolger 2002), Jenssen et al. (2012), and Sanga et al. (2001) are cited in Section 5.6 as part of the basis for the range in mercury intakes:*

> Total diet studies conducted in Asia, United States, and Europe suggest that intakes of total mercury ranging from 1 to 10 µg/day are typical (Carrington and Bolger 2002; EFSA 2014; EPA 1999b; Jenssen et al. 2012; Kim et al. 2016b; Sanga et al. 2001; WHO 1990).

Sirot et al. (2008) and You et al. (2018) have been included in Section 5.6:

> The dominant source of mercury intake and absorption from the diet derives from consumption of fish (Bloom 1992; Davis et al. 2014; De Winter-Sorkina et al. 2003; EFSA 2012; EPA 1999b; Kim et al. 2016b; Lescord et al. 2018; Mahaffey et al. 2004; Nielsen et al. 2015; Sirot et al. 2008; WHO 1990; You et al. 2014, 2018),…

Chapter 4

COMMENT 24: The tables of values and information are correctly reproduced. Sufficient information is provided on the physical and chemical properties in the tables. I would have liked that the chemical formula for the compounds to be included in the table.

RESPONSE: *Chemical formulas of mercury compounds are provided in Table 4-1.*

Chapter 5

COMMENT 25: Section 5.3.1 Might include more information about the mercury emission from creamtions. Cremations were according to the EPS responsible for 5.5% of the nation's mercury emissions in 2017—mostly from dental fillings, which are vaporized but not consumed during the 1,800-degree cremation process. Dental amalgam represents an understudied area of global mercury pollution that includes cremation, sewage sludge, burial, and small-scale gold mining. The exposure of mercury from crematories is a concern in areas close to crematoriums.
Mercury Contamination from Dental Amalgam. Anita Vazquez Tibau 1, Blanche D Grube 2 Review J Health Pollut. 2019 Jun

RESPONSE: *The Tibau and Grube (2019) publication was added to Section 5.3.1 as follows.*

> Mercury emissions from cremation, which contributed 0.17% of the total global anthropogenic mercury emissions in 2019 (UNEP 2018), are expected to increase as global cremations increase (Tibau and Grube 2019).

Chapter 6

COMMENT 26: No comments

RESPONSE: *No response needed.*

Chapter 7

COMMENT 27: No comments

RESPONSE: *No response needed.*

Appendices

COMMENT 28: No comments

RESPONSE: *No response needed.*

Annotated Comments

The Reviewer suggested a number of editorial revisions. The suggested revisions were made to the profile. Responses to Reviewer comments that were not considered editorial or stylistic are presented below.

COMMENT 29: Referring to the statement in Section 2.4, lines 15-19 on page 147 – Studies in children and adolescents reported conflicting results, with one study reporting no associations between mercury exposure and body weight measures at low SHg and one study reporting positive associations at higher BHg. The apparent discrepancy in these findings may be related to exposure levels. However, findings have not been corroborated. – the Reviewer commented "Findings from the MoBa study by Papadopoulou et.al. child weight growth trajectory were negatively associated with high prenatal maternal blood mercury levels."

RESPONSE: *Results of the Papadopoulou et al. (2021) study were added to beginning of Section 2.4. The citation is not included in this statement because citations are not included in the overview summary at the beginning of the sections in Chapter 2.*

> Results of studies in children and adolescents are inconsistent, with one study reporting no associations between mercury exposure and body weight measures at low SHg, one study reporting positive associations at higher BHg, and one study reporting inverse associations in girls, but not in boys, at higher BHg.

COMMENT 30: Referring to the subsection titled *Predominant Mercury Form Unknown (General Populations)* in Section 2.4, line 18 on page 151, the Reviewer commented **"**Findings from the MoBa study by Papadopoulou et.al. child weight growth trajectory were negatively associated with high prenatal maternal blood mercury levels."

RESPONSE: *Results of the Papadopoulou et al. (2021) publication were added to Section 2.4 and Table 2-6.*

The prospective study of mother-child pairs assessed associations between maternal BHg and BMI in children from 1 month through 8 years of age (Papadopoulou et al. 2021). Inverse associations were observed between maternal BHg in the top 10th percentile and BMI in girls at ages 4, 5, 6, 7, and 8 years of age (range of top 10th percentile was not reported); no associations were observed for boys or for boys and girls combined at any assessment age.

COMMENT 31: Referring to the two studies discussed in Section 2.15 on page 273 (Hui et al. 2016; Monastero et al. 2017), the Reviewer commented "In Utero Exposure to Mercury Is Associated With Increased Susceptibility to Liver Injury and Inflammation in Childhood. Chatzi 2021. investigated the effect of prenatal exposure to Hg on childhood liver injury by combining epidemiological results from a multicenter mother-child cohort with complementary in vitro experiments on monocyte cells that are known to play a key role in liver immune homeostasis and NAFLD. We used data from 872 mothers and their children (median age, 8.1 years; interquartile range [IQR], 6.5-8.7) from the European Human Early-Life Exposome (HELIX) cohort. We measured Hg concentration in maternal blood during pregnancy (median, 2.0 μg/L; IQR, 1.1-3.6). We also assessed serum levels of alanine aminotransferase (ALT), a common screening tool for pediatric NAFLD, and plasma concentrations of inflammation-related cytokines in children. We found that prenatal Hg exposure was associated with a phenotype in children that was characterized by elevated ALT (≥22.1 U/L for females and ≥25.8 U/L for males) and increased concentrations of circulating interleukin (IL)- 1β, IL-6, IL-8, and tumor necrosis factor α (TNF-α). Consistently, inflammatory monocytes exposed in vitro to a physiologically relevant dose of Hg demonstrated significant up-regulation of genes encoding these four cytokines and increased concentrations of IL-8 and TNF-α in the supernatants. Conclusion: These findings suggest that developmental exposure to Hg can contribute to inflammation and increased NAFLD risk in early life."

The Reviewer also commented "Association of Fish Consumption and Mercury Exposure During Pregnancy With Metabolic Health and Inflammatory Biomarkers in Children. Chatzi 2020. The study included 805 mothers and their singleton children. Among mothers, the mean (SD) age at cohort inclusion or delivery of their infant was 31.3 (4.6) years. A total of 400 women (49.7%) had a high educational level, and 432 women (53.7%) were multiparous. Among children, the mean (SD) age was 8.4 (1.5) years (age range, 6-12 years). A total of 453 children (56.3%) were boys, and 734 children (91.2%) were of white race/ethnicity. Fish intake consistent with health recommendations (1 to 3 times per week) during pregnancy was associated with a 1-U decrease in metabolic syndrome score in children (β = −0.96; 95% CI, −1.49 to −0.42) compared with low fish consumption (<1 time per week) after adjusting for maternal mercury levels and other covariates. No further benefit was observed with fish intake of more than 3 times per week. A higher maternal mercury concentration was independently associated with an increase in the metabolic syndrome score of their offspring (β per 2-fold increase in mercury concentration = 0.18; 95% CI, 0.01-0.34). Compared with low fish intake, moderate and high fish intake during pregnancy were associated with reduced levels of proinflammatory cytokines and adipokines in children. An integrated analysis identified a cluster of children with increased susceptibility to metabolic disease, which was characterized by low fish consumption during pregnancy, high maternal mercury levels, decreased levels of adiponectin in children, and increased levels of leptin, tumor necrosis factor α, and the cytokines interleukin 6 and interleukin 1β in children."

RESPONSE: *The correct citation for the first publication noted in the Reviewer's comment is Stratakis et al. (2021). Results of this study were added to the discussion of associations between BHg and plasma cytokine levels in children (Section 2.15), with additional results added to Table 2-37.*

Three studies examined the relationship between BHg and plasma cytokine levels in children (Hui et al. 2016; Monastero et al. 2017; Stratakis et al. 2021). A prospective study evaluated associations between maternal BHg and child plasma cytokine levels at age 8 years (Stratakis et al. 2021). Children were stratified into two groups: those at low risk and those at high risk for

nonalcoholic fatty liver disease (NAFLD). This study found positive associations between maternal BHg and cytokine levels (IL-1β, IL-6, IL-8, and tumor necrosis factor-alpha [TNFα]) in children with high risk of NAFLD.

The correct citation for the second publication noted in the Reviewer's comment is Stratakis et al. (2020). This study was not added to the profile. Although a median maternal blood mercury concentration was reported for the study population, results were on inflammatory mediators were stratified based on the number of fish meals per week. The blood mercury concentration of these groups was not reported. Therefore, results cannot be correlated to a mercury biomarker level.

COMMENT32: Referring to the entry in Table 2-40 in Section 2.16.1 on page 295 for Strain et al. 2012, the Reviewer commented "New follow up study at 7 years in the SCDNS Associations of prenatal methylmercury exposure and maternal polyunsaturated fatty acid status with neurodevelopmental outcomes at 7 years of age: results from the Seychelles Child Development Study Nutrition Cohort 2 *Am J Clin Nutr. 2021."*

RESPONSE: *Strain et al. 2021 has been added to Table 2-40 and related text in Section 2.16.1.* Neurobehavioral endpoints were re-examined at age 7 years in a second cohort from the SCDNS (Strain et al. 2021). The study did not find associations between maternal hair levels (mean 2.91 µg/g; range 0.01, 31.66) and scores of tests that evaluated executive function, cognition and linguistic skills. The study found improved scores in association with maternal serum omega-3 levels and no interaction between serum omega-3 levels and maternal hair mercury.

COMMENT 33: Referring to the statement in Section 2.16.1, lines 10-12 on page 330 – The absence of an association with maternal blood mercury may represent variance in blood mercury levels that is unrelated to dietary methylmercury intake (e.g., mercury from amalgam restorations). – the Reviewer commented "A 2020 study in the Norwegian Mother and Child cohort study found increased risk of ASD and ADHD in children associated with maternal mercury blood levels in pregnancy. Metal and essential element concentrations during pregnancy and associations with autism spectrum disorder and attention-deficit/hyperactivity disorder in children. Skogheim et al."

RESPONSE: *Skogheim et al. (2021) has been added to Table 2-48 and related text in Section 2.16.1.* Skogheim et al. (2021) found a nonlinear relationship between maternal blood mercury concentrations and OR for autism spectrum diagnosis, with elevated ORs at maternal blood mercury levels (<1 µg/L) but not at levels from >1 to 5 µg/L. The OR for ADHD diagnosis was negative.

COMMENT 34: Referring to the *in utero* studies listed in Table 2-72 in Section 2.18 on page 411, the Reviewer commented "I suggest including a study by Ramon from a Spanish cohort study 2009. Fish consumption during pregnancy, prenatal mercury exposure, and anthropometric measures at birth in a prospective mother-infant cohort study in Spain. Cord blood total mercury was measured in 554 newborns in a population-based cohort born from 2004 to 2006. Fish consumption was classified in 4 frequency categories (<1 portion/mo, 1-3 portions/mo, 1 portion/wk, and > or =2 portions/wk). When multivariate models were adjusted, newborns in the higher quartile of total mercury weighed 143.7 g less (95% CI: -251.8, -35.6; P for trend = 0.02) and had higher odds of being SGA for length (odds ratio: 5.3; 95% CI: 1.2, 23.9; P from likelihood ratio test = 0.03) without a linear relation (P for trend = 0.13) compared with those in the lowest quartile. Mothers consuming >/=2 portions/wk of canned tuna had newborns who weighed more than those who consumed <1 portion/mo (P for trend = 0.03) and a lower risk of having infants who were SGA for weight (P for trend = 0.01). Consumption of > or

=2 portions/wk of large oily fish was associated with a higher risk of being SGA for weight and consumption of lean fish with a lower risk of being SGA for length compared with the consumption of <1 portion/mo, but in neither case was there a linear relation (P for trend >0.05)."

RESPONSE: *The Ramon et al. (2009) study was not added to the profile. Although a mean blood mercury concentration was reported for the study population, results were stratified as quartiles. However, the blood mercury concentration for the quartiles was not reported. Therefore, results cannot be correlated to a mercury biomarker level.*

COMMENT 35: Referring to the studies summarized in Table 2-73 in Section 2.18 and described on page 414, the Reviewer commented "I suggest to include a study from the Norwegian Mother and child cohort study (MoBa) on prenatal mercury exposure and child growth - Association between exposure to mercury as a predominant unknown form and child body mass index -Maternal seafood intake during pregnancy, prenatal mercury exposure and child body mass index trajectories up to 8 years Eleni Papadopoulou et.al. This study found that Higher prenatal mercury exposure (top decile) was associated with a reduction in child's weight growth trajectory, with the estimates ranging from -130 g [95% Confidence Intervals (CI) ¼ -247, -12 g] at 18 months to -608 g (95% CI ¼ -1.102, -113 g) at 8 years. This study can be included at page 414. A copy of the study is attached."

RESPONSE: *A discussion of the Papadopoulou et al. (2021) study was added to Section 2.18, with additional details added to Table 2-72.*

A prospective study of mother-child pairs assessed associations between maternal BHg and BMI in children from 1 month through 8 years of age (Papadopoulou et al. 2021). No associations were observed between maternal BHg in the top 10th percentile and BMI in girls ages 1 month through 3 years. However, inverse associations were observed between maternal BHg in the top 10th percentile and BMI in girls at ages 4, 5, 6, 7, and 8 years of age; no associations were observed for boys or for boys and girls combined at any assessment age.

COMMENT 36: Section 2.20 –

Unscheduled DNA synthesis was not induced in cats following oral exposure to methylmercury (Miller et al. 1979). – the Reviewer commented "Urine mercury levels correlate with DNA methylation of imprinting gene H19 in the sperm of reproductive-aged men. Lu 2018 Plos One.

Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. Cardenas 2017 Sci Rep.

Prenatal Exposure to Mercury: Associations with Global DNA Methylation and Hydroxymethylation in Cord Blood and in Childhood. Cardenas 2017 Environ Health Perspect.

The relevance of the individual genetic background for the toxicokinetics of two significant neurodevelopmental toxicants: mercury and lead. Gundacer 2010 Mutat Res.

Gestational blood levels of toxic metal and essential element mixtures and associations with global DNA methylation in pregnant women and their infants Kjell Vegard F. Weyde 2021 Science of total environment."

