DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR ALPHA-, BETA-, GAMMA-, AND DELTA-HEXACHLOROCYCLOHEXANE

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

March 2022

Peer reviewers for the third pre-public comment draft of the Toxicological Profile for Alpha-, Beta-, Gamma-, and Delta-Hexachlorocyclohexane were:

Lisa Kamendulis, PhD
Department of Environmental and Occupational Health
Indiana University Bloomington
Bloomington, Indiana

Russell C. Cattley, VMD, PhD, Dipl. ACVP, Fellow IATP
Tyler & Frances Young Professor of Pathology
Room 166 Greene Hall
College of Veterinary Medicine
Auburn University, Alabama

Stephen M. Roberts, PhD
Director, Center for Environmental & Human Toxicology
Professor, College of Veterinary Medicine
College of Medicine, and College of Public Health and Health Professions
University of Florida
2187 Mowry Road
Box 110885
Gainesville, Florida

Comments provided by Reviewer #1

GENERAL COMMENTS

COMMENT 1: Section 1.2 page 2. "…increased liver enzymes…" (p 2, line 34) is not very precise since it is known that gamma-HCH actually decrease the levels of some liver enzymes in the tissues. This phrase would be improved by re-stating as "increased serum enzymes indicative of hepatocellular injury"

RESPONSE: *Section 1.2 was revised as suggested.*

> After oral exposure to γ-HCH for acute, intermediate, and chronic durations, liver effects in rats, mice, and rabbits have included increased serum enzymes indicative of hepatocellular injury, increased serum lipids, increased liver weight, hepatocellular hypertrophy, vacuolar degeneration, necrosis, and congestion (Ali and Shakoori 1998; Amyes 1990; Attia et al. 2011; Boll et al. 1995; Cerón et al. 1995; EPA 1991a, 2000a; Fatih Fidan et al. 2008; Fitzhugh et al. 1950; Grabarczyk et al. 1990; Hfaiedh et al. 2012; Kamal El-Dein et al. 2016; Kopec-Szlezak et al. 1989; Matsuura et al. 2005; Parmar et al. 2003; Singh and Sharma 2011; Sumida et al. 2007; Suter 1983; Vijaya Padma et al. 2011).

COMMENT 2: Section 2.14 page 133. Shouldn't data of Seth et al (pg. 133, line 25-31)" appear in Table 2.13 (pg. 134)

RESPONSE: *The study by Seth et al. (2005) was added to Table 2-13 as suggested.*

COMMENT 3: Section 2.20 page 193. For alpha HCH results of Hitachi et al (1975) (pg. 193, line 9-11) should not be characterized as genotoxicity, instead should appear under hepatic effects as evidence of cell proliferation.

RESPONSE: *The description of Hitachi et al. (1975) was moved to Section 2.9 Hepatic under the α-HCH mechanisms subsection:*

> In male Donryu rats, a 3-week dietary exposure to α-HCH resulted in mitotic disturbances including an increased mitotic rate and an increased frequency of polyploid hepatic cells (Hitachi et al. 1975).

COMMENT 4: Section 5.5.2 page 246. Change "sue" to "due (pg. 246, line 30).

RESPONSE: *Section 5.5.2 was revised as suggested.*

> A decrease of γ HCH in surface water can be observed, possibly sue due to use limitations; trends for drinking water and groundwater are not as clear.

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1. Relevance to Public Health

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

COMMENT 5: Yes.

RESPONSE: *No response needed.*

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

COMMENT 6: Yes.

RESPONSE: *No response needed.*

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

COMMENT 7: Yes.

RESPONSE: *No response needed.*

Minimal Risk Levels (MRLs)

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

COMMENT 8: Yes.

