# DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR CHLOROFORM

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 Chloroform were:

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## Comments provided by Reviewer #1

## ATSDR Charge Questions and Responses and Reviewer Comments

## GENERAL COMMENTS

**COMMENT 1:** This is a well-composed, comprehensive, and timely monograph providing an in-depth toxicological profile of the chemical entity 'chloroform'. Toxicological data and their interpretation, together with a large body of authoritative references, provide an objective and succinct coverage of this specific toxicant relevant to human health effects with inclusion of relevant animal data, and the monograph promises to be useful to a large readership including health care professionals and informed citizens alike.

Environmental exposure levels and routes are addressed adequately, and the potential significance of specific exposure situations is presented with much detail and scientific balance, including 'No-observed-adverse-effect levels' (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) with specific reference to the relevant literature of published studies. There is only a limited number of typos and inaccuracies that can easily be addressed. Likewise, addition of more contemporary studies published in the peer-reviewed original literature is recommended as specified in detail below.

***RESPONSE:*** *ATSDR appreciates these comments from the Reviewer.*

### *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 2:** Compilation of human toxicity data needs to refer to additional endpoints quoting more contemporary references:
Human reproductive toxicity:
Liu C, Chen YJ, Sun B, Chen HG, Mustieles V, Messerlian C, Sun Y, Meng TQ, Lu WQ, Pan XF, Xiong CL, Hou J, Wang YX. Blood trihalomethane concentrations in relation to sperm mitochondrial DNA copy number and telomere length among 958 healthy men. Environ Res. 2023 Jan 1;216(Pt 4):114737. doi: 10.1016/j.envres.2022.114737. Epub 2022 Nov 11. PMID: 36372149. (see attached)
Insert as #1 (as annotated in 'chloroform toxicological profile')

Human intrauterine/prenatal toxicity:
Liu C, Sun Y, Mustieles V, Chen YJ, Huang LL, Deng YL, Wang YX, Lu WQ, Messerlian C. Prenatal Exposure to Disinfection Byproducts and Intrauterine Growth in a Chinese Cohort. Environ Sci Technol. 2021 Dec 7;55(23):16011-16022. doi: 10.1021/acs.est.1c04926. Epub 2021 Nov 23. PMID: 34813313.
Insert as #2 (as annotated in 'chloroform toxicological profile')

Prostate as a chloroform target in humans:
Wei C, Chen Y, Yang Y, Ni D, Huang Y, Wang M, Yang X, Chen Z. Assessing volatile organic compounds exposure and prostate-specific antigen: National Health and Nutrition Examination Survey, 2001-2010. Front Public Health. 2022 Jul 29;10:957069. doi: 10.3389/fpubh.2022.957069. PMID: 35968491; PMCID: PMC9372286.

Insert as #3(as annotated in 'chloroform toxicological profile')

Donat-Vargas C, Kogevinas M, Castaño-Vinyals G, Pérez-Gómez B, Llorca J, Vanaclocha-Espí M, Fernandez-Tardon G, Costas L, Aragonés N, Gómez-Acebo I, Moreno V, Pollan M, Villanueva CM. Long-Term Exposure to Nitrate and Trihalomethanes in Drinking Water and Prostate Cancer: A Multicase-Control Study in Spain (MCC-Spain). Environ Health Perspect. 2023 Mar;131(3):37004. doi: 10.1289/EHP11391. Epub 2023 Mar 8. : 36883836; PMCID: PMC9994181.
Insert as #4 (as annotated in 'chloroform toxicological profile').

***RESPONSE:***

***#1:*** *The human reproductive study by Liu et al. (2023) was added to the Mechanisms of Reproductive Toxicity section in Section 2.16. It was not included in Table 2-17 because no apical health endpoints were evaluated.*

Liu et al. (2023) proposed that reduced sperm quality associated with exposure to trihalomethanes, including chloroform, in some studies may be attributable to reductions in sperm mitochondrial deoxyribonucleic acid (DNA) telomere length. In support, an inverse association was observed between blood chloroform levels and sperm mitochondrial DNA telomere length in 958 sperm donors. The study authors propose that oxidative damage may contribute to the observed association.

***#2:*** *The human developmental study by Liu et al. (2021) was added to Section 2.17, as shown in the text below. An entry was also added to Table 2-18.*

In a cohort study of 1,516 singleton pregnancies, no exposure-related associations were observed between maternal blood levels (median of 10.2 ng/L) and intrauterine measures of fetal growth (abdominal or head circumference, biparietal diameter, femur length, estimated fetal weight) during the 2nd or 3rd trimesters (Liu et al. 2021).

| | | | |
|---|---|---|---|
| **Liu et al. 2021**<br><br>Longitudinal cohort, 1,516 singleton births, mothers recruited during first trimester between 2014 and 2017, maternal age range 18–40 years (China) | Maternal blood chloroform levels over entire pregnancy (ng/L)<br>T1:<7<br>T2: 7–13<br>T3: >13<br>Median: 10.2<br><br>*Note: the study authors attributed blood levels to drinking water exposure* | Ultrasound fetal growth measurements ($2^{nd}$ and $3^{rd}$ trimester) | |
| | | Abdominal circumference | ↓ (T2 versus T1)<br>↔ (T3 versus T1) |
| | | Head circumference | ↔ |
| | | Biparietal diameter | ↔ |
| | | Femur length | ↔ |
| | | Estimated fetal weight | ↔ |

***#3:*** *The human study on prostate-specific antigen (PSA) by Wei et al. (2022) was added to the Mechanisms of Reproductive Toxicity section in Section 2.16. It was not included in Table 2-17 because no apical health endpoints were evaluated. Due to its relevance to prostate cancer, Wei et al. (2022) is also discussed in Section 2.19. It was not included in Table 2.19 because cancer incidence/status was not determined.*

Section 2.16: Wei et al. (2022) reported a positive association between blood chloroform and prostate-specific antigen (PSA) levels in 2,016 men recruited from the general population (NHANES 2001–2010). Since PSA is a key component in prostatic fluid, which mediates coagulation and liquefaction of semen, alterations in PSA levels could impact sperm fertility.

Section 2.19: In a cross-sectional study of NHANES data from 2001 to 2010, a significant association was observed between blood levels of chloroform and PSA, an early biomarker of prostate cancer; however, the incidence of prostate cancer was not evaluated in this study (Wei et al. 2022).

***#4:*** *The prostate cancer study by Donat-Vargus et al. (2023) was added to Section 2.19 and Table 2-19.* An increased risk of prostate cancer was associated with chloroform levels >18.4 µg/L in a case-control study from Spain after controlling for several confounders, including other disinfection byproducts (Donat-Vargas et al. 2023). However, when the study authors calculated mean lifetime waterborne ingested chloroform levels for study participants, no associations were observed between estimated lifetime exposure and risk of prostate cancer.

| | | | |
|---|---|---|---|
| **Donat-Vargas et al. 2023**<br><br>Case-control study, 697 cases (including 590 low- to medium-grade tumor cases and 97 high-grade tumor cases) and 927 controls, 20–80 years of age (Spain) | Mean chloroform in residential drinking water (µg/L)<br>Cases: 21.4<br>Controls: 20.7<br>T1: <18.7[c]<br>T2:18.4–25.5<br>T3: 25.5<br><br>Calculated mean adult lifetime waterborne ingested chloroform levels (µg/day)<br>Cases: 15.4<br>Controls: 15.1<br>T1: <5.4<br>T2: 5.4–19.1<br>T3: >19.1 | Prostate cancer | ↔ (lifetime ingestion)<br>↑ (drinking water levels, T2 or T3 versus T1) |

[c]As reported in Table 5 of study report; there appears to be a typographical error in the primary report. It is likely that either this value should be 18.4 µg/L or the lower value for the second tertile should be 18.7 µg/L.

**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 3:** There should be a separate, specialized list summarizing effects observed only in animals (i.e. effects observed in animals but not in humans). 'Effects observed in animals only' are of concern to humans.

***RESPONSE:*** *The toxicological profiles are developed in accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles. ATSDR does not include a separate list of "effects observed only in animals," as it may lead to unnecessary confusion between effects observed in animals and evaluated, but not observed, in humans and effects observed in animals but not evaluated in humans. Rather, ATSDR presents data (and makes hazard determinations) from both human and animal streams of evidence in a synthesized manner. Hazard determinations for endpoints that undergo systematic review are clear regarding the level of evidence from human and animal data streams, and sections in Chapter 2 clearly present available data from each data stream. For readers who want a quick overview of the balance of human versus animal data, Figures 2-1 and 6-1 show what endpoints have been evaluated in humans and animals, and Tables C-4 and C-5 in Appendix C show the number of studies evaluating effects per organ tissue/system in humans and animals, respectively, as well as the number of studies showing adverse effect per data stream.*

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

**COMMENT 4:** Exposure conditions described adequately.

***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 5:** Agreed. Level of evidence is specified adequately (e.g. 'low level of evidence from human studies; high level of evidence from animal studies; A-13; line 37).