RESPONSE: *The Reviewer requested that the references listed in the comment above be added to the genotoxicity section (Section 2.20). The studies noted above provide data on DNA methylation. In this profile, DNA methylation is considered as an epigenetic mechanism and not as a genotoxicity endpoint. The studies have been added to the mechanisms sections of the profile as shown below. For more detailed information, see Responses for Comment numbers indicated below.*

Lu et al. 2018: Section 2.17 (Comment 17)
Cardenas 2017a: Section 2.16 (Comment 18)

Cardenas 2017b: Section 2.18 (Comment 19)
Gundaker 2010: Section 3.2 (Comment 20)
Weyde et al. 2021: Section 2.18 (Comment 20)

COMMENT 37: Referring to the statement in Section 5.1, line 16 on page 504 – The general population is primarily exposed to mercury through the ingestion of foods. – the Reviewer commented "Headline – summary of routes of exposure."

RESPONSE: *Section 5.1 is an overview of the information discussed in the Chapter 5. A brief statement regarding potential routes of exposure is included in Section 5.1. A more detailed discussion in presented in Section 5.6.*

Comments provided by Reviewer #2

GENERAL COMMENTS

COMMENT 1: I have annotated specific comments in the text and used Track Change for any typographical errors detected (although I did not intend to conduct a review on the grammar). In the annotated feedback, I have provided both major and minor comments in the text, but I have not distinguished between what I consider major vs. minor.

RESPONSE: *All typographical errors identified by the Reviewer were corrected.*

COMMENT 2: In this report, I attempt to provide my overall *major* comments for the Tox Profile as well as *major* comments for each chapter. To the extent possible, I have tried to note where in the text my major comments are relevant, but they do not always refer to a specific section.

RESPONSE: *No response needed.*

COMMENT 3: I first want to recognize the effort required to put together this profile – it is an amazing consolidation of scientific data and I commend the authors for their hard work. I have listed major comments below, but I have not ordered by importance.

RESPONSE: *No response needed. The profile authors appreciate the Reviewer's comment.*

COMMENT 4: 1. There is a lack of definitions, common criteria and common judgements to evaluate the relationship between exposure and health outcomes, biological mechanisms and exposure routes, and calculation of MRLs. This includes a lack of description regarding the criteria used to select studies as well as an apparent difference in the criteria used to evaluate whether select studies inform a particular relationship. There are many examples of this throughout Chapter 2, 5 and MRL calculations. For example, several sections of Chapter 2 include a statement that "studies did not meet the criteria for inclusion"; however, these criteria are never described. In addition, the beginning of Chapter 2 (p 19, lines 5-10) states that ATSDR uses judgement` to establish whether an endpoint is a NOAEL or LOAEL. What is this judgment? If you compare, for example, cardiovascular vs. hematological vs. renal relationships with Hg, there seems to be different judgement used. See annotated comments in these sections

a. Related to point #1, in the Appendix or in each chapter (2 and 3), it would be useful to see a figure that shows how many papers were identified, how many were excluded, and how many were used to infer effects for each section of chapters 2 and 3. This would be similar to a flow diagram
b. Related to above, it would be useful to create a global map of all the studies reviewed for this profile (i.e., number of studies conducted by country). Since this is not a full systematic review, there would be obvious gaps; however, a map would help readers understand where well-studies populations exist.

RESPONSE: *Regarding the comment on inclusion criteria, Section 2.1 discusses the criteria for inclusion of literature in the profile. Text has been revised to refer to this section when criteria are mentioned.*

Section 2.5: Epidemiological studies evaluating effects of elemental mercury in respiratory effects meeting inclusion criteria were not identified (see inclusion criteria, Section 2.1).

Data are not sufficient to determine if exposure to mercury in populations with high fish diets produces adverse respiratory effects, with only one study meeting inclusion criteria (see inclusion criteria, Section 2.1).

Section 2.7: Gastrointestinal effects of mercury have not been well-studied in humans or animals. No epidemiological studies meeting inclusion criteria were identified for any form of mercury (see inclusion criteria, Section 2.1).

Epidemiological studies evaluating gastrointestinal effects of elemental mercury and meeting inclusion criteria were not identified (see inclusion criteria, Section 2.1).

Epidemiological studies evaluating gastrointestinal effects of exposures to methylmercury from high fish diets and meeting inclusion criteria were not identified (see inclusion criteria, Section 2.1).

Section 2.8: Furthermore, few epidemiological studies on hematological effects meet the inclusion criteria for this toxicological profile (see inclusion criteria, Section 2.1).

Little information is available regarding effects of elemental mercury on the hematological system in humans, with only two studies meeting inclusion criteria for this toxicological profile (see inclusion criteria, Section 2.1);…

Data are not sufficient to determine if exposure to mercury in populations that consume high fish diets produces adverse hematological effects, with only one study meeting inclusion criteria for this toxicological profile (see inclusion criteria, Section 2.1).

Section 2.14: Data are not sufficient to determine if exposure to mercury in populations with high fish diets produces adverse effects to the endocrine system, with only one study meeting inclusion criteria for this toxicological profile (see inclusion criteria, Section 2.1).

*Section 2.*15: Immune effects of elemental mercury have not been well studied and few epidemiological studies meeting inclusion criteria were identified (see inclusion criteria, Section 2.1).

Few studies have evaluated immunological effects in populations with high fish diets; studies meeting inclusion criteria are summarized in Table 2-33 (see inclusion criteria, Section 2.1).

Section 2.17: Few studies meeting inclusion criteria were identified for workers and populations with high fish diets, whereas the database for general populations was more robust (see inclusion criteria, Section 2.1).

Regarding the comment on judgment applied to selecting NOAELs and LOAELs, this statement is made in all profiles and ATSDR will consider revising this boilerplate language. However, judgments made are specific to the chemical and study and, for studies selected for supporting MRLs, these judgements are described in detail in Appendix A.

Regarding study counts, Section B.1.2 in Appendix B provides a summary of studies identified from searching and that were selected for further consideration and citing in the profile. A flow

diagram of the selections is provided in Figure B-1. Counts of studies cited in support of discussion of health effects are provided in Figures 2-1, 2-2, 2-3 and 2-4. ATSDR will consider revising this format along the lines of the Reviewer's suggestion for future updates of ATSDR's Guidance for the Preparation of Toxicological Profiles.

Regarding inclusion of maps of studies by country, ATSDR does not see the merit in this, because that would imply that country of origin is somehow relevant to study quality or its contribution to the weight of evidence. Country of origin of populations in epidemiology studies is relevant to the exposure sources and levels and, for this reason, the country origin of each epidemiology study is provided in the relevant summaries of the studies (e.g., see Table 2-39).

COMMENT 5: 2. Relationships between exposure and health are dependent on animal models. However, with the potential exception of the calculations of the MRLs in the Appendix, there appears to be inconsistency in how animal data is used to weigh the strength of a relationship between exposure and health effects, and Ch 3 even cites that there are inter-species variation (p 439, line 26). As I compared sections within Chapter 2 and section between Chapter 2 and 3, it was apparent that the application (i.e,. consideration of results, weighing of effects, etc.) of animal model results was not consistent. To remedy this, I would suggest including the following information either at the beginning of chapter 2 or in the appendix: (i) A summary description of each animal model, specifically describing the advantages and disadvantages of each animal model as they are applied to risk assessment (this is particularly useful for scientists and professionals not familiar with the use of animal models); (ii) For each health effect evaluated in chapter 2, include a short bullet point in the overview that clearly describes how data from animal models contribute to our understanding of the exposure-health effect in humans, which is alluded to in the MRL Appendix A in reference to an uncertainty factor (UF), but the description of why a particular UF size is used is not clear; and (3) report whether any specific criteria were used to select/identify animal or epidemiological studies for discussion, i.e., sample size, study design, similarity of mechanism, etc.

RESPONSE: *Regarding the Reviewer's suggestion of providing a summary description of each animal model: Given the range of endpoints that have been studied in the mercury literature, a critical review of the strengths and weaknesses of the various animal models would be a huge undertaking and not within the scope and objectives of the ATSDR Toxicological Profiles. The overview of each health effects section provides a summary of the important findings in specific animal models but does not critically review the strengths and weakness of the models. As noted in Section 2.1, ATSDR's approach for assessing study quality and weight-of-evidence evaluation is described in ATSDR's Guidance for the Preparation of Toxicological Profiles.*

Regarding the Reviewer's suggestion of providing, for each health effect evaluated in Chapter 2, a short bullet point in the overview that clearly describes how data from animal models contribute to our understanding of the exposure-health effect in humans: This information is provided in bullet form in the overview of each health effect section. For example, in Section 2.4, the profile states: Studies evaluating respiratory effects in animals are available for inhalation exposure to mercury vapor and oral exposure to mercuric chloride or methylmercury. Consistent with human data, respiratory distress and lung damage has been reported following exposure to acute lethal air concentrations of mercury vapor.

Regarding the Reviewer's suggestion of reporting whether any specific criteria were used to select/identify animal or epidemiological studies for discussion (i.e., sample size, study design, similarity of mechanism, etc.): Section 2.1 discusses the criteria for inclusion of literature in the profile. As noted in the response to Comment 4, text has been revised to refer to this section when criteria are mentioned.

COMMENT 6: 3. Although Chapters 2 and 3 focus on organ-specific health effects and biomarkers, it is widely accepted that mercury exposure has a multi-organ impact and that organs do not function in a vacuum. Namely, an acute or chronic MeHg exposure might have immediate observable effects on neurological and/or renal function, the cascading effect is equally detrimental and is not currently being measured. By presenting the effects in a compartmentalized way prevents clinicians, policy-makers and researchers from understanding the systematic impact of Hg exposure. Therefore, I suggest Chapter 1 include an overview of the systematic impacts that mercury can have on humans to emphasize both the multi-health clinical impact of exposure and overall net health improvement that can occur if exposures are reduced. In addition to a summary paragraph(s), I would suggest a figure highlighting where different species of Hg impact different parts of the body.

RESPONSE: *Section 1.2 includes a summary of the major systemic effects of mercury described in Section 2 of the profile. All ATSDR Toxicological Profiles present the more detailed discussion of health effects (Chapter 2) organized by health effect category. If an effect described on one category may also be an outcome of an effect on another category, the text refers the reader to the other section. For example, in Section 2.8:* The anemia observed in this study may have been secondary to blood loss associated with the ulcerative lesions in the large intestine seen at this dose (see Section 2.7, Gastrointestinal).

While a study-by-study format could provide an easier way for the reader to understand all aspects of every study, it would make it much harder to quickly access the information on any given health effect provided by multiple studies.

Regarding the Reviewer's comment on emphasizing multi-organ impacts of mercury: The topic of response interactions (e.g., additivity, synergy, inhibition) is complex and is the subject of ATSDR Interaction Profiles, noted in Section 3.4.

Regarding the Reviewer's comment on including a figure highlighting where different species of mercury impact different parts of the body: ATSDR will consider this in any upcoming review of the profile format. However, for mercury (and most chemicals), the distribution of health effects is dependent on dose. For example, our highest concern for methylmercury exposure is neurodevelopmental; however, at sufficient doses, methylmercury is also nephrotoxic. For this reason, rather than presenting a figure that highlights regions of the body, ATSDR provides a graphical overview of the dose-effect relationships in Figures 1-1 through 1-6.

COMMENT 7: 4. In addition to #3 above, Chapter 1 should succinctly define major sources of mercury and major routes of exposure to the general public and, separately, to specific occupations. For example, Chapter 5 provides some description of ASGM as an important source of mercury, but it is barely mentioned in Chapter 1. Sources of exposure by occupation could benefit from the creation of a table of occupations that shows the level of mercury measured (by species) for each population subgroup. This table could also show some comparisons of mercury exposure in the general population, comparing US populations to Europe, Canada, S. America, etc.

RESPONSE: *Section 1.1 is intended to be a brief overview and as such, cannot provide the level of detail the Reviewer is recommending. Measured occupational exposure levels vary hugely depending on the occupation, data collection methods, and date of the study (e.g., reflecting changing industrial hygiene). This is one reason why ATSDR has relied on biomarkers to represent exposures in the discussion of epidemiological studies and in the derivation of MRLs based on epidemiological studies.*

Regarding reporting of exposure levels by mercury species, studies of occupational exposures rarely report anything but total mercury without speciation.

Regarding the Reviewer's comment on providing a table on sources of exposure: Section 1.1 has been revised to refer the reader to Sections 5.6 and 5.7 for a discussion of sources of mercury exposure.

> The general population is exposed to all forms of mercury. However, exposure of the general population is primarily to organic mercury from dietary exposure to methylmercury (e.g., fish, seafood, rice) and elemental mercury from dental amalgams. Relative to organic and elemental mercury, exposure of the general population to inorganic mercury compounds is minimal. Occupational exposures are primarily to elemental mercury (e.g., dentistry, chloralkali process). Predominant sources of exposure to the general population and occupational exposures are described in greater detail in Sections 5.6 and 5.7.

COMMENT 8: 5. There is no discussion of Mercury(II) cyanide in this profile. This is a major exclusion particularly with the use of cyanide in Nevada for mining as well as the general proliferation (and expected increase) of cyanide in ASGM operations. Please see the suggested citation Drace et.al. In addition, there are studies showing the health effects of Mercury-cyanide exposure (Seney et.al 2020). Suggested text to include in the profile:

a. Solvated mercury complexes react with cyanide to form mercury-cyanide complexes of the general formula: $Hg(CN)_n^{2-n}$.
b. $Hg(CN)_2$ affects the kidneys at low concentrations, with damage occurring at concentrations as low as 0.2 mg/kg body weight. Hg is found in the blood and blood-rich organs, such as the spleen and liver. Splenocyte proliferation increased significantly following exposure to all concentrations of $Hg(CN)_2$, indicating the presence of an inflammatory process that may result in chronic inflammation even at low concentrations of $Hg(CN)_2$. In addition, in the presence of LPS decreased cellular proliferation of splenocytes isolated from rats treated with $Hg(CN)_2$ was noted. This indicates that exposure to $Hg(CN)_2$ may potentially impair the immune system, limiting its ability to address microbial infection and exposure to other toxicants
c. Based on these data, individuals exposed to $Hg(CN)_2$ may be unable to mount an adequate immune response following exposure to other toxicants, bacteria, viruses, etc., leaving them susceptible to a number of disease conditions.
d. Exposure to $Hg(CN)_2$ appeared to be more nephrotoxic than exposure to a corresponding dose of $HgCl_{2.60}$

RESPONSE: *ATSDR agrees that mercuric cyanide is a potential form of exposure to mercury. However, ATSDR has refrained from profiling the toxicology of mercuric cyanide because the exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in great detail in the profile. A description of the toxicology of cyanide is beyond the scope of the mercury profile and is covered in the Toxicological Profile for Cyanide. The following was added to Section 2.1 of the profile:*

> The toxicology of mercuric cyanide is not discussed in this profile because the exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in this profile. The toxicology of cyanide is described in the ATSDR Toxicological Profile for Cyanide.