RESPONSE: *No response needed.*

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

COMMENT 9: Generally Yes. Regarding Intermediate/Oral MRL for gamma-HCH: There is concern over using the results of Sauviat et al (2005) as the principal study for calculation of MRL because this study characterized a LOAEL that is >1000 times lower than all other studies of gamma-HCH. The reporting of exposure in this study was limited to an estimation of dose following "beverage" (presumed drinking water) and the amount consumed was not reported, raising concern that dams may not have consumed adequate water (resulting in maternal effects not addressed in the paper). Consider using gavage study of Johri et al, (2007) or Srivastava et al (2019) instead.

RESPONSE: *Although the study by Sauviat et al. (2005) does not explicitly state that γ-HCH was administered in drinking water, it is assumed that the "beverage" was drinking water based on another*

study of γ-HCH in the same species by the same authors (Breton et al. 2005), which reported exposure "through beverage" but also reported that "Lindane was dissolved in acetone… and diluted to the appropriate concentrations in tap water."

Sauviat et al. (2005) did not report maternal water intake. However, none of the three studies conducted by this group (Breton et al. 2005; Sauviat et al. 2005, 2007) noted any exposure-related effects on water intake, nor did Hfaiedh et al. (2011) in their study of drinking water exposure at a much higher dose (50 mg/kg/day).

Finally, ATSDR notes that, while the difference between the estimated LOAEL (0.00015 mg/kg/day) in Sauviat et al. (2005) and the LOAELs identified for Johri et al. (2007) and Srivastava et al. (2019) are indeed >1000-fold, another study identified a LOAEL much closer to that identified for Sauviat et al. (2005): the mouse study by Meera et al. (1992) that was used as the basis for the intermediate-duration oral MRL in 2005 identified a LOAEL of 0.012 mg/kg/day, which is 80-fold higher than the Sauviat et al. (2005) LOAEL. No change was made to the MRL.

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

COMMENT 10: Agree.

RESPONSE: *No response needed.*

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

COMMENT 11: None.

RESPONSE: *No response needed.*

Chapter 2. Health Effects

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

COMMENT 12: Yes.

RESPONSE: *No response needed.*

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

COMMENT 13: Human studies very limited but adequately addressed. Yes.

RESPONSE: *No response needed.*

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

COMMENT 14: Yes.

RESPONSE: *No response needed.*

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

COMMENT 15: Yes.

RESPONSE: *No response needed.*

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

COMMENT 16: Yes.

RESPONSE: *No response needed.*

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

COMMENT 17: No.

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 18: No.

RESPONSE: *No response needed.*

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

COMMENT 19: Yes.

RESPONSE: *No response needed.*

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

COMMENT 20: Yes.

RESPONSE: *No response needed.*

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

COMMENT 21: Yes. While there may be indirect evidence that HCH causes oxidative stress, it is not clear that this constitutes the mechanism for the toxicity. Some references only provide evidence of reactivity in vitro (homogenates and isolated microsomes). Other indirect evidence included changes in tissues enzyme activities (for examples catalase, glutathione peroxidase, and/or glutathione peroxidase). As noted under Adequacy of the Database, "these are nonspecific effects induced by a wide range of compounds". Other studies attribute the effect of dietary supplements/plant extracts to reduction of oxidative stress, but do not account for changes in HCH metabolism caused by these treatments as an alternative mechanism. As a result, many studies suggesting of oxidative stress as the mechanism of HCH toxicity are not sufficiently conclusive.

RESPONSE: *ATSDR notes that the evidence for oxidative stress induced by HCH in most organ systems includes direct measurement of lipid peroxidation, superoxide anion production, and /or nitric oxide production in tissues from animals exposed in vivo. Based on these data, IARC (2018) concluded that there was strong evidence that γ-HCH induces oxidative stress. ATSDR agrees that oxidative stress is a nonspecific effect, and the language in the Toxicological Profile is intended reflect the understanding that oxidative stress may be one of several mechanisms (e.g., "oxidative stress may contribute to" or "may be involved in"). ATSDR agrees that that the uncertainty in the mechanisms by which dietary supplements/ plant extracts influence HCH toxicity should be noted. Section 2.9 (Hepatic) was revised to place greater focus on direct evidence of oxidative stress and to note the uncertain mechanisms by which dietary supplements and plant extracts influence the effects of HCH.*