***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.

**COMMENT 6:** Agreed; including uncertainty factors applied to $NOAEL_{HEC}$ etc.

***RESPONSE:*** *No response needed.*

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

**COMMENT 7:** When summarizing 'acute' versus 'intermediate' versus 'chronic' duration-associated MRL, the title of the respective front page (A-3; A-15; A-19) could include these terms (i.e. 'acute','intermediate','chronic') in the title line (MRL WORKSHEET) enhancing readability. The same applies to specification of exposure route ('inhalation'; 'oral'; etc.)

***RESPONSE:*** *ATSDR thanks the Reviewer for the suggestion and will consider it in future updates of* ATSDR's *Guidance for the Preparation of Toxicological Profiles*.

***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 8:** Adequate overall representation of published literature.

Table 2.2., p. 54: 'Acute toxic hepatitis in does that died.' 'does' seems to be a typo.

***RESPONSE:*** *No change is required. The study was conducted in rabbits; a pregnant rabbit is referred to as a doe.*

**COMMENT 9:** In chapter 2.15 (NEUROLOGICAL), the term 'inhalant addiction' or 'inhalant dependence' could be referred to - given the significant role that chloroform plays in that context. There is a need to include more references that expand on the topic of volatile substance abuse that involves chloroform. See for example:
Howard MO, Bowen SE, Garland EL, Perron BE, Vaughn MG. Inhalant use and inhalant use disorders in the United States. Addict Sci Clin Pract. 2011 Jul;6(1):18-31. PMID: 22003419; PMCID: PMC3188822.

***RESPONSE:*** *Primary studies reporting neurological results from recreational use of chloroform are reported in Section 2.15. Discussion of individuals with "inhalant addiction" was added to Section 5.7 (Populations with Potentially High Exposure).*

> Individuals with inhalant addiction that repeatedly and intentionally self-administer inhalants to achieve intoxication may have increased risk of exposure to chloroform depending upon the products abused. Chloroform is among the many chemicals in commonly abused products (Howard et al. 2011).

**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:** Additional exposure data documenting a role of chloroform exposure in total carcinogenic risk in large human urban populations should be included:
Zhao S, Gong Y, Yang S, Chen S, Huang D, Yang K, Cheng H. Health risk assessment of heavy metals and disinfection by-products in drinking water in megacities in China: A study based on age groups and Monte Carlo simulations. Ecotoxicol Environ Saf. 2023 Aug 10;262:115330. doi: 10.1016/j.ecoenv.2023.115330. Epub ahead of print. PMID: 37572625.

***RESPONSE:*** *This study was not added to the profile. In accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles, exposure data included in the profile are focused on domestic (U.S.) exposure levels (when available); therefore, the drinking water exposure data from China reported by Zhao et al. (2023) were not added. Cancer risk data were also not added, because Zhao et al. (2023) did not evaluate cancer outcomes. Instead, this public health assessment estimated cancer risk based on measured levels of chloroform (and other chemicals) in drinking water and estimated daily water intake levels. In accordance with ATSDR's Guidance for the Preparation of Toxicological Profiles, studies included in Chapter 2 (including Section 2.19. Cancer) are restricted to health effects studies evaluating both exposure and health outcome.*

**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:** Adequate studies identified, included, and carefully interpreted.

***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:** Adequate attention has been applied.

***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:** See studies referenced above.

***RESPONSE:*** *Please see previous responses to studies referenced above.*

**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:** Not applicable.

***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:** No comment provided by peer reviewer.

***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:** Agreed.

***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:** MOA covered extensively and satisfactorily.

***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:** Conclusions justified.

***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:** Adequate coverage.

Additional reference referring to hepatic CYP2E1 metabolism and toxification should be included: Gemma S, Vittozzi L, Testai E. Metabolism of chloroform in the human liver and identification of the competent P450s. Drug Metab Dispos. 2003 Mar;31(3):266-74. doi: 10.1124/dmd.31.3.266. PMID: 12584152.

***RESPONSE:*** *Gemma et al. (2003) was added to Section 3.1.3.*

The dominant isozyme mediating chloroform metabolism in rats and humans is CYP2E1 (Constan et al. 1999; Gemma et al. 2003; Lipscomb et al. 2004; Testai et al. 1996). However, other isoenzymes contribute to the low-affinity phase of oxidative metabolism, including CYP2A6 in humans and CYP2B1/2 in rats (Gemma et al. 2003; Testai et al. 1996).

**COMMENT 21:** In the following paragraph (p. 181; line 10 ) a significant typo occurs: 'Chinery and Gleason (1993) used a shower model for chloroform-contaminated water …. The model also predicted a steady-state stratum corneum permeability of chloroform in human skin in the range of 0.16–3.6 cm/hour, with the most likely value being 0.2 cm/hour. The study authors suggested that the results predicted by this model could be used to estimate household exposures to chloroform or other exposures which include dermal absorption.'
'0.16–3.6 cm/hour' is incorrect; corrected: '0.16–0.36 cm/hour'

***RESPONSE:*** *The typographical error was corrected.*

The model also predicted a steady-state stratum corneum permeability of chloroform in human skin in the range of 0.16–0.36 cm/hour, with the most likely value being 0.2 cm/hour.

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

**COMMENT 22:** Presented adequately.

***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 23:** Adequate discussion.

***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 24:** None.

***RESPONSE:*** *No response needed.*

**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 25:** Agreed.

***RESPONSE:*** *No response needed.*

***Biomarkers of Exposure and Effect***

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

**COMMENT 26:** Yes; direct detection of chloroform in biospecimens is unequivocal.

***RESPONSE:*** *No response needed.*

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

**COMMENT 27:** Not specific due to mechanistic overlap with many other toxicants and environmental exposure-induced biomedical outcomes and pathologies.

***RESPONSE:*** *No changes requested. The Reviewer's comment is consistent with data presented in Section 3.4 of the toxicological profile.*

***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 28:** Adequate discussion provided.

Draft Interaction Profile - August 2007: Chloroform, 1,1-Dichloroethylene, Trichloroethylene, and Vinyl Chloride (ATSDR) could be included (https://www.atsdr.cdc.gov/interactionprofiles/ip13.html)

***RESPONSE:*** *A final Interaction Profile for Chloroform, 1,1-Dichloroethylene, Trichloroethylene, and Vinyl Chloride has not been published. As per the ATSDR policy, a draft document cannot be cited. The draft interaction profile was reviewed, and it was confirmed that all relevant primary studies regarding binary interactions are cited in Section 3.4.*

**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 29:** Explained. No additional references.

***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 30:** Values as provided are correct.
'Molecular weight: 119.37' should include unit, i.e.: 119.37 $g \cdot mol^{-1}$ (page 203)

***RESPONSE:*** *The units for molecular weight were added to Table 4-2.*

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

**COMMENT 31:** Not applicable (i.e. no 'various forms' of the substance).

***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?

**COMMENT 32:** Complete.

***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 33:** Coverage of this topic is adequate.

***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 34:** Coverage is adequate.

***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?

**COMMENT 35:** Coverage (with inclusion of proper units) is adequate.

Additional exposure data contained in the more recent literature should be included:
Wickliffe JK, Stock TH, Howard JL, Frahm E, Simon-Friedt BR, Montgomery K, Wilson MJ, Lichtveld MY, Harville E. Increased long-term health risks attributable to select volatile organic compounds in residential indoor air in southeast Louisiana. Sci Rep. 2020 Dec 10;10(1):21649. doi: 10.1038/s41598-020-78756-7. PMID: 33303920; PMCID: PMC7730171.

***RESPONSE:*** *Exposure data from Wickliffe et al. (2020) were added to Section 5.5.1.*
Median indoor chloroform levels were 0.39 ppbv in 99 private residences monitored in southeast Louisiana between 2013 and 2015 (Wickliffe et al. 2020).

**COMMENT 36:** Also, Latina women may be at particular risk of exposure to cleaning product chemicals. In California, 81% of maids and housecleaners in the formal sector are Latina, and this proportion may be even higher when informal workers are considered (Wolfe J, et al. 2020. Domestic Workers Chartbook. Washington, DC: Economic Policy Institute):

Harley KG, Calderon L, Nolan JES, Maddalena R, Russell M, Roman K, Mayo-Burgos S, Cabrera J, Morga N, Bradman A. Changes in Latina Women's Exposure to Cleaning Chemicals Associated with Switching from Conventional to "Green" Household Cleaning Products: The LUCIR Intervention Study. Environ Health Perspect. 2021 Sep;129(9):97001. doi: 10.1289/EHP8831. Epub 2021 Sep 1. PMID: 34468180; PMCID: PMC8409434.