COMMENT 9: 6. Global vs. US inference. There are often statements in the overview sections of chapters that make very general statements about trends or relationships, which are only relevant for the US context. For example, p 513, line 25-26.

RESPONSE: *The Section 5.3 has been revised:*

> Anthropogenic releases have historically been primarily to the atmosphere; however, in the United States, these levels have been decreasing as regulations and engineering controls on point source and fugitive emissions limit the amount of mercury released to air.

COMMENT 10: 7. Environmental Justice issues. In Chapters 3 and 5 there is discussion about who is more susceptible (section 3.2) and what populations have potentially high exposure (section 5.7). While these are technically correct, there are also important vulnerability issues for subpopulations, particularly indigenous (e.g., Native populations in Alaska, Canada and the Amazon) and populations with poor access to health care living near resource extractive industries (e.g., Appalachia). These populations, regardless of their age, genetic predispositions, or other factors, have greater risk of exposure and greater risk of adverse outcomes due to the overall social, environmental and economic issues surrounding them. I highly recommend a paragraph devoted to vulnerable populations from an environmental justice perspective be added to Chapter 5.

RESPONSE: *Section 3.2 is intended to discuss populations that are more susceptible as a result of unique behavioral, toxicokinetic, or toxicodynamic factors that can shift the exposure-response relationship for the major health effects of mercury. ATSDR does not discuss societal issues that affect access to health care services or other resources, as these factors would affect susceptibility to any chemical as well as to a host of diseases. The discussion of genetic predispositions is restricted to those that have been shown to interact with mercury in a way that may change the mercury dose-response relationship. Populations that live near sources of environmental mercury may have higher exposures (see Section 5.7); however, this does not necessarily make them more susceptible to mercury toxicity as a result of a change in the exposure-response relationship. ATSDR may consider including discussion of environmental justice issues in future profiles, pending revision of ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT 11: Add to section 2.15:

> Wyatt L, Permar SR, Ortiz E, Berky A, Woods CW, Amouou GF, Itell H, Hsu-Kim H, Pan W. Mercury Exposure and Poor Nutritional Status Reduce Response to Six Expanded Program on Immunization Vaccines in Children: An Observational Cohort Study of Communities Affected by Gold Mining in the Peruvian Amazon. Int J Environ Res Public Health. 2019 Feb 21;16(4):638. doi: 10.3390/ijerph16040638. PMID: 30795575; PMCID: PMC6406457.

RESPONSE: *Table 2-33 and related text in Section 2.15 have been revised to include Wyatt et al. (2019).*

> Studies consist of two prospective studies in children (Hui et al. 2016; Oulhote et al. 2017a), one cross-sectional study in mother-infant pairs (Nyland et al. 2011), one cohort study in pregnant women (McSorley et al. 2018), and a cohort of children (Wyatt et al. 2019). Endpoints examined include serum levels of cytokines (Hui et al. 2016; McSorley et al. 2018; Nyland et al. 2011) and immunoglobulins (Hui et al. 2016; Nyland et al. 2011), immune cell counts (Oulhote et al. 2017a), and antibody response to vaccinations (Wyatt et al. 2019).
>
> A study of children who resided in the Amazonian River Basin, where exposure to dietary methylmercury occurs as a result of wastes from gold mining operations, found decreased antibody response to diphtheria, measles, and pertussis vaccinations in association with a combination of malnutrition and increasing hair mercury levels (Wyatt et al. 2019).
>
> Table 2-33: *see Wyatt et al. (2019) inser*t.

COMMENT 12: Add to section 2.16, Table 2-43:

Reuben A, Frischtak H, Berky A, Ortiz EJ, Morales AM, Hsu-Kim H, Pendergast LL, Pan WK. Elevated Hair Mercury Levels Are Associated With Neurodevelopmental Deficits in Children Living Near Artisanal and Small-Scale Gold Mining in Peru. Geohealth. 2020 May 21;4(5):e2019GH000222. doi: 10.1029/2019GH000222. PMID: 32490301; PMCID: PMC7240868.

RESPONSE: *Section 2.16.1 has been revised to include Reuben et al. (2020).*

Amazonian riverine populations. Studies of methylmercury exposure and neurodevelopmental outcomes have been conducted in populations living in Amazon River basins (Amazonian studies). These include several cross-sectional studies of children from birth cohorts who resided in various river basins, with neurodevelopmental assessments in infancy and various later ages, with the oldest cohort being 14 years of age (Chevrier et al. 2009; Cordier et al. 2002; Dorea et al. 2012, 2014; dos Santos Freitas et al. 2018; Hoshino et al. 2015; Marques et al. 2007, 2015; Reuben et al. 2020).

Studies of Amazonian populations have found associations between prenatal (maternal) or child hair mercury levels and performance on tests of cognitive ability (Chevrier et al. 2009; Cordier et al. 2002; Reuben et al. 2020). A study of families residing in an artisanal and small-scale gold mining region of Amazonian Peru evaluated associations between child (mean age 8 years, N=163) hair mercury and visual-motor coordination, general cognitive ability, and physical health (Reuben et al. 2020). The mean hair mercury was 2.06 µg Hg/g (range 0.08, 14.61). Increasing hair mercury was associated with decreasing scores of the Spanish language Woodcock-Johnson Tests of Cognitive Abilities (β = -2.59 points per ln[µg Hg/g hair], 95% CI -4.52, -0.66).

Artisanal gold mining. Studies have been conducted of neurodevelopment outcomes in populations exposed to mercury released from artisanal gold mining operations (Counter 2003; Counter et al. 1998, 2002, 2006, 2012; Ramirez et al. 2000, 2003 Reuben et al. 2020).

Reuben et al. (2020) was also added to Table 2-43.

COMMENT 13: Add to section 2.20, genotoxicity studies, p. 426, lines 16

Berky AJ, Ryde IT, Feingold B, Ortiz EJ, Wyatt LH, Weinhouse C, Hsu-Kim H, Meyer JN, Pan WK. Predictors of mitochondrial DNA copy number and damage in a mercury-exposed rural Peruvian population near artisanal and small-scale gold mining: An exploratory study. Environ Mol Mutagen. 2019 Mar;60(2):197-210. doi: 10.1002/em.22244. Epub 2018 Oct 5. PMID: 30289587; PMCID: PMC6452630.

RESPONSE: *Berky et al. (2019) was added to Table 2-78. The following text was added to Section 2.20:*

The potential association between exposure to mercury and mitochondrial DNA copy number or damage in WBCs was assessed in Peruvian subjects living various distances from artisanal and small-scale gold mining operations outside the capital city of Puerto Maldonado (Berky et al. 2019). Exposure to mercury in these populations was attributed to consumption of methylmercury contaminated fish. Overall, hair mercury levels were similar across regions and no associations were observed between hair mercury levels and mitochondrial DNA copy number or damage. Additionally, no associations were found when the data were stratified by

relationship to mining operations (upriver, near Puerto Maldonado, downriver). However, when evaluated just in individuals who lived >20 miles outside of the capital city, hair mercury levels were significantly associated with increased mitochondrial DNA damage.

COMMENT 14: For justifying the use of creatinine adjusted urine mercury testing (p 491 lines 5-15):

- Lee E, Park HK, Kim HJ. Adjustment of urinary mercury in health risk assessment of mercury. J Korean Med Sci. 1996 Aug;11(4):319-25. doi: 10.3346/jkms.1996.11.4.319. PMID: 8878800; PMCID: PMC3054087.
- MacPherson, S., Arbuckle, T.E. & Fisher, M. Adjusting urinary chemical biomarkers for hydration status during pregnancy. J Expo Sci Environ Epidemiol 28, 481–493 (2018). https://doi.org/10.1038/s41370-018-0043-z
- Trachtenberg, F., Barregård, L., & McKinlay, S. (2010). The influence of urinary flow rate on mercury excretion in children. Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS), 24(1), 31–35. https://doi.org/10.1016/j.jtemb.2009.06.003
- Martin MD, McCann T, Naleway C, Woods JS, Leroux BG, Bollen AM. The validity of spot urine samples for low-level occupational mercury exposure assessment and relationship to porphyrin and creatinine excretion rates. J Pharmacol Exp Ther. 1996 Apr;277(1):239-44. PMID: 8613926.

RESPONSE: *The text in Section 3.3.1 has been revised to include the references noted in the comment.*

The creatinine and specific gravity adjustments are intended to standardize a measured concentration for variations in urine volume, which by itself can result in concentration or dilution of urinary mercury (Diamond 1988; Lee et al. 1996; MacPherson et al. 2018; Martin et al. 1996; Trachtenberg et al. 2010).

COMMENT 15: References for the inclusion of Mercury Cyanide compounds

- Drace K, Kiefer AM, Veiga MM. Cyanidation of Mercury-Contaminated Tailings: Potential Health Effects and Environmental Justice. Curr Environ Health Rep. 2016 Dec;3(4):443-449. doi: 10.1007/s40572-016-0113-0. PMID: 27696224.
- Seney CS, Bridges CC, Aljic S, Moore ME, Orr SE, Barnes MC, et al. 2020. Reaction of cyanide with hg0-contaminated gold mining tailings produces soluble mercuric cyanide complexes. Chemical Research in Toxicology 33:2834-2844. https://doi.org/10.1021/acs.chemrestox.0c00211

RESPONSE: *ATSDR agrees that mercuric cyanide is a potential form of exposure to mercury. However, ATSDR has refrained from profiling the toxicology of mercuric cyanide because the exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in great detail in the profile. A description of the toxicology of cyanide is beyond the scope of the mercury profile and is covered in the ATSDR Toxicological Profile for Cyanide. The following was added to Section 2.1 the profile:*

The toxicology of mercuric cyanide is not discussed in this profile because the exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in this profile. The toxicology of cyanide is described in the ATSDR Toxicological Profile for Cyanide.

COMMENT 16: Add to p 506, lines 20-21 on the exposure of mercury emissions around gold shops
Moody KH, Hasan KM, Aljic S, Blakeman VM, Hicks LP, Loving DC, Moore ME, Hammett BS, Silva-González M, Seney CS, Kiefer AM. Mercury emissions from Peruvian gold shops: Potential ramifications for Minamata compliance in artisanal and small-scale gold mining communities. Environ Res. 2020 Mar;182:109042. doi: 10.1016/j.envres.2019.109042. Epub 2019 Dec 24. PMID: 32069769.

RESPONSE: *Text in Section 5.2.1 has been revised to include Moody et al. (2020).*
This process can result in direct exposures of mine workers, gold shop merchants, and nearby households to mercury vapor and can release mercury to the environment where it can be converted to methylmercury (Counter et al. 1998; Diringer et al. 2015; Moody et al. 2020; Ramirez et al. 2000; Salazar-Camacho et al. 2021).

COMMENT 17: P 528-529 lines 5-6 and 1-4. Add to compare concentrations of mercury in fish in the amazon
Diringer S, Feingold B, Ortiz E, Gallis J, Araujo-Flores J, Berky A, et al. 2015. River transport of mercury from artisanal and small-scale gold mining and risks for dietary mercury exposure in madre de dios, peru. Environmental Science: Processes and Impacts 17:478-487.

RESPONSE: *Text in Section 5.4.1 has been revised to include Diringer et al. (2015).*
Mercury levels in freshwater fish have been shown to be elevated in areas impacted by gold mining operations (Diringer et al. 2015; Salazar-Camacho et al. 2021).

COMMENT 18: Articles on Environmental Justice (see point 7 above). These articles include populations with both high exposure and high susceptibility to health effects. I can suggest SEVERAL similar publications if needed

- Weinhouse C, Gallis JA, Ortiz E, Berky AJ, Morales AM, Diringer SE, Harrington J, Bullins P, Rogers L, Hare-Grogg J, Hsu-Kim H, Pan WK. A population-based mercury exposure assessment near an artisanal and small-scale gold mining site in the Peruvian Amazon. J Expo Sci Environ Epidemiol. 2021 Feb;31(1):126-136. doi: 10.1038/s41370-020-0234-2. Epub 2020 May 28. PMID: 32467625; PMCID: PMC8281380.
- Golzadeh N, Barst BD, Basu N, Baker JM, Auger JC, McKinney MA. Evaluating the concentrations of total mercury, methylmercury, selenium, and selenium:mercury molar ratios in traditional foods of the Bigstone Cree in Alberta, Canada. Chemosphere. 2020 Jul;250:126285. doi: 10.1016/j.chemosphere.2020.126285. Epub 2020 Feb 21. PMID: 32114346.
- Jerome, Nriagu, Basu, Niladri and Charles, Simone. "CHAPTER 15. Environmental Justice: The Mercury Connection". Mercury in the Environment, edited by Michael S. Bank, Berkeley: University of California Press, 2012, pp. 301-316. https://doi.org/10.1525/9780520951396-019
- Adamou TY, Riva M, Muckle G, Laouan Sidi EA, Lemire M, Ayotte P. Blood mercury and plasma polychlorinated biphenyls concentrations in pregnant Inuit women from Nunavik: Temporal trends, 1992-2017. Sci Total Environ. 2020 Nov 15;743:140495. doi: 10.1016/j.scitotenv.2020.140495. Epub 2020 Jul 3. PMID: 32758811.
- Wheatley, M. (1981). The effect of eating Habits on mercury Levels among Inuit residents of Sugluk, P.Q. Études/Inuit/Studies, 5(1), 27-43. Retrieved July 19, 2021, from http://www.jstor.org/stable/42870506

RESPONSE: *ATSDR does not discuss in toxicological profiles societal issues that affect access to health care services or other resources, as these factors would affect susceptibility to any chemical as well as to*

a host of diseases. ATSDR may consider including discussion of environmental justice issues in future profiles, pending revision of ATSDR's Guidance for the Preparation of Toxicological Profiles.