> There is some evidence that oxidative stress may contribute to the hepatic effects of γ HCH. Significant increases in hepatic microsomal superoxide anion production and cytoplasmic superoxide dismutase activity and lipid peroxidation were found in the livers of Wistar rats fed diets containing 1.8 mg/kg/day γ-HCH for 15 or 30 days (Barros et al. 1991). Groups of 10 male rats (strain not reported) were administered a single dose of γ-HCH (98% purity) in corn oil at a dose of 0 or 12 mg/kg and then sacrificed 24 hours later in a study aimed at evaluating the ameliorating effects of co-treatment with the antioxidants nigella sativa oil and omega 3 fatty acids. Co-treatment with nigella sativa oil and omega 3 fatty acids attenuated the effects of γ-HCH on lipid parameters, clinical chemistry parameters, lipid peroxidation, and antioxidant enzyme activities (Attia et al. 2011). The mitigating effects of nigella sativa oil and omega 3 fatty acids could have resulted from their antioxidant activity or from effects on the absorption or metabolism of γ-HCH.

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 22: Yes. There is concern over conclusions based upon the results of Sauviat et al (2005). The reporting of exposure in this study was limited to an estimation of dose following "beverage" (presumed drinking water) and the amount consumed was not reported, raising concern that dams may not have consumed adequate water (resulting in maternal effects not reported).

RESPONSE: *See response to Comment 9.*

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 23: Yes.

RESPONSE: *No response needed.*

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

COMMENT 24: Yes.

RESPONSE: *No response needed.*

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

COMMENT 25: Yes but only limited information seems available.

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 26: Not aware of any missing relevant data.

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 27: Yes. Yes.

RESPONSE: *No response needed.*

Biomarkers of Exposure and Effect

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

COMMENT 28: Yes.

RESPONSE: *No response needed.*

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

COMMENT 29: NA- There are no biomarkers specific for HCH, and this is stated appropriately.

RESPONSE: *No response needed.*

Interactions with Other Chemicals

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

COMMENT 30: Yes. The discussion addresses some interactions with other substances found at hazardous waste sites (such as chlorinated hydrocarbons, cadmium). However it extends to substances that, while important, are unlikely to be associated with hazardous waste sites (acetaminophen, ethanol, vitamin C, natural plant extracts).

RESPONSE: *No response needed. As indicated in ATSDR guidance, the section on Interactions is intended to "discuss the influence of other substances on the toxicity of the profile substance," where "Other substances include pharmaceuticals, hazardous substances, and substances not designated as hazardous substances."*

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 31: Some mechanisms such as cytochrome P450 induction are appropriately discussed. However, the discussion of natural plant extract may be inappropriately linked to reduction of oxidative stress, as the cited studies do not account for alterations in absorption, distribution, metabolism and elimination that may be occurring in these studies.

RESPONSE: *The discussion of natural plant extracts in Section 3.4 was revised to reflect the uncertainties noted by the Reviewer.*

> Natural plant extracts (i.e., ajwain extract, Hyrtios aff. Erectus sponge extract) have been shown to reduce rodent liver toxicity of HCH isomers administered individually (Anilakumar et al.

2009) or as a mixture with other organochlorine compounds (Abd El-Moneam et al. 2017). The mechanisms by which these plant extracts mitigate HCH toxicity may include antioxidant activity and/or alterations in HCH absorption, distribution, metabolism, or elimination.

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 32: No.

RESPONSE: *No response needed.*

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

COMMENT 33: Yes.

RESPONSE: *No response needed.*

Chapter 5. Potential for Human Exposure

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

COMMENT 34: Yes.

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 35: Yes. Not answered. No.