***RESPONSE:*** *A discussion of increased risk of exposure for domestic house cleaners, as well as other cleaning occupations, was added to Section 5.7. The particular risk for Hispanic women discussed in Wolfe et al. (2020) was added; the percentage of workers for the entire United States was reported in the profile rather than the California-specific value suggested by the Reviewer. Findings regarding exposure from tradition versus green cleaning products reported by Harley et al. (2021) were added.*

> Individuals employed as cleaners (e.g., janitors, hotel housekeeping, domestic staff) form an occupational group that may have increased risk of exposure to chloroform (Lin et al. 2022; Wolfe et al. 2020). The potential for chloroform exposure in these occupations is elevated not only due to increased usage of disinfected water but also from chloroform generated during use of chlorine-containing disinfectants, such as bleach (Bruchard et al. 2023; Odabasi 2008; Lin et al. 2022). A particularly vulnerable group may be Hispanic women, who make up 58.9% of domestic house cleaners in the United States (Wolfe et al. 2020). Switching from traditional cleaning products to products labelled as "green" cleaning products has been shown to reduce chloroform exposure during domestic cleaning (Harley et al. 2021). Geometric mean personal air concentrations of chloroform while cleaning were 0.5 and 0.066 ppb while using traditional and "green" cleaning products, respectively.

**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?

**COMMENT 37:** Agreed.

***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 38:** See references as quoted/ listed above.

***RESPONSE:*** *Please see responses to references as quoted/listed above.*

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

**COMMENT 39:** Agreed.

***RESPONSE:*** *No response needed.*

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

**COMMENT 40:** No bias.

***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 41:** No comment provided by peer reviewer.

***RESPONSE:*** *No response needed.*

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

**COMMENT 42:** No addendum/edits.

***RESPONSE:*** *No response needed.*

***Appendices***

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

**COMMENT 43:** No comment provided by peer reviewer.

***RESPONSE:*** *No response needed.*

***Unpublished Studies***

There are no unpublished studies to review for the chloroform toxicological profile.

**COMMENT 44:** No comment provided by peer reviewer.

***RESPONSE:*** *No response needed.*

## Annotated Comments

The Reviewer provided annotated comments on the toxicological profile. All comments are identical or nearly identical to specific comments above.

## Comments provided by Reviewer #2

## ATSDR Charge Questions and Responses and Reviewer Comments

## GENERAL COMMENTS

**COMMENT 1:** Overall, the update to the Toxicological Profile for Chloroform is comprehensive and includes the published literature since the 1997 update. The only deficiency that I see in the document is a lack of discussion surrounding the use of the default approach (i.e., NOAEL/LOAEL and default UF of 100) for the oral MRLs. The review of the available PBPK models as presented indicates that there are acceptable biological models for both animal and human that could be employed for the cross-species extrapolation; however, it appears that there was no consideration given to their use or that information has been omitted. While there may be justification for the default approach for oral MRLs, there should be a discussion as to why the biological models were not used for animal to human extrapolation of the POD.

***RESPONSE:*** *Rationale for not utilizing available PBPK models for oral MRLs was added to Appendix A.*

Acute MRL: Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al (1990) and Reitz et al (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Both studies relied on data from studies of a single gavage dose (or in the case of humans, gelatin capsule dosing) of chloroform in oil-based vehicles. The models have not been validated for simulating dosimetry of repeated continuous exposures, such as daily ingestion of chloroform in drinking water. Application of either model to dosimetry extrapolation in the derivation of the acute MRL would be highly uncertain. The major uncertainty would be in extrapolating the internal doses from delivery of a large bolus dose to the liver from an oil gavage dose to the internal dose expected for repeated ingestion of chloroform in water. This extrapolation has not been validated. Therefore, the models were not used for dosimetry extrapolation in deriving the acute-duration MRL.

Intermediate and Chronic MRLs: Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al (1990) and Reitz et al (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Neither of these studies evaluated dogs and are therefore not suitable for dose extrapolation.

### *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 2:** I agree with the reported effects know to occur in humans and am unaware of additional references that could be cited.

Correction: Figure 1-3 is inhalation exposure but refers to doses in mg/kg/day in the title “Numbers in triangles and circles are the lowest LOAELs (mg/kg/day) among health effects in humans and animals, respectively.” while the acute, intermediate, and chronic figures denote ppm. Please double check the exposure units and correct accordingly.

***RESPONSE:*** *The units were corrected to ppm.*

Numbers in triangles and circles are the lowest LOAELs (ppm) among health effects in humans and animals, respectively.

**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 3:** It is likely that the effects only observed in animals are of concern to humans.

***RESPONSE:*** *No response needed.*

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

**COMMENT 4:** Exposure conditions are adequately described for both human studies where exposures are less well characterized and animals.

***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 5:** This question is not applicable for chloroform as MRLs were derived for both oral and inhalation routes and exposure durations (i.e, acute, intermediate, and chronic).

***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.

**COMMENT 6:** For the inhalation MRL values, the approach is reasonable with the RGDRet with the reduced animal to human UF as this considers the point of contact tissue PK. It is disappointing that the PBPK models were not used for the oral MRLs based on hepatic effects in rodents. Based on the PBPK model summaries in Chapter 3, it appears that there is some apprehension to use the models as there is no unified model that covers all species and routes of exposure. Further discussion as to why the animal and human PBPK models were unacceptable for oral dosing extrapolation is warranted.

***RESPONSE:*** *Available oral PBPK models were not suitable for dose-extrapolation for the principal studies selected for oral MRLs. The available models (Corley et al 1990; Reitz et al 1990) relied on data from studies of a single gavage dose (or in the case of humans, gelatin capsule dosing) of chloroform in oil-based vehicles. They have not been validated for simulating dosimetry of repeated continuous exposures, such as daily ingestion of chloroform in drinking water; therefore, the models were not used for dosimetry extrapolation in deriving the acute-duration MRL. The intermediate- and chronic-duration MRLs were based on studies conducted in dogs. There are no PBPK models for extrapolating dosimetry from dogs to humans. The model summary in Section 3.1.5.2 was revised for clarity regarding the*

*usefulness of available models in risk assessment, and relevant discussions were added to Appendix A clarifying why PBPK models were not used for dose extrapolation in each MRL worksheet.*

Section 3.1.5.2: Based on the information presented in these models, there is evidence to suggest that PBPK models for chloroform are fairly refined and have the potential for use in human health risk assessments when key conditions are met (e.g., exposure route and duration, evaluated species, target tissue).

Appendix A, Acute MRL: Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al (1990) and Reitz et al (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Both studies relied on data from studies of a single gavage dose (or in the case of humans, gelatin capsule dosing) of chloroform in oil-based vehicles. The models have not been validated for simulating dosimetry of repeated continuous exposures, such as daily ingestion of chloroform in drinking water. Application of either model to dosimetry extrapolation in the derivation of the acute MRL would be highly uncertain. The major uncertainty would be in extrapolating the internal doses from delivery of a large bolus dose to the liver from an oil gavage dose to the internal dose expected for repeated ingestion of chloroform in water. This extrapolation has not been validated. Therefore, the models were not used for dosimetry extrapolation in deriving the acute-duration MRL.

Appendix A, Intermediate and Chronic MRLs: Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al (1990) and Reitz et al (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Neither of these studies evaluated dogs and are therefore not suitable for dose extrapolation.

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

**COMMENT 7:** I agree with the basis of the inhalation UF with 3 for animal to human and 10 for intrahuman. It is acceptable to use a factor of 3 for the LOAEL to NOAEL conversion as well. For the oral endpoints, it is disappointing that the PBPK model were not used for animal to human POD extrapolation along with the reduction in UF to 3 for animal to human.

***RESPONSE:*** *Please see response to Comment 6 regarding the lack of an appropriate PBPK model for oral MRLs. In the absence of an appropriate PBPK model, the default UF of 10 for animal to human extrapolation was used for oral MRLs.*

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

**COMMENT 8:** I have nothing to add.

***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:** The health effect conclusions are consistent with the published literature and provide a concise resource for understanding potential effects from chloroform exposure in both humans and animals.

**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:** The human studies are generally retrospective epidemiological studies. The studies were reasonably described including their strengths and limitations.

***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:** There were adequate animal studies for both the inhalation and oral routes of exposure for the three exposure durations (acute, intermediate, and chronic). The study summaries as well as the justification for the POD study included in Appendix A provide a solid basis for the POD study.

***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:** The animal species were appropriate for the significant toxicological endpoints and there appeared to be good agreement between rat and mouse for endpoints that served as the basis of the MRLs.

***RESPONSE:*** *No response needed.*

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

**COMMENT 13:** Adequate attention was paid to the dose-response relationships. BMD analysis was conducted on continuous endpoints under consideration for the POD and NOAEL/LOAEL was used where appropriate.

***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:** I am unaware of any additional studies that should be considered for the POD.

***RESPONSE:*** *No response needed.*

**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:** I am unaware of any additional studies that may be relevant to deriving the MRLs.