Chapter 1

COMMENT 19: I suggest including paragraphs describing the multi-organ impact of metal exposure to communicate the idea that exposure can affect different organ systems simultaneously. MINAMATA includes this in their report. This profile should have one as well.

RESPONSE: *ATSDR agrees that, as with nearly all chemicals, toxicity can involve many different organ systems and these effects can have independent actions or interactions; and interactions may vary with dose level. Section 1.2 includes a summary of the major systemic effects of mercury described in Chapter 2 of the profile.*

COMMENT 20: To present system by system effect, is perhaps not the right way to communicate to clinicians – this will lead to compartmentalized treatment. Risk needs to be considered from both a cumulative and interactive point of view. i.e., cumulative effects and interactions between multi-affected organ systems can guide how to treat an individual

RESPONSE: *The purpose of the ATSDR Toxicological Profile is to provide a summary of the health effects of mercury, not to provide guidance on how to clinically manage mercury toxicity. All ATSDR Toxicological Profiles present the discussion of health effects, organized by organ system. This format is followed to make it easier for the reader to quickly access the information on any given health effect. ATSDR develops educational and informational materials for health care providers categorized by hazardous substance, clinical condition, and/or by susceptible population. See* https://www.atsdr.cdc.gov/emes/health_professionals/index.html *and Appendix D, QUICK REFERENCE FOR HEALTH CARE PROVIDERS, for more information on resources for clinicians.*

COMMENT 21: MRLs: It is not clear what criteria was used to judge whether studies focused on one particular exposure is sufficient. For example, the calculation of MRLs for inorganic Hg salts (oral, intermediate duration) cites 12 animal studies for LOAELs; however, the MRL calculations for elemental Hg (inhalation, acute exposure) states there is insufficient data, citing 7 "selected" studies reporting NOAEL and LOAEL results. What is the criteria used to state that one MRL has sufficient information and one does not? None of this is clear in the text or appendix.

RESPONSE: *ATSDR uses a number of criteria for determining whether a database is adequate to support derivation of an MRL. The criteria include the quality of the studies, whether a wide range of endpoints were evaluated, and whether data can be used to establish dose-response relationships; the number of studies in the database is not a criterion used to assess the adequacy of the database. A discussion of why a particular database was not considered adequate for MRL derivation is included in Appendix A.*

COMMENT 22: MRLs: It is not clear why the values were assumed for uncertainty factors. This includes for the UF of 10 and 3. It is hard to agree or disagree with these values without knowing how they were derived.

RESPONSE: *ATSDR, the U.S. Environmental Protection Agency (EPA), and other agencies use a default uncertainty factor of 10. An uncertainty factor of 3 is used to account for the species to species extrapolation if dosimetric adjustments are made to the point of departure from animal inhalation studies.*

Chapter 2

COMMENT 23: Most of the major comments for chapter 2 are listed in overall comments and in the annotated version of the text

RESPONSE: *No response needed.*

Chapter 3

COMMENT 24: Section 3.1, line 20 and p 446 line 1 – it is confusing to say 2% of dermal exposure is from inhalation. It isn't clear what this means.

RESPONSE: *The text in Section 3.1 has been revised.*

Dermal: Systemic absorption of mercury during a full-body immersion in mercury vapor was estimated to be 2% of the amount absorbed from inhalation during the immersion.

Systemic dermal absorption
Section 3.1.1. Based on the measured rate of dermal absorption and 80% absorption of inhaled mercury vapor, the relative contributions of the dermal and inhalation absorption routes during a full body immersion in mercury vapor were estimated to be 2.6 and 97.4% respectively (Hursh et al. 1989).

COMMENT 25: Section 3.3.1 – the discussion of nails as a biomarker was not provided prior to the paragraph written on nails. This paragraph also does not mention the validity of mercury in nails as a measurement of mercury body burden. The recommendation of nails for organic Hg exposure should be described in the context of nails being correlated with actual health outcomes (i.e., nails were generally not discussed as a valid biomarker in Chapters 1 or 2 either)

RESPONSE: *Section 3.3.1 is intended to be a discussion of biomarkers that have been used to assess exposure and the basis for their uses. The section is not intended to critically evaluate the validity of the use of any specific biomarker. Use of nail mercury as an exposure biomarker is noted in Section 1.2 and in various studies described in Section 2, where it has been used in cited epidemiological studies.*

COMMENT 26: Urine is not a good biomarker of total mercury (p 490, lines 10-11). It is not valid for organic (methyl) mercury. This sentence is confusing and contradicts statements in other parts of chapter 3 (i.e., p. 490, p 467 line 14, etc.).

RESPONSE: *ATSDR agrees with the statement that urine total mercury is not a good biomarker of exposure to methylmercury. Total urine mercury reflects total exposure and provides no information about the form of exposure. The next sentence states this directly:* As discussed below, biomarkers that are more strongly correlated to methylmercury exposure are methylmercury concentration in whole blood, or total mercury concentration in RBCs or hair; these are more significant depots for accumulation of methylmercury than inorganic mercury.

COMMENT 27: Urine also needs to be creatinine-adjusted to be valid. P. 491 line 12 implies that non-adjusted urine mercury is equivalent simply because it explains variance. Note, it is impossible for me to review the Park & Chung 1980 article since it is written in Korean. There are several, more recent articles that demonstrate the recommendation that creatinine-adjusted urinary mercury is required. See citations listed above.

RESPONSE: *The text in Section 3.3.1 has been revised to include the reference suggested by the Reviewer. The Park and Chung (1980) reference was deleted from the profile.*

> The creatinine and specific gravity adjustments are intended to standardize a measured concentration for variations in urine volume, which by itself can result in concentration or dilution of urinary mercury (Diamond 1988; Lee et al. 1996; MacPherson et al. 2018; Martin et al. 1996; Trachtenberg et al. 2010).

COMMENT 28: P. 492 line 23 – how do we define what the dominant source of exposure is *prior to* evaluating mercury exposure? This is circular. The profile should state that biomarker testing should be accompanied with a risk factor survey that can more accurately identify the source of exposure so that the biomarker result can be interpreted correctly (i.e., as inorganic, elemental or organic Hg).

RESPONSE: *The text in Section 3.3.1 has been revised to include the following statement:*

> Total blood mercury should be interpreted as a biomarker of exposure to methylmercury only if other information is available that supports methylmercury being the dominant form of exposure in the population.

COMMENT 29: Section 3.1.5 – PBPK models. I suggest including an overview table of the models presented that include a general description of their application (what they are used for), who are they used for (adults, children, pregnant women, etc.), etc, to provide more organization for the reader. I also suggest that the section provide an explanation for which models are the predominant models used for specific applications as well as some kind of graphics showing how well the models have fit past data (to demonstrate goodness of fit). There is also an insufficient amount of text provided to describe the differences between human and human toxicokinetic models (i.e., the relevance of animal toxicokinetics is not put into context)

RESPONSE: *The intent of Section 3.1.5 is to allow the reader to be aware of existing published PBPK models and offer a brief description of the structure of each model. The section includes a discussion of the basis for calibration and evaluation of each model and provides references to published applications of the models. A critical evaluation of each model or a comparison and contrasting of models is beyond the scope of Section 3.1.5. Information on toxicokinetics in humans and other species is provided in Section 3.1.*

COMMENT 30: Section 3.4. This section is weakly written. See annotated comments in text.

RESPONSE: *The intent of Section 3.4 is to provide a brief summary of the interactions between mercury and other chemicals that have been published. The focus of the section is to discuss chemical interactions that may alter the toxicity of mercury. The topic of dose and response interactions (e.g., additivity, synergy, inhibition) is complex and is the subject of ATSDR interaction profiles, noted in Section 3.4 (ATSDR 2004).*

Chapter 4

COMMENT 31: Add Mercury (II) Cyanide as a mercury compound

RESPONSE: *ATSDR agrees that mercuric cyanide is a potential form of exposure to mercury. However, ATSDR has refrained from profiling the toxicology of mercuric cyanide for the following reasons. The exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in great detail in the profile. A description of the toxicology of cyanide is beyond the scope of this mercury profile and is covered in the ATSDR Toxicological Profile for Cyanide. Given that the toxicology of mercuric cyanide is not a topic of the profile, it is not included in Section 4.* Section 2.1 has been revised to include:

> The toxicology of mercuric cyanide is not discussed in this profile because the exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in this profile. The toxicology of cyanide is described in the ATSDR Toxicological Profile for Cyanide.

Chapter 5

COMMENT 32: Why are dental amalgams such as large part of the discussion and characterized as a "major" source of exposure? Many countries are phasing out their use and the exposure is inorganic, which is much less impactful than organic. See annotated comments

RESPONSE: *ATSDR agrees that use of mercury amalgams in dental restorations is in decline; however, mercury amalgam will continue to be a source of exposure in people who have dental amalgams, during their replacement, and during processing of amalgam by dental practitioners. As noted in Section 5.2, in 2018, approximately 9,287 pounds of elemental mercury were used to produce dental amalgam in the United States (EPA 2020b).*

COMMENT 33: P. 503-504: see annotated comments suggesting creation of a table showing the bullet points on lines 16-24 (p 503) and lines 1-2 (p 504)

RESPONSE: *Figure 5-1 appears in all ATSDR Toxicological Profiles and is derived from the EPA National Priorities List (NPL). This source does not provide data on mercury species.*

COMMENT 34: P. 505 – US has stopped production of mercury since 1992; however, in tables 5-1 and 5-2, facilities are listed as "producing" mercury. This is confusing. Is this meant to mean "Recycling?"

RESPONSE: *Text in Section 5.2.1 has been revised to include an explanation of the designation "produce" in Tables 5-1 and 5-2.*

> Data from the TRI is intended to meet the requirements of the Emergency Planning and Community Right-to-Know Act (EPCRA) Section 313 for mercury and the mercury compounds category. If a facility processes or imports mercury or mercury containing compounds, it is required to report these data to the TRI and this meets the definition of the reporting category, Produce.

COMMENT 35: Section 5-3: there is a specific description of how facilities are defined as TRI facilities; however, the listing of these codes and classifications are obtuse. How is anyone supposed to know what these mean? I suggest including: (1) a statement of the types of facilities typically included and the types of facilities excluded from TRI, with examples; and (2) listing a table or flowchart showing the total number of Hg-releasing facilities and how many facilities are excluded from being classified in TRI data based on the exclusion criteria provided.

- Also modify Tables 5-6 to show "total annual emissions", not just a percentage of total, and create another table (i.e., Table 5-6a) showing annual emissions from natural sources

RESPONSE: *The text introducing the TRI data is included in all ATSDR Toxicological Profiles. ATSDR will consider revising the format of this section in its periodic review of the profile format and ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT 36: Section 5-4:

- Add a figure showing the environmental cycling of Hg (natural and anthropogenic)
- P. 528-529: include Diringer et.al. 2015 paper to compare with the Salazar paper for bioaccumulation of Hg. The Diringer paper is more complete than the Salazar-Camacho paper, demonstrating bioaccumulation

RESPONSE: *A figure (Figure 5-3) showing the environmental cycling of Hg (natural and anthropogenic) was added to Section 5.4.*

Text in Section 5.4.1 has been revised to include Diringer et al. (2015).

> Mercury levels in freshwater fish have been shown to be elevated in areas impacted by gold mining operations (Diringer et al. 2015; Salazar-Camacho et al. 2021).

COMMENT 37: Section 5-5: Tables 5-19, 5-20 and 5-21: modify to report quartiles (min, 25th, 50th, 75th, maximum) rather than min, mean and maximum

RESPONSE: *The data are presented as reported in the source document (EPA 1999); the 25th and 75th percentiles are not reported.*

COMMENT 38: Section 5-7: Add subsection on environmental justice (see major comments above)

RESPONSE: *ATSDR does not discuss in toxicological profiles societal issues that affect access to health care services or other resources, as these factors would affect susceptibility to any chemical as well as to a host of diseases. ATSDR may consider including discussion of environmental justice issues in future profiles, pending revision of ATSDR's Guidance for the Preparation of Toxicological Profiles.*

Chapter 6

COMMENT 39: P. 604-605 lines 31-33 and 1-2: Need to specifically state that better integrated surveillance is needed such that mercury measurements are directly included in existing health systems. For example, in the US, all newborns should receive a heel stick to test for both blood lead and blood mercury levels. Alternatively, these mercury testing can be conducted on hair. Urine and hair mercury testing can be conducted at age 1, 5 and 12. These exposure assessments need to be integrated into regular clinical practice and surveillance reporting.

RESPONSE: *ATSDR does not provide in toxicological profiles recommendations or proposals regarding public health surveillance or diagnostic procedures.*

Chapter 7

COMMENT 40: This chapter should have a summary table showing the results of the MRL calculations. Such a summary table either belongs here or in Chapter 1 (or both)

RESPONSE: *ATSDR does not include MRL values in Chapter 7. Information on the calculation of the MRLs is described in detail in Appendix A. Chapter 1 includes a table (Table 1-1 through 1-3) that summarizes MRLs, including the derived MRL value, critical effect, point of departure, uncertainty factors, and critical study or studies. A footnote in this table refers the reader to Appendix A for more information on MRL derivation.*

Annotated Comments

The Reviewer suggested a number of editorial revisions. The suggested revisions were made to the profile. Responses to Reviewer comments that were not considered editorial or stylistic are presented below.

COMMENT 41: Referring to the statement in Section 1.1, lines 11-13 on page 1 – Anthropogenic emissions of mercury have typically been to the atmosphere, but these emissions have been declining for the past several decades in North America. – the Reviewer commented "Reference?
This sentence is misleading – it implies we are not impacted by global mercury emissions. I would suggest rewriting to add:
"; although global mercury emissions continue to rise due to activities such as artisanal gold mining and fossil fuel burning"".