RESPONSE: *No response needed.*

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

COMMENT 36: Yes. No.

RESPONSE: *No response needed.*

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

COMMENT 37: Yes. Yes. Yes. Yes. No.

RESPONSE: *No response needed.*

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

COMMENT 38: Yes - This section appears accurate and complete, but may be improved by some summary statement (bold type at the beginning of section 5.6, and/or a graphic representation, to increase emphasis on the declining environmental (non-medical) exposure that are indicated by monitoring survey results. Yes.

RESPONSE: *The following summary statement was added to the beginning of Section 5.6:*

Exposure of the general population to HCH has declined steadily since its use as a pesticide was discontinued.

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 39: No.

RESPONSE: *No response needed.*

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

COMMENT 40: Yes.

RESPONSE: *No response needed.*

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

COMMENT 41: Yes.

RESPONSE: *No response needed.*

Chapter 7. Regulations and Guidelines

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

COMMENT 42: No.

RESPONSE: *No response needed.*

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

COMMENT 43: No.

RESPONSE: *No response needed.*

Appendices

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

COMMENT 44: None.

RESPONSE: *No response needed.*

Comments provided by Reviewer #2

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1. Relevance to Public Health

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

COMMENT 1: No effects known to occur in humans were identified. Information on potential health effects of HCH isomers and technical HCH is humans is very limited. I agree with the characterization of this information in the document in terms of both observations and limitations in interpretation.

RESPONSE: *No response needed.*

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

COMMENT 2: I agree that there are some effects observed in animals that are likely to be of concern in humans. Factors that can contribute to this conclusion include consistency with which the effect is seen in different animal species, mechanism(s) of action relevant to humans, and on the other side, absence of information from human studies to suggest that the effect does not occur in humans. Specific to this profile, I agree with the conclusions in this section regarding effects that can be considered to be "likely to be of concern to humans."

RESPONSE: *No response needed.*

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

COMMENT 3: Exposure conditions are concisely and adequately described in this section.

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 4: In each case where no MRL was derived, I agree that the data (or lack thereof) support that decision.

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? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 5: My only concern with the MRLs is with the oral intermediate MRL for beta-HCH and the absence of an oral chronic MRL. It appears to me that the dose-response relationships for liver effects of beta-HCH are almost the same for intermediate and chronic exposure. NOAELs are not available for either duration, and the LOAEL appears to be 0.9 mg/kg/day for both intermediate and chronic exposure based on results from Van Velsen and Fitzhugh studies, respectively. By identifying 0.18 mg/kg/day as a minimal MRL based on slight changes in histopathology, development of an oral chronic MRL is precluded. I would consider designating the same LOAEL for both durations (0.9 mg/kg/day) and applying the same uncertainty and modification factors to derive both intermediate and chronic MRLs.

RESPONSE: *Hepatic effects consisting of hyalinization of centrilobular cells were seen at 0.18 mg/kg/day after intermediate-duration exposure in the study by Van Velsen et al. (1986). The effects were considered to be minimal, and the uncertainty factor of 3 for extrapolation from a LOAEL to a NOAEL reflects the lower severity of the effects. The LOAEL of 0.7–0.9 mg/kg/day identified in the study by Fitzhugh et al. (1950) corresponded to the lowest dose tested; a NOAEL was not identified. Thus, it is not clear that this LOAEL would adequately protect against hepatic effects after chronic exposure, given the finding of effects at 0.18 mg/kg/day after a shorter exposure duration in the study by Van Velsen et al. (1986). No change was made to the MRLs.*

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

COMMENT 6: The MRL database assessment succinctly captures where information is, or is not, sufficient to develop an MRL, and suits its purpose well. My only disagreement with content is discussed in my response to the previous question.