***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:** For inhalation, the appropriate NOAELs/LOAELs were identified; however, it is unclear why the hepatic based oral NOAELs/LOAELs only included oral bolus gavage dosing studies when distributed dosing studies (i.e., drinking water dosing) clearly showed higher PODs for heptic toxicity. Even a discussion as to what the POD would be for a distributed oral dosing study would provide the public with valuable information to understand how the oral MRLs are based on conservative assumptions and studies that do not represent their own exposure patterns to chloroform.

***RESPONSE:*** *NOAEL/LOAEL determinations for hepatic endpoints in animal studies were not restricted to gavage studies. The LSE table is comprehensive, including NOAEL/LOAEL determinations for hepatic endpoints from gavage, drinking water, and capsule studies in animals. The text in Section 2.9 discusses the increased susceptibility of hepatotoxicity in rodents following gavage exposure and identifies the lowest doses associated with effects following gavage exposure in the text preceding Table 2-7 and the lowest doses associated with effects following drinking water exposure in the text immediately after Table 2-10.*

*Regarding identification of PODs and the basis of MRLs, there are no oral MRLs based on bolus gavage studies. The acute-duration oral MRL is based on a drinking water study in mice (Larson et al. 1994b) and the intermediate- and chronic-duration oral MRLs are based on capsule administration studies in dogs (Heywood et al. 1979). The oral MRLs do the opposite of what is suggested by the Reviewer—oral gavage studies in rodents are removed from consideration as candidate POD, acknowledging that increased toxicity is likely due to saturation of detoxification pathways following bolus gavage exposure. Due to this, findings from drinking water studies were considered more relevant to environmental exposure levels and scenarios (as noted above by the Reviewer). Therefore, in rodents, only drinking water studies were considered for the basis of the MRL. The capsule studies in dogs were not excluded because dogs were exposed to chloroform in toothpaste via gelatin capsules (to evaluated exposure via toothpaste, because at the time of the study, that was a relevant human exposure condition). While these are not drinking water studies, dose delivery in a toothpaste vehicle via a capsule is not expected to be rapid or to replicate the conditions of a single bolus dose.*

**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:** I agree with the categorization used in the LSE tables.

***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:** The MOAs for chloroform has been adequately discussed. I am unaware of additional studies that provide additional information regarding the MOAs.

***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:** Chapter 2 does not appear to have a conclusion section per say; however, the database is extensive and representative of the known critical endpoints for chloroform.

***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:** There is adequate discussion of chloroform ADME including differences that have been reported between animals and humans.

***RESPONSE:*** *No response needed.*

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

**COMMENT 21:** The available PK/PD models for chloroform appear to be included. The reviews appear to be very positive and indicate that the model should be used for a chloroform risk assessment. It is difficult to rectify the descriptions of the models with the lack of used, especially for the hepatic endpoints that served as the basis of the oral MRLs. Is this due to a lack of guidance to incorporate quantitative methods for Toxicological Profiles?

***RESPONSE:*** *Available oral PBPK models were not suitable for dose-extrapolation for the principal studies selected for oral MRLs. The available models (Corley et al 1990; Reitz et al 1990) relied on data from studies of a single gavage dose (or in the case of humans, gelatin capsule dosing) of chloroform in oil-based vehicles. They have not been validated for simulating dosimetry of repeated continuous exposures, such as daily ingestion of chloroform in drinking water; therefore, the models were not used for dosimetry extrapolation in deriving the acute-duration MRL. The intermediate- and chronic-duration MRLs were based on studies conducted in dogs. There are no PBPK models for extrapolating dosimetry from dogs to humans. The model summary in Section 3.1.5.2 was revised for clarity regarding the usefulness of available models in risk assessment, and relevant discussions were added to Appendix A clarifying why PBPK models were not used for dose extrapolation in each MRL worksheet.*

Section 3.1.5.2: Based on the information presented in these models, there is evidence to suggest that PBPK models for chloroform are fairly refined and have the potential for use in human health risk assessments when key conditions are met (e.g., exposure route and duration, evaluated species, target tissue).

Appendix A, Acute MRL: Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al (1990) and Reitz et al (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Both studies relied on data from studies of a single gavage dose (or in the case of humans, gelatin capsule dosing) of chloroform in oil-based vehicles. The models have not been validated for simulating dosimetry of repeated continuous exposures, such as daily ingestion of chloroform in drinking water. Application of either model to dosimetry extrapolation in the derivation of the acute MRL would be highly uncertain. The major uncertainty would be in extrapolating the internal doses from delivery of a large bolus dose to the liver from an oil gavage dose to the internal dose expected for repeated ingestion of chloroform in water. This extrapolation has not been validated. Therefore, the models were not used for dosimetry extrapolation in deriving the acute-duration MRL.

Appendix A, Intermediate and Chronic MRLs: Available PBPK models were evaluated for potential suitability for oral dose extrapolation. Corley et al (1990) and Reitz et al (1990) are the only published reports of validation of models for predicting chloroform dosimetry from the oral exposure route. Neither of these studies evaluated dogs and are therefore not suitable for dose extrapolation.

**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:** There is adequate discussion of the concordance between animals and humans related to toxicokinetics for chloroform.

***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:** All data relevant to child and developmental effects for chloroform have been covered.

***RESPONSE:*** *No response needed.*

**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:** The discussion of potential sensitive populations is reasonable.

***RESPONSE:*** *No response needed.*

***Biomarkers of Exposure and Effect***

**QUESTION:** Are the biomarkers of exposure specific for the substance?

**COMMENT 25:** As the biomarkers for chloroform are the measurement of chloroform in blood or exhaled breath, it is possible that at least some of the biomarker is attributable to carbon tetrachloride as discussed in section 3.3.1.

***RESPONSE:*** *No response needed.*

**QUESTION:** Are the biomarkers of effect specific for the substance?

**COMMENT 26:** As discussed in section 3.3.2, the primary effects reported for chloroform are shared by many chemicals, especially other trihalomethanes and halogenated solvents.

***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?

**COMMENT 27:** There is adequate discussion of potential chemical interactions between chloroform and other chemicals. There is extensive discussion of the potential interactions with other trihalomethanes and chlorinated solvents with regards to hepatic and renal toxicity. The section also includes discussion of potential interactions with cadmium, alcohol and co-administered drugs such as morphine.

***RESPONSE:*** *No response needed.*

**QUESTION:** If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions?

**COMMENT 28:** There is adequate discussion of the mechanism of interaction between chloroform and other chemicals.

***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:** The chemical and physical information appears to be complete. It would be useful to include the SMILES for chemicals in Table 4-1.

***RESPONSE:*** *The SMILES for chloroform was added to Table 4-1.*

**QUESTION:** Is information provided on the various forms of the substance?

**COMMENT 30:** Yes, there is information provided for both liquid and gas forms of chloroform.

***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:** There is adequate discussion of the current and historical uses of chloroform including available information on import/export.

***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:** To my knowledge, the discussion is sufficient regarding environmental fate of chloroform and the extent of occurrence at NPL sites.

***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:** There is extensive text on chloroform transport, partitioning, transformation and degredation. I am unaware of additional information pertinent to the discussion.

***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:** The discussion surrounding environmental concentrations is comprehensive to the best of my knowledge. I am unaware of any additional relevant information.

***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?

**COMMENT 35:** The text surrounding sources and pathways of human exposure are extensive although the available data for occupationally exposed appears to be lacking as discussed in Chapter 6.

***RESPONSE:*** *Data needs for occupational human exposure levels were added to Chapter 6.*

Data regarding occupational exposure levels in humans are incomplete and are usually the result of limited, special studies. Studies designed to obtain better, current estimates of expected chloroform exposures in various workplace settings would be useful, including industrial settings (facilities that manufacture or use chloroform, drinking-water plants, wastewater-treatment plants, paper and pulp plants), indoor pools and spas, and industrial and domestic cleaning scenarios.

***Chapter 6. Adequacy of the Database***

**QUESTION:** Do you know of other studies that may fill a data gap?

**COMMENT 36:** I am unaware of other studies that would fill the data gaps.

***RESPONSE:*** *No response needed.*

**QUESTION:** Do you agree with the identified data needs?

**COMMENT 37:** I agree with most of the discussion; however, I take issue with the request for additional drinking water studies. Where studies were available and clearly showed higher NOAEL/LOAEL, they were ignored and the POD was based on the gavage study which produces a lower POD even the agency clearly states that distributed dosing studies are more relevant to human exposure scenarios. Why would stakeholders spend the substantial amounts of money on more relevant exposure route studies when agencies do not use the information in derivation of acceptable intakes for human risk assessment?