RESPONSE: *For Chapter 1 of the profile, references are only included for very specific information (for example, National Health and Nutrition Examination Survey [NHANES] biomarker data), but not for general information. The revision suggested by the reviewer was included in Section 1.1.*

> Anthropogenic emissions of mercury have typically been to the atmosphere; although these emissions have been declining for the past several decades in North America, global emissions continue to rise due to activities such as artisanal gold mining and fossil fuel burning.

COMMENT 42: Referring to the statement in Section 1.2, line 13 on page 4 – Animal studies focus on the same exposure routes as epidemiological studies. – the Reviewer replaced 'the same' with 'similar' and commented "That are not the same. i.e., animal models don't use dental amalgams."

RESPONSE: *The sentence in Section 1.2 was revised as follows:*

> Animal studies generally focus on similar exposure routes as epidemiological studies.

COMMENT 43: Referring to the statement in Section 1.2, lines 19-20 on page 6 – However, relative to neurological effects, it does not appear that these effects are sensitive targets for environmental exposures to methylmercury. – the Reviewer commented "I don't agree with this sentence. It is also not consistent with many of the relationships discussed in chapter 2."

RESPONSE: *The sentence noted by the Reviewer in Section 1.2 was deleted from the profile.*

COMMENT 44: Referring to Figure 1-1 in Section 1.4 on page 10, the Reviewer commented "Check the units on the dose. Is there a time unit?"

RESPONSE: *For Figure 1-1 in Section 1.3 (not Section 1.4), there is no time unit for the dose. The exposure duration (time) is indicated as "Acute" and "Intermediate" in the boxes under "Effects in Animals."*

COMMENT 45: Referring to Figure 1-4 in Section 1.4 on page 13, the Reviewer commented "Check if dose requires a time unit."

RESPONSE: *For Figure 1-4 in Section 1.3 (not Section 1.4), there is no time unit for the dose. The exposure duration (time) is indicated in the figure as "Acute," "Intermediate," and "Chronic."*

COMMENT 46: Referring to Figure 1-6 in Section 1.4 on page 15, the Reviewer commented "Figure 1-4 uses a filled in triangle. Why is this an open triangle?"

RESPONSE: *The open triangle was changed to a closed triangle per the Reviewer's comment.*

COMMENT 47: Referring to the sub-heading in Table 1.1 in Section 1.4 on page 16 – Inhalation exposure (μg Hg/m^3) – the Reviewer commented "Time unit?"

RESPONSE: *For Figure 1-4 in Section 1.3 (not Section 1.4), there is no time unit for the dose. The exposure duration (time) is indicated in the Table as "Acute," "Intermediate," and "Chronic."*

COMMENT 48: Referring to the statements in Section 2.1, lines 5-9 on page 19 – ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. – the Reviewer commented "What judgement is used? Where is this described? Is it consistent for each health effect?"

RESPONSE: *ATSDR defines less serious LOAELs and serious LOAELs in the sentences prior to the referenced sentence. Additional guidance on categorizing the adversity to health effects is contained in ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT 49: Referring to Figure 2-1 in Section 2.1 on page 33, the Reviewer commented "If this is not supposed to be a complete systematic review as previously described, what is the purpose of this figure? It could have significant bias based on the judgement applied to include or exclude a study (with said judgement not actually described in this profile).
The same comment applies to Figures 2-1 to 2-24

I would suggest an edit to these figures stating that these are the studies used in this profile, which is not an exhaustive list"

RESPONSE: *The title of Figures 2-1, 2-2, 2-3, and 2-4 were revised to "Overview of the Number of Studies Examining Elemental Mercury Health Effects in Chapter 2." The following statement in Section 2.1 notes the extensive nature of the literature databased on mercury:* The literature database on health effects of mercury is enormous, with a large number of epidemiological studies, including studies in children, and studies in laboratory animals. Due to the extent of the literature database, it is not practical or realistic to cite all, or even most, of the studies on health effects of mercury. Thus, this profile does not attempt to provide a comprehensive review of all literature; instead, the profile summarizes the major lines of evidence regarding health effects.

COMMENT 50: Referring to Figure 2-5 in Section 2.1 on page 43, the Reviewer commented "The Sprague-Dawley rats appear to have a slightly lower LSE – why? What is the difference between these animal models? Can a section be added to include a description that might explain sensitivity of a specific genetic animal model vs another?"

RESPONSE: *Discussions of general differences between animal strains and potential reasons for these differences are beyond the scope of the profile. Throughout Chapter 2, strain differences in the response to mercury exposure are noted in the text; however, the possible basis for the strain differences is only included if discussed by study authors.*

COMMENT 51: Referring to the statement in Section 2.4, lines 9-12 on page 146 – Epidemiological studies in adults reported positive associations between mercury exposure of general populations and body weight outcomes, including BMI, percent body fat, visceral adipose tissue, waist circumference, and overweight; however, data are limited. – the Reviewer commented "This comment applies to many sections that fine limited epidemiological data. If there are only 2-3 studies discussed for this exposure route, it would be nice to show the citation here."

RESPONSE: *All sections of Chapter 2 (Sections 2.2 through 2.20) begin with an overview intended to provide the reader with concise, high-level summaries of available data and health effects. Citations are only included in the overview if highly specific information is included or to provide citations for background information. The overviews are followed by detailed discussions of effects, with citations included.*

COMMENT 52: Referring to the statement in Section 2.5, lines 24-26 on page 155 – Data are not sufficient to determine if exposure to mercury in populations with high fish diets produces adverse respiratory effects, with only one study meeting inclusion criteria. – the Reviewer commented "? what is the criteria?"

RESPONSE: *Inclusion criteria are provided in Section 2.1, under the heading "Literature Search Strategy and Inclusion Criteria." As stated in Section 2.1:* Quality criteria were considered in selecting studies to include in the mercury profile and, in particular, for consideration as support for MRLs. In general, epidemiological studies that attempted dose-response assessments (e.g., regression models) were included in the profile if the following criteria were met: (1) reported estimates of variance in the dose-response metrics (e.g., standard error [SE], confidence level [CL]); (2) included adjustments for confounding; and (3) reported biomarker data. For studies used to derive MRLs, reporting of quality assurance of analytical methods was also required.

COMMENT 53: Referring to the statement in Section 2.6, lines 12-13 on page 158 – Taken together, results of epidemiological studies do not provide conclusive evidence that the cardiovascular system is a sensitive target for mercury. – the Reviewer commented "This is an example of where it is not clear how this judgement was made. For example, in this subsection, there are a number of studies cited that demonstrate a significant relationship between Hg exposure and SBP or DBP. On P. 169, lines 5-7, there is even a dose response that appears to be described. In Section 2.11 (Renal) there appears to be an equal amount of epi data, yet that section says there IS conclusive evidence for a relationship
I would recommend either re-reviewing this section for evaluating the effect or reducing the strength of this sentence by stating that current epidemiological studies do not provide conclusive evidence."

RESPONSE: *The sentence in Section 2.6 noted in the comment was revised to include recommendations by the Reviewer.*

> Taken together, results of current epidemiological studies do not provide conclusive evidence that the cardiovascular system is a highly sensitive target for mercury.

COMMENT 54: Referring to the statements in Section 2.11 on page 207 about the mechanism for mercury-induced glomerulonephritis and renal effects, the Reviewer commented "This is another example of different judgement used to evaluate the strength of an effect. How does the strength of this data differ from those given in the hematological or cardiovascular sections, which concluded no conclusive relationship?"

RESPONSE: *The intent of the overview of Section 2.11 is to summarize the major contributing evidence for renal effects of mercury, which are described in greater detail elsewhere in the section. As noted in the overview, evidence that mercury is nephrotoxic derives from extensive study in animal models, clinical cases histories, and epidemiological studies of exposure in workers. This general approach to weight-of-evidence discussion is used in all of the heath effect sections.*

COMMENT 55: Referring to the statement in Section 2.11, lines 22-30 on page 215 – These inconsistencies may be attributed to differences in study designs or exposure markers utilized; however, it may also indicate that the kidney is not a sensitive target organ at the levels of exposure to elemental mercury observed in these study populations. – the Reviewer commented "Why an undeniable effect if having inconsistencies?"

RESPONSE: *The epidemiology is inconsistent with respect to finding or not finding associations between mercury exposure biomarkers and renal effects. The text in Section 2.11 has been revised to emphasize the dose rather than study design as a potential contributor to these inconsistencies.*

> Some studies have found associations between exposure to mercury and decreased GFR or tubular damage; however, these outcomes were not consistently observed across studies at similar exposures (based on exposure biomarkers). These inconsistencies may reflect differences in exposure levels as well as differences in study designs or the exposure markers utilized.

COMMENT 56: Referring to the statement in Section 2.11, lines 11-13 on page 234 – Studies indicate that mercury exposure of the general population may be associated with glomerular and tubular damage, but few studies have been conducted; thus, data are not sufficient to draw firm conclusions regarding associations. – the Reviewer commented "Again—why the statement that the evidence is conclusive that

Hg exposure affects renal function? This is another example of different judgement used in each section."

RESPONSE: *Text in Section 2.11 has been revised to note that the data are limited because of the number of studies corroborating associations.*

> Few epidemiological studies in general populations have been conducted of renal outcomes associated with exposure to mercury. The results of these studies indicate that mercury exposure of the general population may be associated with glomerular and tubular damage.

COMMENT 57: Referring to the statement in Section 2.15, lines 7-8 on page 259 – Few studies have evaluated immunological effects in populations with high fish diets; studies meeting inclusion criteria are summarized in Table 2-33. – the Reviewer commented "? what is the criteria
For example, what is this paper excluded:

> Wyatt L, Permar SR, Ortiz E, Berky A, Woods CW, Amouou GF, Itell H, Hsu-Kim H, Pan W. Mercury Exposure and Poor Nutritional Status Reduce Response to Six Expanded Program on Immunization Vaccines in Children: An Observational Cohort Study of Communities Affected by Gold Mining in the Peruvian Amazon. Int J Environ Res Public Health. 2019 Feb 21;16(4):638. doi: 10.3390/ijerph16040638. PMID: 30795575; PMCID: PMC6406457.

Shows declines in immune response with elevated HHg."

RESPONSE: *Inclusion criteria provided in Section 2.1, under the heading "Literature Search Strategy and Inclusion Criteria," were expanded for clarification:*

> For epidemiological studies, well-conducted and reported studies were considered for inclusion in the profile. Quality criteria were considered in selecting studies to include in the mercury profile and, in particular, for consideration as support for MRLs. In general, epidemiological studies that attempted dose-response assessments (e.g., regression models) were included in the profile if the following criteria were met: (1) reported estimates of variance in the dose-response metrics (e.g., standard error [SE], confidence level [CL]); (2) included adjustments for confounding; and (3) reported biomarker data.

The Wyatt et al. (2019) paper was not previously included in the profile because it was published after the literature search was conducted for the updated profile (literature search dates were conducted in January 2019). An updated literature search will be conducted following receipt of public comments. Results of the Wyatt et al. (2019) study were added to Section 2.15, with additional details in Table 2-33.

> Studies consist of two prospective studies in children (Hui et al. 2016; Oulhote et al. 2017a), one cross-sectional study in mother-infant pairs (Nyland et al. 2011), one cohort study in pregnant women (McSorley et al. 2018), and a cohort of children (Wyatt et al. 2019). Endpoints examined include serum levels of cytokines (Hui et al. 2016; McSorley et al. 2018; Nyland et al. 2011) and immunoglobulins (Hui et al. 2016; Nyland et al. 2011), immune cell counts (Oulhote et al. 2017a), and antibody response to vaccinations (Wyatt et al. 2019).
>
> A study of children who resided in the Amazonian River Basin, where exposure to dietary methylmercury occurs as a result of wastes from gold mining operations, found decreased antibody response to diphtheria, measles, and pertussis vaccinations in association with a combination of malnutrition and increasing hair mercury levels (Wyatt et al. 2019).

COMMENT 58: Referring to the statement in Section 2.15, lines 14-17 on page 269 – Studies of general populations have examined associations between mercury biomarkers and several immune endpoints including immunological diseases, ANAs (an indicator of autoimmunity), serum cytokines, and

immune cell counts (Table 2-37). – the Reviewer commented "Awkward to state this here since it is stated at the beginning of the subsection..

RESPONSE: *The parenthetical phrase "an indicator of autoimmunity" was removed from the sentence in Section 2.15 noted by the Reviewer as follows:*

> Studies of general populations have examined associations between mercury biomarkers and several immune endpoints including immunological diseases, ANAs, serum cytokines, and immune cell counts (Table 2-37).

COMMENT 59: Referring to the geometric mean cord blood lead levels discussed in Section 2.16.1, lines 8-10 on page 304, the Reviewer commented "Is this supposed to be Hg and not Pb?"

RESPONSE: *The levels reported are of blood lead; Kjellstrom et al. measured blood lead levels because of concerns that exposure to lead could be a confounder of associations between hair mercury and neurodevelopmental outcomes.*

COMMENT 60: Referring to Table 2-43 in Section 2.16.1 on page 309, the Reviewer commented "Table is missing:

> Reuben A, Frischtak H, Berky A, Ortiz EJ, Morales AM, Hsu-Kim H, Pendergast LL, Pan WK. Elevated Hair Mercury Levels Are Associated With Neurodevelopmental Deficits in Children Living Near Artisanal and Small-Scale Gold Mining in Peru. Geohealth. 2020 May 21;4(5):e2019GH000222. doi: 10.1029/2019GH000222. PMID: 32490301; PMCID: PMC7240868."

RESPONSE: *Table 2-43 has been revised to include Reuben et al. (2020).*

COMMENT 61: Referring to the sub-heading in Section 2.16.2 on page 343 – Studies of fluorescent lamp production workers-neurological effects in adults – the Reviewer suggested removing '-neurological effects in adults' and commented "Unnecessary title."

RESPONSE: *The title of this subheading in Section 2.16.1 was revised to:* Studies of fluorescent lamp production workers.