RESPONSE: *See response to Comment 6.*

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 7: I agree in general with the health effect conclusions in Section 2.1. However, these conclusions seem to focus almost exclusively on non-cancer effects, and it would be useful to also include some conclusions here regarding the carcinogenicity of the HCH isomers. Also, consider adding hepatic effects as a sensitive target for gamma-HCH – LOAEL doses for hepatic effects are in the same ballpark as developmental and immune system effects.

RESPONSE: *Section 2.1 is a summary of noncancer effects occurring at the lowest doses and therefore subjected to systematic review of pertinent studies for use in MRL derivation. Hepatic effects were not considered for MRL derivation and the corresponding studies were not reviewed systematically. ATSDR does not derive MRLs based on carcinogenicity. No change was made to Section 2.1.*

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 8: The results of several epidemiological studies of HCH isomers are presented in tables separated based upon HCH isomer and type of health effect (e.g., cardiovascular, cancer). Overviews of the findings of the studies and major limitations, if any, are discussed briefly in the text. In addition, the results of case reports of effects from accidental poisonings and suicide attempts are presented, which provide information on potential acute effects of exposure in humans. With this many isomers, health effects, and studies to be considered, the level of presentation of the human studies is appropriate for the purposes of the document.

RESPONSE: *No response needed.*

QUESTION: W ere 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 9: Numerous animal studies of HCH toxicity were identified and summarized in the report. Some were relatively comprehensive in the endpoints examined and some were limited in various ways, such as numbers of doses, endpoints, etc. Summary presentations of the animal studies in tables in the document, which include key experimental details, make it easy for the reader to readily identify what each study offers. The document does a reasonably good job of evaluating the animal study literature collectively for each of the routes so that the most important types of toxicity can be identified.

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 10: The vast majority of the animal studies of HCH isomers were conducted using mice and rats, which are appropriate for the toxicological endpoints being examined. There are no issues or concerns with the animal species from which HCH toxicological data are available.

RESPONSE: *No response needed.*

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

COMMENT 11: As is the case with most chemicals, the value of human studies is principally for hazard identification, and only very limited dose-response information can be obtained. Dose-response relationship information must therefore come primarily from animal data. Given that the MRLs are derived based on the NOAEL/LOAEL approach (see Appendix A), presentation of the dose-response data derived from animal studies in this section is appropriate. From the MRL worksheets in Appendix A, it is clear that benchmark dose modeling was considered in deriving the point of departure for the critical

effect, but in each case was not possible for reasons that are provided. I agree with these decisions. In the introduction to Appendix A, it states that MRLs are derived using the NOAEL/uncertainty factor approach. While that happened to be the case for the HCH isomers, it gives the impression that this is the only approach considered. This may be "boilerplate" language for these profiles, but I would encourage modification to indicate both NOAEL/LOAEL and benchmark dose modeling approaches are considered for assessing dose-response relationships and identification of a point of departure.

RESPONSE: *The text in the introduction to Appendix A is boilerplate. ATSDR will consider this suggestion in future revisions to the Toxicological Profile guidance.*

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 12: I am not aware of any studies not included in the profile that would be important in evaluating the toxicity of HCH isomers.

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 13: I am not aware of any studies not included in the profile that would be important in deriving MRLs for the isomers.

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 14: I didn't find any NOAELs or LOAELs that were mistaken or missing from the text and LSE tables and figures. As a minor comment, the studies at the beginning of Table 2-1 are mis-numbered creating a disconnect with the associated LSE figure.

RESPONSE: *The numbering for studies in Table 2-1 was corrected.*

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 15: There are a few categorizations that might merit re-consideration. They are:
1) Table 2-3, Study 15, repro effect "slight testicular atrophy" as Serious (Less Serious might fit better);
2) Table 2-4, Study 78 and Study 79, developmental and neuro effects, respectively, "Ultrastructural changes in the brain" and "Reduced locomotor activity and spatial memory; ultrastructural changes in the hippocampus and substantia nigra …" as Serious (Less Serious might fit better); 3) Table 2-4, Study 96, repro, 40% reduction in litter size as Less Serious (Serious might be more consistent with other calls).