***RESPONSE:*** *The data need request in question is for "Additional, multi-dose, acute-duration drinking water studies could decrease uncertainty in the acute-duration oral MRL." The Reviewer indicates that ATSDR should not request more drinking water studies since they were ignored, and the POD was based on a gavage study. However, in contrast to the statements made by the Reviewer, the POD was based on a drinking water study by Larson et al. (1994b). As discussed in the response above to Comment 16, and detailed in Appendix A, rodent gavage studies were not considered as candidate principal studies during*

*MRL derivation due to the clear increased sensitivity of this route that was attributable to toxicokinetic overwhelm due to bolus dosing. Instead, drinking water studies were considered more relevant to human exposure scenarios. However, since only two acute-duration drinking water studies were identified, ATSDR maintains that additional multi-dose, acute-duration drinking water studies could decrease uncertainty in the acute-duration oral MRL.*

**QUESTION:** Are the data needs presented in a neutral, non-judgmental fashion?

**COMMENT 38:** Yes, there are some inconsistencies between the data needs and what is used for the oral POD as described above.

***RESPONSE:*** *Please see response to Comment 37. Since the acute-duration oral MRL is based on a drinking water study, not a gavage study (as stated by the Reviewer), there is no inconsistency between the data needs and what is used for the oral POD.*

***Chapter 7. Regulations and Guidelines***

**QUESTION:** Are you aware of any additional regulations or guidelines that should be included?

**COMMENT 39:** The discussion is comprehensive. I am unaware of additional regulations that are not reported.

***RESPONSE:*** *No response needed.*

**QUESTION:** Are there any that should be removed?

**COMMENT 40:** No, all should be presented.

***RESPONSE:*** *No response needed.*

***Appendices***

**COMMENT 41:** The appendices were very informative of the endpoints and calculations considered for the MRLs. The expansion to include multiple potential POD evaluations is helpful. For the oral POD, it would be useful to include a distributed study POD evaluation to provide the public with an understanding of just how conservative the use of oral bolus gavage studies is to human health risk assessment.

***RESPONSE:*** *As discussed in previous responses, none of the oral MRLs are based on oral bolus gavage studies. Therefore, no additional PODs require evaluation.*

***Unpublished Studies***

There are no unpublished studies to review for the chloroform toxicological profile

**COMMENT 42:** No comment provided by peer reviewer.

***RESPONSE:*** *No response needed.*

## Annotated Comments

The Reviewer did not provide annotated comments on the toxicological profile.

## Comments provided by Reviewer #3

***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 1:** Figures 1.1 and 1.2 are well-designed and informative.

I agree with the known health effects in humans as reported.

An area overlooked is the potentially susceptible populations including pregnant women, and individuals with diseases that lead to fat accumulation in the liver such as alcoholic liver disease, metabolism associated fatty liver disease (MAFLD, formerly called NAFLD) and obese individuals. There is an increased potential for these individuals to retain chloroform for longer periods due to increased fat content.

***RESPONSE:*** *A discussion regarding a potential increased susceptibility in individuals with increased fat content was added to Section 3.2. At this time, there is no clear evidence that pregnant women have increased susceptibility to chloroform toxicity compared to nonpregnant women, and no supporting references were provided by the Reviewer. Therefore, pregnant women were not specifically called out as a susceptible population. If pregnancy results in a large maternal weight gain, the potential increase in susceptibility would be covered in the discussion regarding increased fat content.*

> Obese individuals and those with diseases that lead to fat accumulation in the liver such as alcoholic liver diseases or metabolism associated fatty liver disease may be at increased risk of toxicity since chloroform preferentially distributes to fat (see Section 3.1.2). The kinetics of exposure for lipophilic compounds will be altered in obese individuals, compared to lean individuals. Clearance from blood may be quicker, leading to lower blood levels due to increased uptake in body fat, resulting in an overall extension of half-life and thus increasing cumulative exposure potential (Merrill and Birnbaum 2011). Increased activity of CYP2E1 has also been observed in obese individuals, especially those with type II diabetes (Brill et al. 2012; Wang et al. 2003), which could also contribute to increase susceptibility to chloroform toxicity in target organs with CYP2E1-mediated effects (e.g., liver and kidney).

**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 2:** All the adverse effects of chloroform observed in animals are also observed in human at certain dose. Thus, this is not a concern.

***RESPONSE:*** *No response needed.*

**QUESTION:** Have exposure conditions been adequately described?

**COMMENT 3:** The exposure conditions have been adequately described for the most part. One specific omission is GI exposure via contaminated drinking water, which seems like a possible source. Dermal exposure via bathing in contaminated water has been considered but drinking contaminated potable water is not considered.

***RESPONSE:*** *The general population exposure overview in Section 1.1 was revised to clearly indicate drinking water as a possible source of exposure, consistent with Section 5.6.*

The general population is most likely to be exposed to chloroform through inhalation of indoor and outdoor air, ingestion of food or disinfected water, or dermal contact with disinfected water.

***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 4:** MRL have been developed for all exposure scenarios and they are scientifically justified.

***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. Do you agree/disagree with each component of the total uncertainty factor?

**COMMENT 5:** I am not an expert in MRL determination but from my limited knowledge the MRLs determined are scientifically justified. I have some specific concerns about use of uncertainty factors. Why is the use of uncertainty factors variable between exposure scenarios? For example, in 'Intermediate-Duration Inhalation MRL' the uncertainty factor for using LOEAL is 3 but for "Chronic-Duration Inhalation MRL' it is 10.

***RESPONSE:*** *Uncertainty factors have a default value of 10. In the case of the uncertainty factor for use of a LOAEL, if the adverse effect observed at the POD is considered a minimal effect (small magnitude, mild severity, and/or transient effect), it is standard ATSDR practice to use an uncertainty factor of 3 rather than the full uncertainty factor of 10. For chloroform, the POD for the intermediate-duration inhalation MRL is considered a minimal LOAEL because the observed nasal lesions were categorized as minimal severity (which progressed in severity with increased concentration in the principal study). This justification for use of an uncertainty factor of 3 is shown in Appendix A in the Uncertainty Factors section of the MRL derivation. For the chronic-duration inhalation MRL, the POD is based on a LOAEL for nasal lesions that are more severe in nature (atrophy, respiratory metaplasia); therefore, a full uncertainty factor of 10 is warranted. No justification is required when the default uncertainty factor of 10 is used.*

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

**COMMENT 6:** What is the procedure for adjusting NOEL for continuous exposure duration? Has this been described in some part of the document?

***RESPONSE:*** *If exposure is intermittent (x hours/day, y days/week), it is adjusted to continuous (i.e., 24 hours/day exposure) using the following equation:*

$$NOAEL_{ADj} = NOAEL \times \frac{x\ hours/day}{24\ hours} \times \frac{y\ days/week}{7\ days}$$

*All MRL calculations, including adjustments for continuous exposure, are shown in the MRL worksheets in Appendix A. The footnote in Table 1-1 refers the reader to Appendix A for additional information regarding MRLs.*

***Chapter 2. Health Effects***

**QUESTION:** Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature?

**COMMENT 7:** The health effect conclusions are adequately supported by current scientific evidence.

Are there any studies or reports of either nasal or orphareangeal damage in humans due to inhalation exposure?

***RESPONSE:*** *As discussed in Section 2.4 and indicated in Appendix A, no data pertaining to potential nasal effects in humans following exposure to chloroform were identified.*

**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?

**COMMENT 8:** It is advisable to list the criteria used for inclusion and exclusion of studies in this report. They can be described briefly in the text and citations provided. At this time, it is not clear why the agency chose some studies and not others for this TP.

***RESPONSE:*** *The inclusion/exclusion criteria are documented in Appendix B of the toxicological profile.*

**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)?

**COMMENT 9:** The studies listed in the tables in chapter 2 are adequate. They are well-designed and have all the relevant information extracted from the actual publications.

***RESPONSE:*** *No response needed.*

**QUESTION:** Were the animal species appropriate for the most significant toxicological endpoint of the study?

**COMMENT 10:** The animal species used for tox endpoints include mainly rodents (rats and mice) and occasionally higher mammals including cats, and dogs. A summary of this in pictorial format will be helpful. It can follow the same template as of Fig. 2-1.

***RESPONSE:*** *ATSDR thanks the Reviewer for the suggestion and will consider it in future updates of* ATSDR's *Guidance for the Preparation of Toxicological Profiles*.

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

**COMMENT 11:** No comment from the peer reviewer was received.

***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?

**COMMENT 12:** An important consideration for final outcome of acute toxicity is liver regeneration, which is dose dependent. Chloroform acute exposure causes significant liver and kidney regeneration and the extent of, or the lack of regeneration is a critical factor in death as an outcome. This should be noted. In fact, a lot of this work was conducted using ATSDR funding via the office of Dr. Moise Mumtaz. This link has most of those references: https://pubmed-ncbi-nlm-nih-gov.kumc.idm.oclc.org/?term=Mehendale+HM%2C+chloroform&sort=pubdate

***RESPONSE:*** *Information pertaining to the role of liver and kidney regeneration in chloroform toxicity from the following references were added to Section 2.9 and 2.10.*

Anand SS, Murthy SM, Vaidya VS, et al. 2003. Tissue repair plays pivotal role in final outcome of liver injury following chloroform and allyl alcohol binary mixture. Food Chem Toxicol 41(8):1123-1132.