COMMENT 62: Referring to the sub-heading in Section 2.16.2 on page 344 – Thermometer production workers – the Reviewer commented "This paragraph (section) never summarizes the net total effect of Hg exposure in this population. They style is very different compared to other parts of this nuerological section."

RESPONSE: *The discussion of thermometer workers in Section 2.16.2 has been revised to include the following summary statement, consistent with the discussion of other worker categories:*

> These studies compared signs and symptoms in workers and reference groups and found higher prevalence of tremor, impaired motor coordination, and impaired vision in workers.

COMMENT 63: Referring to the statement in Section 2.17, lines 25-27 on page 363 – Few studies meeting inclusion criteria were identified for workers and populations with high fish diets, whereas database for general populations was more robust. – the Reviewer commented "What are the criteria?"

RESPONSE: *Inclusion criteria are provided in Section 2.1, under the heading "Literature Search Strategy and Inclusion Criteria." As stated in Section 2.1,* Quality criteria were considered in selecting studies to include in the mercury profile and, in particular, for consideration as support for MRLs. In general, epidemiological studies that attempted dose-response assessments (e.g., regression models) were included in the profile if the following criteria were met: (1) reported estimates of variance in the dose-response metrics (e.g., standard error [SE], confidence level [CL]); (2) included adjustments for confounding; and (3) reported biomarker data. For studies used to derive MRLs, reporting of quality assurance of analytical methods was also required.

COMMENT 64: Referring to the header for Section 2.19 on page 415 – CANCER – the Reviewer commented "The challenge with epidemiological studies of cancer && Hg is that there are no studies that can prospectively follow a large cohort of people to look at lifetime exposures and compare exposure rates to observed cancer rates. The only way to evaluate cancer risk is through animal studies or design a Framingham type study."

RESPONSE: *ATSDR agrees with the Reviewer that there are no large, prospective, life-time exposure studies evaluating the potential carcinogenesis of mercury. However, evaluation of available epidemiological data to assess potential carcinogenesis of chemicals is standard practice of several agencies (EPA, International Agency for Research on Cancer [IARC], Department of Health and Human Services [HHS]). Conclusions from these agencies are summarized in Section 2.19 of the profile.*

COMMENT 65: Referring to the subsection Overview in Section 2.20 on page 418, the Reviewer commented "As a general observation – this subsection is organized very differently from other subsections. i.e., there is no separation of discussion of epi vs. animal studies."

RESPONSE: *ATSDR organizes the genotoxicity section of toxicological profiles by general study type –* in vivo *and* in vitro*. This is outlined in ATSDR's Guidance for the Preparation of Toxicological Profiles.* In vivo *studies are not further divided by human versus animal studies. The test species (human or animal) is clearly denoted in the tables in Section 2.20.*

COMMENT 66: Referring to the subsection Organic mercury in Section 2.20 on page 419, the Reviewer commented "One epi study that looked at DNA damage in humans with different levels of HHg (organic) exposure needs to be included:

> Berky AJ, Ryde IT, Feingold B, Ortiz EJ, Wyatt LH, Weinhouse C, Hsu-Kim H, Meyer JN, Pan WK. Predictors of mitochondrial DNA copy number and damage in a mercury-exposed rural Peruvian population near artisanal and small-scale gold mining: An exploratory study. Environ Mol Mutagen. 2019 Mar;60(2):197-210. doi: 10.1002/em.22244. Epub 2018 Oct 5. PMID: 30289587; PMCID: PMC6452630."

RESPONSE: *Berky et al. (2019) was added to Table 2-78. The following text was added to Section 2.20:*

> The potential association between exposure to mercury and mitochondrial DNA copy number or damage in WBCs was assessed in Peruvian subjects living various distances from artisanal and small-scale gold mining operations outside the capital city of Puerto Maldonado (Berky et al. 2019). Exposure to mercury in these populations was attributed to consumption of methylmercury contaminated fish. Overall, hair mercury levels were similar across regions and no associations were observed between hair mercury levels and mitochondrial DNA copy number

or damage. Additionally, no associations were found when the data were stratified by relationship to mining operations (upriver, near Puerto Maldonado, downriver). However, when evaluated just in individuals who lived >20 miles outside of the capital city, hair mercury levels were significantly associated with increased mitochondrial DNA damage.

COMMENT 67: Referring to the subsection Organic Mercury in Section 2.20 on page 426, the Reviewer commented "There needs to be a discussion of the Berky et.al. study here, which looked at DNA damage. See previous comment for citation."

RESPONSE: *See response to Comment 66.*

COMMENT 69: Referring to the statement in Section 3.1, lines 26-27 on page 439 – Inter-species variation in regional deposition of inhaled Hg^0 vapor has been observed (Leggett et al. 2001). – the Reviewer commented "Difference within animals. How does this relate to their relevance in application of human health effects?"

RESPONSE: *Inter-species differences in regional deposition of inhaled mercury vapor could contribute to inter-species differences in respiratory tract dosimetry and inter-species dosimetry extrapolations from animal models to humans. These differences would need to be considered in PBPK models used for this purpose. However, as described in Sections 3.1.5 and 6.2, there currently are no published PBPK data in models for extrapolating internal dosimetry of mercury vapor from animals to humans. Section 6.2 has been revised to:*

Models of mercury vapor and inorganic mercuric mercury in monkeys, mice, and rats would be helpful for extrapolating external dose-response relationships (e.g., NOAELs, LOAELs) observed in these species to equivalent external dose in humans.

COMMENT 70: Referring to the statement in Section 3.2 on page 484-485 – Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age. – the Reviewer removed 'or less' and commented "Why would they be less? None of the data shown indicate a lower susceptibility."

RESPONSE: *The referenced statement is part of boilerplate (template) text included in all ATSDR Toxicological Profiles in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles; it is not specific to mercury.*

COMMENT 71: Referring to the statement in Section 3.2, line 14 on page 485 – Children and the elderly are likely to have increased susceptibility to mercury compared to non-elderly adults as it is generally accepted that developing systems are more susceptible than mature systems. – the Reviewer commented "Define elderly. >65? >70?"

RESPONSE: *The statement in Section 3.2 was revised as follows:*

Children and older adults (≥65 years of age) are likely to have increased susceptibility to mercury compared to younger adults as it is generally accepted that developing and aging systems are more susceptible than mature, but not yet declining, systems.

COMMENT 72: Referring to the statement in Section 3.3.1, lines 10-12 on page 490 – Measurements of total mercury in blood and urine can be considered biomarkers of total exposure to all forms of mercury and do not provide information to confidently estimate the magnitude of exposures specifically to methylmercury, inorganic mercury compounds, or elemental mercury. – the Reviewer commented "This sentence needs to be clarified. Urine is not a good biomarker of organic mercury exposure, so you cannot say it is a biomarker of total exposure when exposure is predominatly organic. This is clearly stated on p. 467 that urine is not a good biomarker."

RESPONSE: *ATSDR agrees with the statement that urine total mercury is not a good biomarker of exposure to methylmercury. Total urine mercury reflects total exposure and provides no information about the form of exposure. The next sentence in Section 3.3.1 states this directly:* As discussed below, biomarkers that are more strongly correlated to methylmercury exposure are methylmercury concentration in whole blood, or total mercury concentration in RBCs or hair; these are more significant depots for accumulation of methylmercury than inorganic mercury.

COMMENT 73: Referring to the paragraph in Section 3.3.1, lines 5-15 on page 491 discussing the measurement of urinary mercury levels, the Reviewer commented "This paragraph needs to clearly state that the recommended biomarker is a creatinine-adjusted Uhg measurement. Not the non-adjustmented measurement. There are several publications that support this statement, particularly in pregnant women and people with low Hg exposure. I don't think you need all the citations, but here are several:

Lee E, Park HK, Kim HJ. Adjustment of urinary mercury in health risk assessment of mercury. J Korean Med Sci. 1996 Aug;11(4):319-25. doi: 10.3346/jkms.1996.11.4.319. PMID: 8878800; PMCID: PMC3054087.

MacPherson, S., Arbuckle, T.E. & Fisher, M. Adjusting urinary chemical biomarkers for hydration status during pregnancy. *J Expo Sci Environ Epidemiol* **28,** 481–493 (2018). https://doi.org/10.1038/s41370-018-0043-z

Trachtenberg, F., Barregård, L., & McKinlay, S. (2010). The influence of urinary flow rate on mercury excretion in children. *Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS)*, *24*(1), 31–35. https://doi.org/10.1016/j.jtemb.2009.06.003

Martin MD, McCann T, Naleway C, Woods JS, Leroux BG, Bollen AM. The validity of spot urine samples for low-level occupational mercury exposure assessment and relationship to porphyrin and creatinine excretion rates. J Pharmacol Exp Ther. 1996 Apr;277(1):239-44. PMID: 8613926."

RESPONSE: *The text in Section 3.3.1 has been revised to include the reference noted in the comment.*
The creatinine and specific gravity adjustments are intended to standardize a measured concentration for variations in urine volume, which by itself can result in concentration or dilution of urinary mercury (Diamond 1988; Lee et al. 1996; MacPherson et al. 2018; Martin et al. 1996; Trachtenberg et al. 2010).

COMMENT 74: Referring to the statement in Section 3.3.1, lines 22-24 on page 492 – It follows that, in populations in which methylmercury is the dominant source of mercury intake, total mercury in blood will be dominated by methylmercury and the blood total mercury concentration will reflect methylmercury intake. – the Reviewer commented "This is a circular statement – how does one know what the predominant course of exposure is?
One suggestion is that this sentence can use the WHO risk factor screening tool (https://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf)."

RESPONSE: *The text in Section 3.3.1 has been revised to include the following:*

> Total blood mercury should be interpreted as a biomarker of exposure to methylmercury only if other information is available that supports methylmercury being the dominant form of exposure in the population.

COMMENT 75: Referring to the discussion of mercury concentrations in nails in Section 3.3.1, lines 24-33 on page 494, the Reviewer commented "Why aren't nails discussed earlier in this chapter as to whether we can conclusively use nails as a biomarker (i.e., p 492, lines 5-17)? This seems out of place. I believe nails need to be mentioned, but they should be referenced earlier as another type of biomarker but without conclusive evidence for linking levels of NHg to health effects through MRLs."

RESPONSE: *Section 3.3.1 is intended to be a discussion of biomarkers that have been used to assess exposure and the basis for their uses. The section is not intended to critically evaluate the validity of the use of any specific biomarker. Use of nail mercury as an exposure biomarker is noted in Section 1.2 and various studies described in Section 2, where it has been used in cited epidemiological studies.*

COMMENT 76: Referring to the header for Section 3.4 on page 495 – INTERACTIONS WITH OTHER CHEMICALS – the Reviewer commented "In general, this section is completely insufficient. Very limited discussion on any interaction of any chemical with mercury. While I agree with keeping this section short, since the discussion of interactions could be quite dense and, overall, not productive since many of the interactions have limted data, I do think there needs to be a table listed on the types of interactions that are currently under study as well as a better discussion of the potential additive, multiplicative or competing interactions that might exist with some chemicals."

RESPONSE: *The intent of Section 3.4 is to provide brief summaries of the interactions between mercury and other chemicals. The topic of dose and response interactions (e.g., additivity, synergy, inhibition) is complex and is the subject of ATSDR Interaction Profiles, noted in Section 3.4 (ATSDR 2004).*

COMMENT 77: Referring to the header for Section 4.1 on page 497 – CHEMICAL IDENTITY – the Reviewer commented "This chapter is missing any mention of cyano-mercury compounds. In fact, there is no discussion of the health impacts of exposure to these compounds even though cyanide is used in gold mining in Nevada."

RESPONSE: *ATSDR agrees that mercuric cyanide is a potential form of exposure to mercury. However, ATSDR has refrained from profiling the toxicology of mercuric cyanide because the exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in great detail in the profile. A description of the toxicology of cyanide is beyond the scope of the mercury profile and is covered in the ATSDR Toxicological Profile for Cyanide. The following was added to Section 2.1 of the profile:*

> The toxicology of mercuric cyanide is not discussed in this profile because the exposure-response relationship for mercuric cyanide will reflect contributions of inorganic mercuric mercury and cyanide. The toxicology of inorganic mercuric mercury is described in this profile. The toxicology of cyanide is described in the ATSDR Toxicological Profile for Cyanide.

COMMENT 78: Referring to the statements discussing general population exposures in Section 5.1 on page 503-504, the Reviewer commented "MAJOR COMMENT (just to stress)

This list should be separarted into 3 bulleted lists by mercury species (inorganic, elemental, organic). By keeping it all in one list, it makes it appear that all species exposures are equal, which they are not. In addition, this will clarify what the sources of each type of exposure might be
The list should also be sorted into a ranking of the importance of exposure –i.e., why would water be listed before dental amalgams?
At the very least – I would rank these by importance and specify the mercury species for each source."

RESPONSE: *Figure 5-1 appears in all ATSDR Toxicological Profiles and is derived from the EPA NPL. This source does not provide data on mercury species.*

COMMENT 79: Referring to the statement in Section 5.1, lines 29-30 on page 504 – Intake of elemental mercury from dental amalgams is another major contributing source to the total mercury body burden in humans in the general population. – the Reviewer removed 'major' and commented "Dental amalgams should not be classified as a "major" source. You could say "important", but it is not equivalent to dietary sources for example. This sentence should also mention that many countries are phasing out (down) the use of dental amalgams. For example, the EU stopped using them in July 2018 in children and pregnant women. (I recognize that the phase-out is without controversy in the US)."

RESPONSE: *Revisions suggested by the Reviewer were made in Section 5.1 as follows:*

> Intake of elemental mercury from dental amalgams is another important contributing source to the total mercury body burden in humans in the general population. This is expected to decline as use of dental amalgams is being phased out in many countries.

COMMENT 80: Referring to the statement in Section 5.2.1, line 17 on page 506 – Artisanal goldmining is practiced in some countries. – the Reviewer replaced 'some' with 'many' and commented "At least 70 countries have ASGM."

RESPONSE: *The revisions suggested by the Reviewer were made as shown in Section 5.2.1:*

> Artisanal goldmining is practiced in many countries.