RESPONSE: *ATSDR considers both testicular atrophy and ultrastructural changes in the brain to be serious effects, so no changes were made to the classification of these effects. Regarding Table 2-4, Study 96, the litter size reduction of 40% does not meet the ATSDR threshold for a serious LOAEL (50% reduction in number of offspring). However, the reductions in F3 male testes size appear to indicate testicular atrophy, which is a serious LOAEL. Therefore, Table 2-4 study 96 and Figure 2-7 were revised to indicate the dose of 1 mg/kg/day in the study by Beard and Rawlings (1998) as a serious LOAEL.*

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 16: Descriptions of mechanisms of action are generally very limited in the document, mostly attributing effects to oxidative stress and citing one or two studies. I don't think that this is a failing of the document as much as a reflection of the time when most of the HCH toxicity research was conducted. HCH isomers have not been a research priority in quite a while and never got the mechanistic attention of other, higher profile chemicals. Consequently, most of the literature available was from a time when measuring a few endpoints related to oxidative stress passed for identifying the mechanism of action. I have no suggestions for changes — this is a limitation in information available.

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 17: The conclusions reached in this chapter are appropriate given the overall database.

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 18: This section provides a thorough and adequate summarization of the literature regarding absorption, distribution, metabolism, and excretion of HCH isomers. The section provides valuable information for understanding some of the differences in effects among the isomers. As a minor comment, lines 30-32 on the first page of Section 3.1.3 Excretion states that the excretion profile follows zero-order kinetics with a half-time for elimination of beta-HCH from breast milk. If the kinetics are zero order, the concept of half life is not applicable.

RESPONSE: *The text of Section 3.1.4 (Excretion) was revised as follows:*

> The excretion profile for β-HCH in milk lipids followed zero-order kinetics and the mean excretion rate was approximately 7% per month.

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

COMMENT 19: The available pharmacokinetics/pharmacodynamic models are presented with a brief but adequate discussion of the supporting data. As a minor comment, the section on the DeJongh and Blaauboer model states, "Simulated gamma-HCH concentrations … compared adequately with experimental results." (line 12, page not numbered). "Adequately" is a little vague and it would be helpful to give the reader a little more information on the comparison. Also, consider clarifying what is meant by the next sentence, ".., the model has not been validated using biological evaluation of kinetics parameters."

RESPONSE: *Section 3.1.5 was revised to provide additional information on the comparison between simulated and measured levels, and the sentence regarding validation was deleted.*

> Simulated γ-HCH concentrations in fat, brain, and muscle compared well with measured values obtained after single intraperitoneal exposure in rats. Simulated levels in blood were slightly higher than measured levels after oral and intraperitoneal exposure.

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 20: Based on the limited information available on toxicokinetics in humans, Section 3.1.6 covers these topics as well as can be expected.

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 21: I am not aware of any data relevant to child health and developmental effects that were not discussed in the profile.

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 22: Several types of individuals that may have higher risk of susceptibility are discussed. Evidence supporting their consideration as sensitive populations is clearly presented and reasonable. I have no disagreement with the information presented in this section.

RESPONSE: *No response needed.*

Biomarkers of Exposure and Effect

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

COMMENT 23: Potential biomarkers for exposure are discussed and critically evaluated. I agree with the points raised that limit the value of each these – the non-specificity of HCH metabolites and the lack of quantitative data to correlate levels of HCH isomers in tissue or fluids with past exposure. Skin lipid concentrations provide some information on adipose tissue levels and relative differences in exposure, but not quantitative exposure estimates.

RESPONSE: *No response needed.*

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

COMMENT 24: I agree with the draft that there are no biomarkers for effect specific to HCH isomers. Effects produced by HCH for which biomarkers are available are all produced by a wide variety of chemicals.