Anand SS, Mehendale HM. 2004. Liver regeneration: a critical toxicodynamic response in predictive toxicology. Environ Toxicol Pharmacol 18(2):149-160.

Anand SS, Mumtaz MM, Mehendale HM. 2005a. Dose-dependent liver tissue repair after chloroform plus trichloroethylene binary mixture. Basic Clin Pharmacol Toxicol 96(6):436-444.

Anand SS, Mumtaz MM, Mehendale HM. 2005b. Dose-dependent liver regeneration in chloroform, trichloroethylene and allyl alcohol ternary mixture hepatotoxicity in rats. Arch Toxicol 79(11):671-682.

Anand SS, Philip BK, Palkar PS, et al. 2006. Adaptive tolerance in mice upon subchronic exposure to chloroform: Increased exhalation and target tissue regeneration. Toxicol Appl Pharmacol 213(3):267-281.

Mehendale HM. 1991. Role of hepatocellular regeneration and hepatolobular healing in the final outcome of liver injury. A two-stage model of toxicity. Biochem Pharmacol 42(6):1155-1162.

Mehendale HM. 2005. Tissue repair: an important determinant of final outcome of toxicant-induced injury. Toxicol Pathol 33(1):41-51.

Philip BK, Anand SS, Palkar PS, et al. 2006. Subchronic chloroform priming protects mice from a subsequently administered lethal dose of chloroform. Toxicol Appl Pharmacol 216(1):108-121.

Rao KN, Virji MA, Moraca MA, et al. 1993. Role of serum markers for liver function and liver regeneration in the management of chloroform poisoning. J Anal Toxicol 17(2):99-102.

*The following text was added to Section 2.9:*

Rao et al. (1993) reported that biomarkers of liver regeneration are key determinants of a favorable prognosis following acute toxicity, including des-γ-carboxy prothrombin, α-fetoprotein, retinol binding protein, and 5-glutamyl-peptide:amino-acid 5-glutamyltransferase.

As reported in a human study by Rao et al. (1993), the rodent liver is capable of regenerative repair after oral or injection exposure to chloroform (Anand et al. 2003, 2005a, 2005b, 2006). This capacity for repair is a key determinant of the final outcome of the hepatoxic effects associated with acute chloroform toxicity, as the capacity for repair can become overwhelmed at high doses resulting in potentially fatal liver injury (Anand and Mehendale 2004; Mehendale 1991, 2005). Mechanistic pathways involved in repair are varied, including various cellular signaling pathways (chemokines, cytokines, growth factors, nuclear receptors) that result in promitogenic gene expression and cell division. Initiation of this repair pathway via repeat, sublethal chloroform exposures in mice can be protective of acute lethal exposures by mitigating, in part, acute hepatotoxic effects (Philip et al. 2006), resulting in tolerance to low-dose repeat exposures (Anand et al. 2006).

*The following text was added to Section 2.10:*

Although data are limited, the rodent kidney appears to be capable of regenerative repair following exposure to chloroform as seen in the liver (Anand et al. 2006; Philip et al. 2005). Mechanistic pathways are likely similar to those proposed for the liver, which include various cellular signaling pathways (chemokines, cytokines, growth factors, nuclear receptors) that result in promitogenic gene expression and cell division (Anand and Mehendale 2004; Mehendale 1991, 2005). Initiation of this repair pathway via repeat, sublethal chloroform exposures in mice can be protective of acute lethal exposures by mitigating, in part, acute renal toxicity (Philip et al. 2006), resulting in tolerance to low-dose repeat exposures (Anand et al. 2006).

**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?

**COMMENT 13:** This seems to be a fairly comprehensive collection of studies that can inform MRL derivation.

***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?

**COMMENT 14:** The tables are highly informative and provide adequate detail of each study, doses used, NOAEL/LOAELs. The figures are good but can be improved for clarity. The idea of showing MRLs and connecting them with dotted lines to studies is a bit hard to figure out.

***RESPONSE:*** *Please refer to the User's Guide in Appendix D of the profile for detailed explanations of the LSE figures. ATSDR thanks the Reviewer for the specific suggestion regarding the graphical depiction of the MRLs and will consider it in future updates of* ATSDR's Guidance for the Preparation of Toxicological Profiles.

**QUESTION:** Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

**COMMENT 15:** An important question is what is considered 'adverse'? When assigning either serious or less serious labels to effects, has the agency considered only those effects that are at gross tissue level, or has it considered changes in gene expression which may not show histopathological changes yet but are signs of molecular damage?

It seems that the categorization is a qualitative rather than quantitative measure. Is there a way to make it more quantitative?

***RESPONSE:*** *ATSDR defines "less serious" adverse effects as effects that "prevent or will prevent an organ or organ system from functioning in a normal manner but is not sufficiently serious to cause an observable effect on the whole animal." "Serious" adverse effects are defined as effects that "evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death)." The Reviewer is correct that, in many cases, this is a qualitative determination based on information laid out in* ATSDR's *Guidance for the Preparation of Toxicological Profiles*. *ATSDR only uses quantitative "cut-offs" to define the boundary between less serious and serious effect calls in instances in which there is concrete biological evidence to support such a "hard-line" determination (e.g., body weight effects, methemoglobinemia levels, acetylcholinesterase inhibition).*

*At this time, ATSDR does not make NOAEL/LOAEL determinations based on gene expression (or similar mechanistic) data due to unknown adversity at the target organ level. These data are considered under mechanisms of action and may contribute to the overall hazard conclusions, particularly if they are part of an established adverse outcome pathway for a critical effect association with the chemical of interest. ATSDR continues to monitor advancements in this field of research and will consider such approaches in future updates of* ATSDR's *Guidance for the Preparation of Toxicological Profiles if these methodologies progress to a stage at which human relevance/extrapolation is established.*

**QUESTION:** Have all possible mechanisms of action been discussed within their relevant health effect section?

**COMMENT 16:** See my comment on CQ6. Extensive work has shown that ability of the liver, the kidneys and to a certain extend the lung as critical determinants of chemical toxicity (Mehendale HM, Tox Pathol 2005). This has been studied extensively in relation to chloroform alone and chloroform as part of chemical mixtures. This vast body of work, partly funded by this agency, should be considered.

This link has most of those references: https://pubmed-ncbi-nlm-nih-gov.kumc.idm.oclc.org/?term=Mehendale+HM%2C+chloroform&sort=pubdate

***RESPONSE:*** *Please see response to Reviewer 3, Comment 12 for references and text added to the profile regarding the well-studied regenerative response in the liver and the less well-studied regenerative response in the kidney. None of the suggested references discussed regenerative responses in the lung.*

**COMMENT 17:** Another concern is lack of any mention of potentially susceptible populations including obese individuals who may have higher body burden. This study is a good starting point- https://pubmed-ncbi-nlm-nih-gov.kumc.idm.oclc.org/25847167/

***RESPONSE:*** *A discussion regarding a potential increased susceptibility in individuals with increased fat content was added to Section 3.2. However, Burch et al. (2015) was not added to the profile because a chloroform-specific analysis was not conducted. It was not added as "supporting evidence" from other trihalomethanes because the study did not find any statistically significant differences in risk for elevated ALT in obese versus non-obese individuals, as shown in the Supplemental materials (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4462191/bin/NIHMS678452-supplement-Suppl_.pdf).*

Obese individuals and those with diseases that lead to fat accumulation in the liver such as alcoholic liver diseases or metabolism associated fatty liver disease may be at increased risk of toxicity since chloroform preferentially distributes to fat (see Section 3.1.2). The kinetics of exposure for lipophilic compounds will be altered in obese individuals, compared to lean individuals. Clearance from blood may be quicker, leading to lower blood levels due to increased uptake in body fat, resulting in an overall extension of half-life and thus, increasing cumulative exposure potential (Merrill and Birnbaum 2011). Increased activity of CYP2E1 has also been observed in obese individuals, especially those with type II diabetes (Brill et al. 2012; Wang et al. 2003), which could also contribute to increase susceptibility to chloroform toxicity in target organs with CYP2E1-mediated effects (e.g., liver and kidney).

**COMMENT 18:** It is clear that at least in case of two major target organs of chloroform including liver and kidney and to a certain extend the nasal epithelium, CYP2E1-mediated metabolism of chloroform is required. As such, this is a common mechanism of toxicity and anything that can affect CYP2E1 expression and/or activity can affect chloroform toxicity. Many physiological factors affect CYP2E1 expression and activity including diabetes, obesity, malnutrition and alcohol consumption, all of which can make an individual highly susceptible to chloroform toxicity. Further, other target organ toxicities including neurotoxicity, developmental toxicity and immunotoxicity may or may not be metabolism dependent. It is advised that a separate section on mechanisms and then discuss CYP2E1-mediated metabolism based and metabolism independent toxicities.