COMMENT 81: Referring to the statement in Section 5.2.1, lines 20-22 on page 506 – This process can result in direct exposures of mine workers to mercury vapor and can release mercury to the environment where it can be converted to methylmercury (Counter et al. 1998; Ramirez et al. 2000) – the Reviewer commented "And gold shop workers
And nearby households

> Moody KH, Hasan KM, Aljic S, Blakeman VM, Hicks LP, Loving DC, Moore ME, Hammett BS, Silva-González M, Seney CS, Kiefer AM. Mercury emissions from Peruvian gold shops: Potential ramifications for Minamata compliance in artisanal and small-scale gold mining communities. Environ Res. 2020 Mar;182:109042. doi: 10.1016/j.envres.2019.109042. Epub 2019 Dec 24. PMID: 32069769."

RESPONSE: *The sentence in Section 5.2.1 was revised as suggested and the Moody et al. (2020) publication was added:*

> This process can result in direct exposures of mine workers, gold shop merchants, and nearby households to mercury vapor and can release mercury to the environment where it can be converted to methylmercury (Counter et al. 1998; Diringer et al. 2015; Moody et al. 2020; Ramirez et al. 2000; Salazar-Camacho et al. 2021).

COMMENT 82: Referring to the table header for Table 5-1 in Section 5.2.1 on page 506 – Facilities that Produce, Process, or Use Mercury – the Reviewer commented "The word produce here is confusing since on the prior page (p 505, line 34) you state that production in the US has ended. I would change this word to Recycled or clarify what you mean by produce."

RESPONSE: *Text in Section 5.2.1 has been revised to include and explanation of the designation "produce" in Tables 5-1 and 5-2.*

> Data from the TRI is intended to meet the requirements of the Emergency Planning and Community Right-to-Know Act (EPCRA) Section 313 for mercury and the mercury compounds category. If a facility processes or imports mercury or mercury containing compounds, it is required to report these data to the TRI and this meets the definition of the reporting category, Produce.

COMMENT 83: Referring to the table header for Table 5-2 in Section 5.2.1 on page 508 – Facilities that Produce, Process, or Use Mercury Compounds – the Reviewer commented "Same comment as table 5-1."

RESPONSE: *Text has been revised to include and explanation of the designation "produce" in Tables 5-1 and 5-2 of Section 5.2.1.*

> Data from the TRI is intended to meet the requirements of the Emergency Planning and Community Right-to-Know Act (EPCRA) Section 313 for mercury and the mercury compounds category. If a facility processes or imports mercury or mercury containing compounds, it is required to report these data to the TRI and this meets the definition of the reporting category, Produce.

COMMENT 84: Referring to the statement in Section 5.2.2, lines 10-12 on page 510 – In 2015, 2016, 2017, 2018, and 2019, mercury imports were reported as 26, 24, 20, 6, and 10 metric tons, respectively (USGS 2020). – the Reviewer commented "It would be useful to state the predominant country from where imports are coming."

RESPONSE: *The following statement was added to Section 5.2.2:*

> According to USGS (2020), imports for the period of 2016−2018 were from Canada (39%), France (32%), Switzerland (13%), China (8%), and other countries (8%) (USGS 2020).

COMMENT 85: Referring to the statement in Section 5.2.3, lines 1-2 on page 512 – In 2018, it was estimated that approximately 290 pounds of mercury were used in vaccine usage in the United States (EPA 2020b). – the Reviewer commented "It would be useful to also estimate the total dose per vaccine. Some estiamtes state that in multi-dose shots, vials only have about 25ug of Hg,".

RESPONSE: *As noted in Section 2.1, toxicity of mercury in medical products (other than dental amalgams) was not considered or evaluated in the ATSDR Toxicological Profile for Mercury. Therefore, data on which to estimate mercury doses in applicable vaccines was not reviewed.*

COMMENT 86: Referring to the SIC codes discussed and listed in Section 5.3, lines 3-15 on page 513, the Reviewer commented "Unless you are a policy expert, it is impossible to know what SIC codes are or what some of these exclusion categories mean.

This section should have a statement that describes the types of facilites that are not included as well as a total number of facilites that are included in 2020 (or 2021), stating how this number has changed since the last Tox Profile."

RESPONSE: *This text is included in all ATSDR Toxicological Profiles. ATSDR will consider expanding the text to define SIC codes in future review and update of ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT 87: Referring to the units used in the statement in Section 5.3, lines 21-23 on page 513 – Studies of 11 western U.S. states between 2000 and 2013 estimated that the average annual emission of mercury due to wildfires in these states was 3,100±1,900 kg/year (Webster et al. 2016). – the Reviewer commented "Please keep the units the same in the paragraph, i.e., 3.1+- 1.9Mg/year.

RESPONSE: *Values converted to common units (metric tons) were added in parentheses following original data using units reported by the cited source.*

> Studies of 11 western U.S. states between 2000 and 2013 estimated that the average annual emission of mercury due to wildfires in these states was 3,100±1,900 kg/year (3.1 metric tons/year) (Webster et al. 2016).

COMMENT 88: Referring to the statement in Section 5.3, lines 25-27 on page 513 – Anthropogenic releases have historically been primarily to the atmosphere; however, these levels have been decreasing as regulations and engineering controls on point source and fugitive emissions limit the amount of mercury released to air. – the Reviewer commented "This is not relevant everywhere – likely just refers to the US. Please qualify this statement as "in the US"."

RESPONSE: *Text in Section 5.3 has been revised to qualify the statement as referring to the United States.*

> Anthropogenic releases have historically been primarily to the atmosphere; however, in the United States, these levels have been decreasing as regulations and engineering controls on point source and fugitive emissions limit the amount of mercury released to air.

COMMENT 89: Referring to the table header for Table 5-3 in Section 5.3 on page 514 – Cumulative Man-made Releases of Mercury to Air, Land, and Water Until 2010 – the Reviewer commented "Is this Global or just the US? Please state."

RESPONSE: *The cumulative releases are global, not just the United States. In Section 5.3, the title of Table 5-3 was revised as follows:*

> Cumulative Worldwide Man-made Releases of Mercury to Air, Land, and Water Until 2010

COMMENT 90: Referring to the statement in Section 5.3.1, line 12 on page 519 – The bulk of these emissions (>90%) arise from stack emissions rather than fugitive emissions. – the Reviewer commented "Do you define fugitive emissions? I haven't seen it."

RESPONSE: *Text in Section 5.3.1 has been revised to state that the Streets et al. (2017) estimates were worldwide anthropogenic emissions, as shown below. The title of Table 5-3 was revised as shown in Comment 89.*

Streets et al. (2017) performed a comprehensive temporal review of worldwide anthropogenic emission sources of mercury…

COMMENT 91: Referring to the units used the statement in Section 5.3.1, lines 17-19 on page 519 - The United Nations Global Mercury Assessment for 2018 estimated that the global inventory of mercury emissions to the atmosphere from anthropogenic sources in 2015 was approximately 2,220 metric tons (UN 2019). – the Reviewer commented "It is annoying that the author keeps switching from pounds to tons to Kg. Please keep one unit—you can put other units in paranetheses."

RESPONSE: *ATSDR presents data in units as reported in the primary source. For ease of comparison, all weight data in the text of Section 5.3 originally reported in units other than metric tons now include a unit conversion to metric tons in parentheses. For Tables 5-4, 5-5, and 5-6, metric ton conversions were only included in the final "totals" row to avoid congestion of the tables.*

COMMENT 92: Referring to the table title for Table 5-6 in Section 5.3.1 on page 520 – Global Anthropogenic Emissions of Mercury to the Atmosphere by Sector – the Reviewer commented "Add a column to report total emissions per year
I also suggest creating table 5-7 that reports sources of natural emissions and the total emissions per year (in the same units)."

RESPONSE: *Table 5-6 has been revised to include mercury emissions.*

COMMENT 93: Referring to the estimate of the natural weathering of mercury-bearing minerals in igneoud rocks (Gavis and Ferguson 1972) in Section 5.3.2, lines 4-5 on page 521, the Reviewer commented "Is this still a relelvant estimate from 50 years ago?
Global Precipitation has increased at a rate of 0.1 inches per decade and in some places, precipitation has increased more. This would be an aveage of 0.5inches more today than 1970, meaning we should have MORE mercury released to surface waters, whch does not take into account the potential for MORE erosion due to less forest and vegetation coverage on land."

RESPONSE: *Text in Section 5.3.2 has been revised as follows:*

Natural weathering of mercury-bearing minerals in igneous rocks can contribute substantially to environmental mercury. An analysis conducted in 1972 estimated that this source directly released about 800 metric tons of mercury per year to surface waters of the earth (Gavis and Ferguson 1972).

COMMENT 94: Referring to Section 5.4 on page 524 – ENVIRONMENTAL FATE – the Reviewer commented "This section would benefit from a figure showing how mercury cycles in the environment. Examples include:
https://science.sciencemag.org/content/341/6153/1457.full
https://www.unep.org/resources/publication/global-mercury-assessment-2018 (page 10)."

RESPONSE: *A figure (Figure 5-3) showing the environmental cycling of Hg (natural and anthropogenic) was added to Section 5.4*

COMMENT 95: Referring to the discussion of the levels of total mercury in fish impacted by gold mining reported in the Salazar-Camacho et al. (2021) study and the correlation with trophic level, the Reviewer commented "This data needs to be compared with the Diringer et.al 2015 data on fish in the Peruvian Amazon. The supplmental data in this study provide additional fish to compare.

At the very least, the Diringer paper should be cited as providing equivalent data on the biomagnification of mercury in fish in the Amazon

> Diringer S, Feingold B, Ortiz E, Gallis J, Araujo-Flores J, Berky A, et al. 2015. River transport of mercury from artisanal and small-scale gold mining and risks for dietary mercury exposure in madre de dios, peru. Environmental Science: Processes and Impacts 17:478-487."

RESPONSE: *Text in Section 5.4.1 has been revised to include Diringer et al. (2015).*

> Mercury levels in freshwater fish have been shown to be elevated in areas impacted by gold mining operations (Diringer et al. 2015; Salazar-Camacho et al. 2021).

COMMENT 96: Referring to the statements in Section 5.4.2, lines10-13 on page 536 – Thus, the transport of methylmercury into plants in wetland environments provides a temporary storage sink and reduces the levels in the surrounding aquatic environments. However, as plant tissues decompose, they typically release stored methylmercury back to the environment. – the Reviewer commented "Not just decomposition. Deforestation, wetland clearing, wildfires, etc., all result in the release of hg from plant storage."

RESPONSE: *Text in Section 5.4.2 has been revised.*

> However, decomposition of plant tissue, deforestation, clearing of wetlands, and fires can release stored methylmercury to the other environmental media.

COMMENT 97: Referring to the title for Table 5-13 in Section 5.5 on page 538 – Summary of Environmental Levels of Mercury – the Reviewer commented "Global or the US?"

RESPONSE: *Text in Section 5.5 and the title of Table 5-13 have been revised.*

> An overview summary of ranges of concentrations detected worldwide in environmental media is presented in Table 5-13.
>
> Table 5-13. Summary of Environmental Levels of Mercury Worldwide

COMMENT 98: Referring to the column header in Table 5-18 in Section 5.5.4 on page 553 – Standard Deviation (ng/g) – the Reviewer commented "Is this SD based on n>1 fish or n>1 samlpe per fish? Please describe in the footnote what the SD is based on
Also please put in the footnote what the sample design was and why there would be missing SD data."

RESPONSE: *Legend of Table 5-18 has been revised to include explanation of SD.*

> CV = coefficient of variation (SD/mean); SD = standard deviation for means based on ≥2 fish

COMMENT 99: Referring to the title for Table 5-19 in Section 5.5.4 on page 557 – Mercury Concentrations (ppm) for Largemouth Bass Collected in Various States Throughout the United States (1990-1995) – the Reviewer commented "For tables 5-19, 5-20 and 5-21:
Is the mean arithmetic or geometric?

Why report the mean? Why not report:
Minium, 25th percentile, median, 75th percentile, maximum?
This would match the data reported in Table 5-30."

RESPONSE: *The data are presented as reported in the source document (EPA 1999); the 25th and 75th percentiles are not reported. The legend of Table 5-19 has been revised to explain the calculation of the mean.*

> [a]Weighted average of composite samples where the weight is the number of fish in each composite ($\Sigma(C_i \times N_i)/N_t$, where C_i and N_i are the concentrations and number of fish in each composite sample, respectively, and N_t is the total number of fish in all composites).

COMMENT 100: Referring to the title for Table 5-20 in Section 5.5.4 on page 558 – Mercury Concentrations (ppm) for Channel Catfish Collected in Various States Throughout the United States (1990-1995) – the Reviewer commented "Edit as suggested in 5-19 --- report quartiles."

RESPONSE: *The data are presented as reported in the source document (EPA 1999); the 25th and 75th percentiles are not reported. The legend of Table 5-20 has been revised to explain the calculation of the mean as follows:*

> [a]Weighted average of composite samples where the weight is the number of fish in each composite ($\Sigma(C_i \times N_i)/N_t$, where C_i and N_i are the concentrations and number of fish in each composite sample, respectively, and N_t is the total number of fish in all composites).

COMMENT 101: Referring to the units used in Section 5.5.4 on page 561, the Reviewer commented "The units in this section keep jumping from ppm to ug/g. Please keep this consistent. I would just keep using ug/g."

RESPONSE: *Since 1 ppm = 1 ug/g = 1 mg/kg, all units in the text of Section 5.5.4 were changed to mg/kg for consistency with the Food and Drug Administration (FDA) Total Diet Study. When referring to the FDA action level, units in ppm were retained with mg/kg in parentheses since the published unit for the FDA action level is ppm.*

COMMENT 102: Referring to the discussion of rice consumption as a source of dietary mercury intake in Section 5.5.4 on page 563, the Reviewer commented "Rice seems to be an important dietary source of Hg, second to fish, but has failed to receive any focused paragraph about it. I would strongly suggest devoting a paragraph to rice exposure (enriched, non-enriched, white vs. brown, etc.) describing the importance of rice as an exposure risk. This can occur here or in the section on food."