RESPONSE: *No response needed.*

Interactions with Other Chemicals

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

COMMENT 25: There is a fairly extensive section on potential interactions between HCH isomers and other substances leading to both increased and decreased toxicity. The discussion is inclusive of chemicals for which co-exposure might occur at a hazardous waste site (e.g., cadmium, pesticides), but also includes literature on dietary constituents and plant extracts.

RESPONSE: *No response needed.*

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

COMMENT 26: Where known, mechanisms of interactions are included in the discussion of interactions. I am not aware of additional references to supplement this section.

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 27: The information provided in the chemical and physical properties tables appears correct to me.

RESPONSE: *No response needed.*

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

COMMENT 28: Information is provided for each of the subject HCH isomers.

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 29: Information on these topics appears complete. I am not aware of any additional references of significance. As a minor comment, it might be helpful to the reader to provide a brief explanation if possible, as to what the use "ancillary" in Table 5-1 might include. This is listed for all the facilities in the table and is in fact the only HCH use at all but one.

RESPONSE: *Section 5.2.1 was revised to add information on the meaning of ancillary uses under TRI, as follows:*

> Most of the uses by these facilities are considered to be ancillary, indicating purposes other than chemical processing or manufacturing. Examples of ancillary uses as defined under TRI include cleaners, degreasers, lubricants, fuels, and waste treatment uses.

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 30: This section comprehensively evaluates all potential completed pathways of environmental exposures. The occurrence of HCH isomers at NPL sites is addressed specifically in this section, including the geographical distribution (by states) of these sites. I am not aware of any additional relevant information regarding HCH contamination at NPL sites, or waste sites in general. As a minor comment, I suggest adding incidental ingestion of contaminated soil to the list of low-level potential exposure pathways for the general population (page 222, lines 3-5). Dermal contact with soil is listed, yet incidental soil ingestion will drive the risk assessment of HCH at soil contaminated sites.

I also have a comment about formatting beginning with Section 5.3. In other sections of the document, a general introduction to the topic may be presented in bold text, with specific information on HCH isomers following without bolding. In Section 5.3, the first paragraph of all the subsections is bolded, even though it is not introductory and proceeds directly to presentation of HCH-specific information.

RESPONSE: *Section 5.1 was revised to include incidental ingestion as a potential exposure pathway for the general population. The bold formatting in the document is consistent with ATSDR formatting guidance, so no change was made.*

> The general public may be exposed to low levels of HCH through inhalation of contaminated ambient air, consumption of contaminated drinking water, or through incidental ingestion of or dermal contact with contaminated soils.

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 31: Extensive information is presented from the literature regarding transport, partitioning, transformation, and degradation of HCHs in all relevant media (air, groundwater, surface water, soil, sediment, and others). I am not aware of any additional relevant studies.

The paragraph on page 235, lines 16-21 seems out of place (dermal bioavailability of HCH isomer-contaminated soil on human skin). It belongs in Chapter 3, and in fact is covered there.

RESPONSE: *The paragraph pertaining to dermal bioavailability was deleted from Section 5.4.1.*

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 32: This section provides information on levels in environmental media. Much of the information is presented in tables that are clearly organized with proper units of measurement. Any limitations in the data available, such as from lower limits of analytical quantitation, are appropriately noted. I am not aware of any studies with information on this topic that have not been cited.

As a minor comment, the results presented from the Smith-Baker and Saleh, 2011 study on page 271 (lines 7-11 is surprising. It is unclear why HCH concentrations in hair representing urban environmental exposure (1,500 ng/g) would be 4-times higher than pesticide applicators (460 ng/g). Did the authors comment on this finding? If they have an explanation or caveat, it might be worth repeating in the document here.