***RESPONSE:*** *As per* ATSDR's *Guidance for the Preparation of Toxicological Profiles, a separate mechanisms of toxicity section is included in Chapter 2 only when there is a generalized mechanisms of toxicity common across all target tissues. As indicated by the Reviewer, mechanisms of toxicity appear to differ among target organs; therefore, relevant mechanisms of toxicity are discussed within specific target/organ sections of Chapter 2 (when data are available). Mechanisms of toxicity sections included in the profile address the comments by the Reviewer, including discussions of CYP2E1-mediated effects in the Mechanisms of Respiratory Toxicity, Hepatotoxicity, and Renal Toxicity sections and discussions of mechanisms of toxicity not dependent upon metabolism (or not clearly established as CYP2E1-mediated) in the Mechanisms of Respiratory Toxicity, Cardiovascular Toxicity, Immunotoxicity, Neurotoxicity, and Reproductive Toxicity sections.*

*Regarding susceptible populations, existing text in Section 3.2 discusses pre-existing conditions, diseases, exposures to other substances, and CYP2E1 polymorphisms that may increase an individual's susceptibility to chloroform toxicity based on altered metabolism. These include liver or kidney disease, alcohol abuse, and malnutrition/starvation. A statement was added to Section 3.2 regarding potential increased risk in diabetic and obese individuals.*

Increased activity of CYP2E1 has also been observed in obese individuals, especially those with type II diabetes (Brill et al. 2012; Wang et al. 2003), which could also contribute to increase

susceptibility to chloroform toxicity in target organs with CYP2E1-mediated effects (e.g., liver and kidney).

**QUESTION:** Are the conclusions appropriate given the overall database?

**COMMENT 19:** The conclusions are appropriate and well supported

***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?

**COMMENT 20:** The discussion of ADME is excellent and substantiated by references.

A section on the oversize role of CYP2E1 is chloroform toxicity should be included in the 'metabolism' section. It would be worth pointing out if there are any reports on CYP2E1-independent metabolism.

***RESPONSE:*** *The intent of Chapter 3 is to discuss toxicokinetics and does not include detailed discussions regarding toxicity. The role of CYP2E1 in chloroform toxicity, where relevant, is discussed in Chapter 2 in target-specific Mechanisms of Toxicity sections. Section 3.1.3 includes discussion of CYP2E1-independent metabolic pathways (minor pathways)—see Figure 3-1. A brief discussion of low-affinity CYP isoenzymes was also added to Section 3.1.3.*

However, other isoenzymes contribute to the low-affinity phase of oxidative metabolism, including CYP2A6 in humans and CYP2B1/2 in rats (Gemma et al. 2003; Testai et al. 1996).

**QUESTION:** Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented?

**COMMENT 21:** The description of various PBPK models seems in depth.

Schematic and detailed parameters of only the Corley et al. model have been included while the other models have been described only in the text. It would be good to have uniformity in this regard and other models should also be described schematically. If there is a specific reason why information/schematic on the other models was not used, that justification should be highlighted in the bold text at the beginning.

***RESPONSE:*** *As indicated in Section 3.1.5.3, the Corley model is the first chloroform model, and most subsequent PBPK models are based on the Corley model. Therefore, provided details were more in-depth for the Corley model. The following statement was added in Section 3.1.5.3 for clarity:*

Therefore, the Corley model is shown schematically in Figure 3-2 and discussed in-depth below; with subsequent models discussed more briefly.

**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:** This discussion is very brief and can be expanded. The statement that 'many laboratory animal models have been used' should be substantiated with at least one reference per species used.

***RESPONSE:*** *This section was expanded to refer the reader to relevant Tables and Chapters to obtain relevant references, as relevant reference strings would be extremely long and cumbersome. It is beyond the scope of the profile to provide an in-depth analysis of extrapolation methods, but Section 3.1.6 was expanded for clarity as shown below.*

> Many laboratory animal models have been used to describe the toxicity of chloroform, including rats, mice, rabbits, dogs, and cats (Tables 2-1, 2-2, and 2-3). By far, rats and mice are the most well-studied laboratory animal species. As discussed in preceding sections of Chapter 3, toxicokinetic data are available from a limited number of human studies, several studies in rats and mice, and a limited number of studies in other laboratory animals (monkeys, guinea pigs). Generally, the pharmacokinetic and toxicokinetic data gathered from rats and mice compare favorably with the limited information available from human studies, with no indication of clear species-related differences that would drastically impact default extrapolation assumptions. PBPK models, such as Corley et al. (2000), have been developed using pharmacokinetic and toxicokinetic data, and some of these have used species-specific information to define model parameters to reduce the amount of extrapolation needed between rodents and humans under some exposure conditions and target tissues (Section 3.1.5). PBPK model conditions, species, and target tissues need to be evaluated for suitability for the selected critical effect prior to use in dose extrapolation for risk assessment in humans.
>
> As mentioned previously, male rodents, particularly mice, have a sex-related tendency to develop severe renal disease when exposed to chloroform, particularly by the inhalation and oral exposure routes. This effect appears to be species-related, since experiments in rabbits and guinea pigs found no sex-related differences in renal toxicity. However, there is no mechanistic data to suggest that the renal disease observed in mice and rats is not relevant to humans.

***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?

**COMMENT 23:** The discussion is brief but seems to be adequate

***RESPONSE:*** *No response needed.*

**QUESTION:** Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations?

**COMMENT 24:** The discussion is very brief and could be expanded to provide details of some of the references cited.

Specific discussion on how chloroform toxicity may change (exacerbate?) in obese individuals is needed. With over 30% of Americans identified as either overweight or obese, it is critical to discuss this issue. Higher body fat has potential to keep more chloroform in the body and possible long term bioavailability even from short term exposure.

***RESPONSE:*** *A discussion of potential increased risk of toxicity in obese individuals was added to Section 3.2. It is beyond the scope of the profile to provide detailed description of the studies included in Section 3.2. Rather, this section is intended as a high-level overview of populations that may have increased susceptibility to chloroform toxicity. Therefore, additional details for existing citations were not added.*

Obese individuals and those with diseases that lead to fat accumulation in the liver such as alcoholic liver disease or metabolism associated fatty liver disease may be at increased risk of toxicity since chloroform preferentially distributes to fat (Section 3.1.2). The kinetics of exposure for lipophilic compounds will be altered in obese individuals, compared to lean individuals. Clearance from blood may be quicker, leading to lower blood levels due to increased uptake in body fat, resulting in an overall extension of half-life and thus, increasing cumulative exposure potential (Merrill and Birnbaum 2011). Increased activity of CYP2E1 has also been observed in obese individuals, especially those with type II diabetes (Brill et al. 2012; Wang et al. 2003), which could also contribute to increased susceptibility to chloroform toxicity in target organs with CYP2E1-mediated effects (e.g., liver and kidney).

***Biomarkers of Exposure and Effect***

**QUESTION:** Are the biomarkers of exposure specific for the substance?

**COMMENT 25:** This discussion is adequate.

***RESPONSE:*** *No response needed.*

**QUESTION:** Are the biomarkers of effect specific for the substance?

**COMMENT 26:** This discussion is adequate.

***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?

**COMMENT 27:** The discussion is adequate but can be improved. Additional references have been suggested. PMID: 21782743 (Chloroform and thioacetamide), PMID: 12842180 (Chloroform and allyl alcohol), PMID: 1711308 (Chloroform, chlordecone in gerbils), PMID: 2475365 (Chloroform and chlordecone), PMID: 2453913 (chloroform and chlordecone).

***RESPONSE:*** *The suggested references were added to Section 3.4.*

The hepatotoxicity of chloroform was also enhanced by co-exposure to CCl4 in rats (Harris et al. 1982), co-exposure to various alcohols (allyl alcohol, methanol, ethanol, isopropanol, t-butanol, pentanol) in rats (Anand et al. 2003; Ray and Mehendale 1990), co-exposure to ethanol in mice (Kutob and Plaa 1962), or co-exposure to chlordecone (Kepone®) in mice and gerbils (Cai and Mehendale 1991; Purushotham et al. 1988).

Anand et al. (2004, 2005b) proposed that trichloroethylene-induced liver injury was also reduced by co-exposure with chloroform due to increased compensatory liver tissue repair. Several other studies have shown increased compensatory liver tissue repair in rats exposed to binary chemical mixtures of chloroform and other known hepatotoxicants, despite varying mechanisms of toxicity, including allyl alcohol, thioacetamide, and chlordecone (Anand et al. 2003, 2004; Mehendale 1991; Mehendale et al. 1989). As seen in chloroform-only studies (Section 2.9), the capacity for regenerative repair in binary mixture studies resulted in a halted progression of hepatotoxicity and reduction in lethality.

**QUESTION:** If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions?

**COMMENT 28:** While most of mechanisms of action has been focused on chloroform metabolism, the toxicodynamic component of regeneration has been ignored. The references mentioned in comment to CQ8 and to CQ10 to Chapter 2 cover this aspect and should be mentioned.