RESPONSE: *Rice as a source of exposure to mercury is noted in Section 5.6 in the following sentence:*
Consumption of rice can also make a substantial contribution to dietary mercury intake and, in some populations, rice has been shown to be the dominant sources of dietary mercury intake (Rothenberg et al. 2016b; Wells et al. 2020; Zhang et al. 2010).

COMMENT 103: Referring to the discussion of total mercury absorption via dental mercury amalgam in Section 5.6 on page 568, the Reviewer commented "13 dental restoratiosn is 3-4 times higher than the average restorations in the US according to NHANES.
https://www.nidcr.nih.gov/research/data-statistics/dental-caries

So—the statement that amalgams contribute to hald of total mercury absoption is inaccurate and only true for people with poor dental hygiene / access to dental care."

RESPONSE: *ATSDR agrees that use of mercury amalgams in dental restorations is in decline; however, mercury amalgam will continue to be a source of exposure in people who have dental amalgams, during their replacement, and during processing of amalgam by dental practitioners. As noted in Section 5.2.,* in 2018, approximately 9,287 pounds of elemental mercury were used to produce dental amalgam in the United States (EPA 2020b).

COMMENT 104: Referring to Section 5.7 starting on page 569 – POPULATIONS WITH POTENTIALLY HIGH EXPOSURES – the Reviewer commented "Add discussion on environmental justice, particularly the elevated levels of exposure among indigenous and populations with limited acces to health care (and living near resource extractive industries)."

RESPONSE: *ATSDR does not discuss societal issues that affect access to health care services or other resources, as these factors would affect susceptibility to any chemical as well as to a host of diseases. ATSDR may consider including discussion of environmental justice issues in future profiles, pending revision of ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT 105: Referring to Table 5-25 in Section 5.7 on page 574 – Geometric Mean and Selected Percentiles of Inorganic Mercury Blood Concentrations (in µg/L) for the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 2003–2010 – the Reviewer commented "For each of these tables that specif y the total mercury species exposure, how is this known? Is this based on an assumption of the predominant form of mercury exposure?"

RESPONSE: *The mercury species are known based on the analytical methods used to measure the species. The analytical methods used to measure inorganic mercury, methylmercury, and ethylmercury are described in the Centers for Disease Control and Prevention (CDC) documentation of the NHANES.*

COMMENT 106: Referring to Section 6.2 on page 590 – IDENTIFICATION OF DATA NEEDS – the Reviewer commented "Unclear what the criteria is for weighing the importance of human vs. animal s studies. i.e., what is needed more?"

RESPONSE: *As noted in Section 2.1, ATSDR's approach for assessing study quality and weight-of-evidence evaluation is described in ATSDR's Guidance for the Preparation of Toxicological Profiles. Appendix B describes the strategy that was used to select literature for inclusion in the profile. Quality criteria were considered in selecting studies to include in the mercury profile and, in particular, for consideration as support for MRLs. In general, epidemiological studies that attempted dose-response assessments (e.g., regression models) were included in the profile if the following criteria were met: (1) reported estimates of variance in the dose-response metrics (e.g., SE, CL); (2) included adjustments for confounding; and (3) reported biomarker data. For studies used to derive MRLs, reporting of quality assurance of analytical methods was also required. All well-conducted and reported studies were considered for inclusion with a focus on routes of exposure most relevant to environmental exposure of humans (inhalation, oral, dermal). Parenteral studies were included only when needed to support understanding of mechanisms, but not for exposure-response relationships (since dose-response relationships observed following parenteral dosing may not accurately reflect exposure-response relationships).*

COMMENT 107: Referring to the statement in Section 6.2, lines 6-9 on page 603 – The major transport and transformation processes involved in the environmental fate of mercury have been fairly well defined; the most important fate process for human exposure, bioaccumulation of methylmercury in aquatic food chains is also well defined (EPA 1979, 1984; Stein et al. 1996; UN 2019). – the Reviewer commented "More work is needed in tropical environment."

RESPONSE: *Section 6.2 has been revised as shown.*

Additional information on mercury transport and flux in waterbodies and in tropical environments, in general, would be helpful.

COMMENT 108: Referring to the statement in Section 6.2, lines 30-32 on page 603 – While bioconcentration in the aquatic food chain is well studied, little is known about the bioaccumulation potential for terrestrial food chains, although it appears to be smaller than in aquatic systems (Lindqvist et al. 1991). – the Reviewer commented "I would argue that we have less knowledge of bioaccumulation in aquatic food chains of tropical and semi-tropical environments. This is where the majority of ASGM activities reside."

RESPONSE: *Section 6.2 has been revised.*

Additional information on mercury transport and flux in waterbodies and in tropical environments, in general, would be helpful.

COMMENT 109: Referring to the statement in Section 6.2 on page 604-605 – Mercury has been measured in human blood, hair, breast milk, urine, feces, and saliva (Bakir et al. 1973; CDC 2019; EPA 1984; Fujita and Takabatake 1977; Galster 1976; Oskarsson et al. 1996; Pitkin et al. 1976; Wheatley and Paradis 1995; WHO 1990). Continued biomonitoring data are needed to determine the temporal trends of mercury exposure to the U.S. population. – the Reviewer commented "Better surveillance to integrate Hg data into existing health information systems. i.e., obtaining regular samples at birth, age 1, age 5, etc."

RESPONSE: *ATSDR does not provide in toxicological profiles recommendations or proposals regarding public health surveillance or sampling criteria.*

COMMENT 110: Referring to the statement in Section 6.2, lines 5-7 on page 605 – The most important pathways appear to be via inhalation of metallic mercury vapors, intake of inorganic mercury associated with dental amalgams in children up to 18 years old, and ingestion of methylmercury in foods, primarily fish and shellfish (FDA 2017a). – the Reviewer commented "I would list these in order of importance. Namely: Diet, dental amalgam, inhalation.
Listing inhalation first implies it is the most important (both in terms of total exposure and the health effect of exposure), which it is not."

RESPONSE: *Section 6.2 has been revised to emphasize dietary sources.*

The most important pathways appear to be ingestion of methylmercury in foods, primarily fish and shellfish (FDA 2017a), intake of inorganic mercury associated with dental amalgams in children up to 18 years old, and inhalation of metallic mercury vapors.

COMMENT 111: The Reviewer added 'ADD TABLE' following Table 7-1 in Chapter 7 on page 612 and commented "I suggest adding a table that summarizes the MRL calculations. This will make it easier for clinicians to find information they need."

RESPONSE: *ATSDR does not include MRL values in Chapter 7. Information on the calculation of the MRLs are described in detail in Appendix A. Chapter 1 includes a table (Tables 1-2 through 1-4) that summarizes MRLs, including the derived MRL value, critical effect, point of departure, uncertainty factors, and critical study or studies. A footnote in this table refers the reader to Appendix A for more information on MRL derivation.*

COMMENT 112: Referring to the statement in Appendix A, lines 3-4 on page A-1 – MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. – the Reviewer commented ""reliable and sufficient" – what is the definition of this?"

RESPONSE: *There are no specific definitions for "reliable and sufficient." If ATSDR considers the database for a specific MRL to be unreliable or insufficient, then the database limitations are discussed in detail in the MRL worksheet in Appendix A.*

COMMENT 113: Referring to the Reference for the Minimal Risk Level Worksheet in Appendix A on page A-8 – Bast-Pettersen et al. 2005; Boogaard et al. 1996; Chapman et al. 1990; Ellingsen et al. 2001; Fawer et al. 1983; Langworth et al. 1992a; Wastensson et al. 2006, 2008 – the Reviewer commented "I am unclear on how these studies overcome some of the weaknesses noted on p A-5, lines 11-15. This goes back to the original question of – what is a reliable and sufficient study?"

RESPONSE: *The seven principal epidemiology studies provide the basis for the chronic duration MRL, because exposures occurred over periods that exceeded 1 year. The animal studies described in Table A-1 were short-term exposures <15 days. As noted in Section 2.1, ATSDR's approach for assessing study quality and weight-of-evidence evaluation is described in the ATSDR's Guidance for the Preparation of Toxicological Profiles.*

COMMENT 114: Referring to the equation under the Uncertainty Factor in Appendix A on page A-22 – Weighted Median$_{95\%LCL}$ ÷ UFs = MRL – the Reviewer commented "Where does this weighted median come from?"

RESPONSE: *The text in Appendix A has been revised to indicate that the weighted median of the seven studies listed in Table A-6 is weighted by number of subjects in each study.*

The 95% lower confidence limit (LCL) of the weighted median of the seven principal studies (Table A-6) is divided by a total uncertainty factor (UF) of 10.

Comments provided by Reviewer #3

GENERAL COMMENTS

COMMENT 1: Overall, I found the draft Tox Profile for Mercury to be very thorough and, in areas where I am familiar with the literature, quite accurate. Chapter 1 provides a clear and useful summary of the effects of mercury and the human exposure conditions of concern. The format for presentation of the toxicity study results in Chapters 1 and 2 has been greatly improved over the years, and I believe the figures and tables summarizing the results are now much more accessible and informative. I gave particular attention to the sections in Chapters 2 and 3 on methylmercury, and was very satisfied with the in-depth evaluation of the epidemiological studies and pharmacokinetic models. With regard to children as a potential sensitive population, I would suggest adding a discussion of a recent PBPK modeling study that was probably published after the cutoff for the draft document:
Pope Q, Rand MD. Variation in Methylmercury Metabolism and Elimination in Humans: Physiological Pharmacokinetic Modeling Highlights the Role of Gut Biotransformation, Skeletal Muscle, and Hair. Toxicol Sci. 2021 Feb 26;180(1):26-37. doi: 10.1093/toxsci/kfaa192. PMID: 33481013; PMCID: PMC7916735.

RESPONSE: *Section 3.15 has been revised to include a description of the Pope and Rand (2021) model. See response to Comment 14.*

COMMENT 2: I agree with the derivations of the MRLs, as well as the decisions that the available data were inadequate for deriving some of the MRLs. In particular, the derivation of the chronic MRL for methylmercury is very clearly described and I believe it makes appropriate use of the best available science.

RESPONSE: *No response needed.*

ATSDR Charge Questions and Responses and Reviewer Comments

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 3: Yes.

RESPONSE: *No response needed.*

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 4: Yes, effects in animals are qualitatively relevant to humans.

RESPONSE: *No response needed.*

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

COMMENT 5: Yes.

RESPONSE: *No response needed.*

Minimal 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 6: Yes.

RESPONSE: *No response needed.*

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.

a. 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 7: Yes. Yes.

RESPONSE: *No response needed.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 8: None.

RESPONSE: *No response needed.*

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 9: Yes.

RESPONSE: *No response needed.*

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 10: Yes. Yes.

RESPONSE: *No response needed.*

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 11: Yes.

RESPONSE: *No response needed.*

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 12: Yes.

RESPONSE: *No response needed.*

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

COMMENT 13: Yes.

RESPONSE: *No response needed.*

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 14: In the section on children as a potential sensitive population, I would suggest adding a discussion of a recent PBPK modeling study that was probably published after the cutoff for the draft document:
Pope Q, Rand MD. Variation in Methylmercury Metabolism and Elimination in Humans: Physiological Pharmacokinetic Modeling Highlights the Role of Gut Biotransformation, Skeletal Muscle, and Hair. Toxicol Sci. 2021 Feb 26;180(1):26-37. doi: 10.1093/toxsci/kfaa192. PMID: 33481013; PMCID: PMC7916735.

RESPONSE: *Section 3.15 has been revised to include a description of the Pope and Rand (2021) model. Section 3.2 was revised to note age differences in elimination kinetics.*

> Differences in elimination kinetics may also contribute to differences in susceptibility of children and adults (Pope and Rand 2021).

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 15: No.

RESPONSE: *No response needed.*

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 16: Yes.

RESPONSE: *No response needed.*

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 17: Yes.

RESPONSE: *No response needed.*

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 18: Yes.

RESPONSE: *No response needed.*

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 19: Yes.

RESPONSE: *No response needed.*

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 20: Yes.

RESPONSE: *No response needed.*

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

COMMENT 21: Yes.

RESPONSE: *No response needed.*

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 22: Yes. Yes.

RESPONSE: *No response needed.*

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 23: In the section on children as a potential sensitive population, I would suggest adding a discussion of a recent PBPK modeling study that was probably published after the cutoff for the draft document:
Pope Q, Rand MD. Variation in Methylmercury Metabolism and Elimination in Humans: Physiological Pharmacokinetic Modeling Highlights the Role of Gut Biotransformation, Skeletal Muscle, and Hair. Toxicol Sci. 2021 Feb 26;180(1):26-37. doi: 10.1093/toxsci/kfaa192. PMID: 33481013; PMCID: PMC7916735.

RESPONSE: *Section 3.15 has been revised to include a description of the Pope and Rand (2021) model. Section 3.2 was revised to note age differences in elimination kinetics.*

> Differences in elimination kinetics may also contribute to differences in susceptibility of children and adults (Pope and Rand 2021).

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 24: Yes. Yes.

RESPONSE: *No response needed.*

Biomarkers of Exposure and Effect

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

COMMENT 25: Yes.

RESPONSE: *No response needed.*

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

COMMENT 26: This is adequately discussed.

RESPONSE: *No response needed.*

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 27: Yes. Yes.

RESPONSE: *No response needed.*

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 28: Yes.

RESPONSE: *No response needed.*

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 29: Not to my knowledge.

RESPONSE: *No response needed.*

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

COMMENT 30: Yes.

RESPONSE: *No response needed.*

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 31: I believe so.

RESPONSE: *No response needed.*

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 32: Yes. Yes. No.

RESPONSE: *No response needed.*

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 33: Yes. No.

RESPONSE: *No response needed.*

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 34: Yes. Yes. Yes. Yes. No.

RESPONSE: *No response needed.*

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 35: Yes. Yes.

RESPONSE: *No response needed.*

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 36: No.

RESPONSE: *No response needed.*

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

COMMENT 37: Yes.

RESPONSE: *No response needed.*

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

COMMENT 38: Yes.

RESPONSE: *No response needed.*

Chapter 7. Regulations and Guidelines

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

COMMENT 39: No.

RESPONSE: *No response needed.*

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

COMMENT 40: No.

RESPONSE: *No response needed.*