***RESPONSE:*** *Please see response to Reviewer 3, Comment 12 for references and text added to the profile. Discussions regarding liver and kidney regeneration were added to the Mechanisms of Toxicity sections of Section 2.9 and 2.10, respectively.*

***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:** Information is adequate.

***RESPONSE:*** *No response needed.*

**QUESTION:** Is information provided on the various forms of the substance?

**COMMENT 30:** Information is adequate.

***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?

**COMMENT 31:** The information is detailed and adequate.

Is EPA CDR the sole source of information on production of chloroform? Are there any other independent sources?

***RESPONSE:*** *Import data from the U.S. International Trade Commission was added.*
The U.S. International Trade Commission reported a total import volume of 357,517 kg for chloroform in 2022 (USITC 2023).

**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?

**COMMENT 32:** This information seems inadequate. While NPL sites have been mentioned, no specific details of levels of chloroform detected in screening has been mentioned.

***RESPONSE:*** *Chloroform levels in water, soil, and air at NPL sites from 1981 to 2022 are reported in Table 5-6.*

**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?

**COMMENT 33:** This information is adequate and detailed.

***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?

**COMMENT 34:** This information is adequate and detailed.

***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?

**COMMENT 35:** This information is adequate and detailed.

***RESPONSE:*** *No response needed.*

***Chapter 6. Adequacy of the Database***

**QUESTION:** Do you know of other studies that may fill a data gap?

**COMMENT 36:** There may be other studies, but the current citations are adequate. Additional references have been identified in this report.

***RESPONSE:*** *Please see comments pertaining to additional references suggested by Reviewer 3 in the preceding comments.*

**QUESTION:** Do you agree with the identified data needs?

**COMMENT 37:** There is a definite and urgent need for data on how obesity affects chloroform toxicity. With over 30% of US population obese or overweight, this is a major issue.

***RESPONSE:*** *The following statement was added to Section 6.2:*

Since toxicokinetics of lipophilic compounds are expected to be different in lean versus obese individuals (Merrill and Birnbaum 2011), epidemiological studies stratifying analyses by BMI may be useful in determining if there is increased risk of chloroform-related toxicity in obese individuals.

**COMMENT 38:** There will be data need in the future on effects of climate change (increasing temperatures mainly) on chloroform exposure, environmental fate and health effects.

***RESPONSE:*** *It is beyond the scope of this document to speculate on the data needs required to evaluate the potential effects of climate change on chloroform exposure, environmental fate, and health effects.*

**QUESTION:** Are the data needs presented in a neutral, non-judgmental fashion?

**COMMENT 39:** This seems adequate.

***RESPONSE:*** *No response needed.*

***Chapter 7. Regulations and Guidelines***

**QUESTION:** Are you aware of any additional regulations or guidelines that should be included?

**COMMENT 40:** None.

***RESPONSE:*** *No response needed.*

**QUESTION:** Are there any that should be removed?

**COMMENT 41:** All information is needed.

***RESPONSE:*** *No response needed.*

## Annotated Comments

The Reviewer provided annotated comments on the toxicological profile. Many of these comments are identical or nearly identical to specific comments above. This section focuses on comments not previously addressed.

**COMMENT 42:** (Section 1.1, page 1, line 30) Are there any specific estimates of the fence line communities? These are defined as communities living next to facilities that either use, produce or

generate chloroform. Many people from these communities may work in those same facilities increasing risk of double exposure.

***RESPONSE:*** *Literature searches did not identify current estimates for fence line community exposure levels. The following statement was added Section 1.1:*

> Individuals who both work in facilities that manufacture/use chloroform and live nearby (e.g., fence line communities) may have increased risk of higher cumulative exposure due to both occupational plus residential exposure.

**COMMENT 43:** (Section 2.5, page 95, line 7) Are there any indication whether metabolic activation of chloroform via CYP2E1 is associated with cardiotoxicity?

***RESPONSE:*** *No information regarding the potential role of CYP2E1 was identified. The structural damage of the transverse tubular system is likely due to lipophilic membrane perturbation (non-metabolism mediated), similar to neurological effects. The role of metabolism has not been evaluated for other proposed mechanisms. The following statements were added to Section 2.5:*

> Damage is likely due to interference with the lipid arrangement of the transverse tubular walls (similar to the lipophilic membrane perturbation mechanism of action proposed for neurotoxicity, discussed in Section 2.15).
>
> It is unknown if proposed cytotoxic and altered cellular communication mechanisms of toxicity are CYP2E1-mediated (reliant on metabolism to reactive metabolites).

**COMMENT 44:** (Section 2.19, page 160, line 1) While the cytotoxicity as mechanism of chloroform induced carcinogenicity is undisputed, it should be noted that both animal and human studies show association of chloroform exposure to tumors in other organs where CYP2E1-mediated metabolism may or may not be involved or at least not been studied. It would be good to discuss this.

***RESPONSE:*** *There is inadequate evidence to identify a target organ for tumor development that would not show CYP2E1-mediated metabolism. Available data do not provide adequate evidence for tumor development in organs other than the liver and the kidney. Animal data do not report tumors in any other organs, and human data are inadequate to clearly identify additional targets (IARC 1999; IRIS 2001; NTP 2016). In fact, the U.S. EPA determined that chloroform is likely carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia; chloroform is not likely to be carcinogenic to humans by any route of exposure under conditions that do not cause cytotoxicity and cell regeneration. Therefore, the cancer determination is directly tied to the cytotoxic mechanism of action.*

**COMMENT 45:** (Section 2.19, page 160, line 8) An extra space was deleted after "(DNA)".

***RESPONSE:*** *The extra space was deleted.*

**COMMENT 46:** (Section 3.1, page 166, line 12) Is this the most correct term to explain CYP2E1?

***RESPONSE:*** *Since these bullets are intended to give a high-level overview to orient the reader, mixed function oxidase is the appropriate term. Cytochrome P450 enzymes are the most widely studied family of mixed-function oxidates.*

**COMMENT 47:** (Section 3.1.3, page 175, Figure 3-1) Why is CYP2E1 not recognized specifically in these figures? Are there any other CYPs involved in metabolism of Chloroform?

***RESPONSE:*** *Figure 3-1 indicates P450s in the major aerobic and anaerobic pathway, CYP2E1 is part of the P450 family. CYP2E1 is the predominant form; however, other low-affinity CYP isoenzymes are involved in metabolism. The text in Section 3.1.3 was revised for clarity.*

The dominant isozyme mediating chloroform metabolism in rats and humans is CYP2E1 (Constan et al. 1999; Gemma et al. 2003; Lipscomb et al. 2004; Testai et al. 1996). However, other isoenzymes contribute to the low-affinity phase of oxidative metabolism, including CYP2A6 in humans and CYP2B1/2 in rats (Gemma et al. 2003; Testai et al. 1996).

**COMMENT 48:** (Section 3.1.6, page 194, line 30) Please cite references to support this statement.

***RESPONSE:*** *The reviewer was referring to "many laboratory animal models". Instead of an extremely long string reference or arbitrarily selected citations, the text was revised to list the species evaluated and the reader is directed to the LSE tables for citations.*

Many laboratory animal models have been used to describe the toxicity of chloroform, including rats, mice, rabbits, dogs, and cats (see Tables 2-1, 2-2, and 2-3).

**COMMENT 49:** (Section 5.1, page 204, line 5) These two sentences are confusing. The confusion is because of the word "identified" and "evaluated" in the first and second sentence, respectively. What is the Agency trying to say?

***RESPONSE:*** *There are 1,868 NPL sites in the Unites States and its territories. Chloroform has been identified in 792 of them; however, it is unknown if it was evaluated for (tested for) at all 1,868 sites. Therefore, it would be inappropriate to assume that it is only at 792 sites, as it could be at other sites not tested for chloroform. ATSDR thanks the Reviewer for highlighting this potential source of confusion in the standardized text of the profile. ATSDR will consider revising the boilerplate text for clarity it in future updates of* ATSDR's *Guidance for the Preparation of Toxicological Profiles*.

**COMMENT 50:** (Section 5.1, page 205, line 22) A comma was deleted to read "pesticides and".

***RESPONSE:*** *The sentence was revised for clarity.*

Chloroform has also been used as a solvent in the pharmaceutical industry, a heat transfer medium in fire extinguishers, an intermediate in the preparation of dyes and pesticides, as well as in various other applications.

**COMMENT 51:** (Section 5.2.2, page 210, line 1) A table similar to 5-1 for import can be generated based on CDR data of import. How many pounds are imported from these sources?

***RESPONSE:*** *A table was not generated. As stated in Section 5.2.2, import volumes were not reported in CDR.*

**COMMENT 52:** (Section 5.7, page 238, line 4) It should be noted that these fence line communities may have higher exposure also because many people from this community also work in the production/use facilities.

***RESPONSE:*** *The following statement was added to Section 5.7:*

Additionally, workers who also live in communities near production/use facilities (e.g., fence line communities) have an even greater potential for exposure.