RESPONSE: *The study authors did not provide any explanation for the discrepancy. The following text was added to Section 5.6.*

> The study authors did not suggest an explanation for the higher levels in the samples from environmentally exposed persons in Houston, Texas compared with levels in pesticide applicators in Atlanta, Georgia; however, the sample sizes were very small (eight applicators and eight each environmentally exposed persons in Atlanta and Houston). In addition, the ages of the volunteers from whom hair samples were collected were not reported, and hair from older individuals could have higher accumulation of γ-HCH. Further, there was no information on whether any volunteers had previous exposure to γ-HCH applied to the scalp for treatment of lice.

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

COMMENT 33: Sources and pathways of exposure for the general population and populations with the greatest contact (specifically, individuals using head lice products and workers occupationally exposed to HCH isomers) are well discussed. Appropriate to this topic, the text makes clear the importance of temporality in considering data on HCH isomer concentrations in individuals given the phase out of use

of these isomers except in prescription products for scabies and head lice. I agree with the populations selected for discussion – all are important, and none is missing.

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 34: I am not aware of any other studies that could fill a data gap.

RESPONSE: *No response needed.*

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

COMMENT 35: I agree with all of the identified data needs. However, there isn't much discussion of mechanisms of toxicity as a data need. As is discussed in this chapter, information on effects in humans is quite limited, and given the phase out of almost all uses of HCH in the U.S., opportunities to get new information will be scarce. As a result, we will continue to rely principally on animal data for our evaluation of human health risks from HCH exposure. Assessment of the human relevance of animal data benefits greatly from the availability of information on mode of action. As noted in comments elsewhere, information on mode(s) of action of HCH is weak. I would encourage adding a bit more discussion of mechanistic studies as a data need in this section.

RESPONSE: *The following paragraph was added to Section 6.2 under Health Effects.*

> For the key health outcomes, especially those shown above, data on the mechanisms by which HCH isomers induce toxicity are limited. Additional mechanistic studies may improve the understanding of the human relevance of toxic effects observed in animals.

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

COMMENT 36: The data needs are presented in a neutral, non-judgmental manner — I detected no bias in the discussion of data needs.

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 37: Some U.S. state regulatory agencies (such as OEHHA in California) have different inhalation unit risk values for the HCH isomers and technical HCH. However, the introduction specifies international and national guidelines, and on that basis the table appears complete.

RESPONSE: *No response needed.*

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

COMMENT 38: All appear appropriate — none should be removed.

RESPONSE: *No response needed.*

Appendices

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

COMMENT 39: The MRL worksheets in Appendix A are well organized, informative, and extremely useful. Appendix B documents the literature search and Appendix C provides the framework, process, and outcomes of systematic literature review for health effects of HCH. Both of these appendices are well constructed, appropriately detailed, and important in providing transparency for the analysis conducted by ATSDR. The Users Guide in Appendix D is a useful addition to the document, particularly for those not already familiar with ATSDR toxicological profiles.

RESPONSE: *No response needed.*

Comments provided by Reviewer #3

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1. Relevance to Public Health

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

COMMENT 1: I agree with the human health effects reported in the profile.

RESPONSE: *No response needed.*

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

COMMENT 2: I agree that the effects observed in animals are likely to be of concern to exposed humans. Liver toxicity observed following α HCH is a toxicity that can occur in humans. Likewise, the neurological, developmental, and immune system effects observed following exposure to β-, γ-HCH isomers are also toxicities that can occur in humans.

RESPONSE: *No response needed.*

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

COMMENT 3: The exposure conditions for animal studies were adequately described in this section.

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 4: Since the composition of HCH isomers in technical HCH is variable, no acute-, intermediate-, and chronic-duration MRLs were derived for technical HCH. I agree with this decision.

Inhalation studies for any other the HCH isomers are not available, there I agree that acute-, intermediate-, or chronic-duration inhalation MRLs for HCH isomers could not be derived.

Further, I agree that no appropriate acute-, intermediate- or chronic-duration oral studies are available for δ-HCH, thus supporting that MRLs for this isomer could not be derived.

I agree that there are insufficient data for derivation of an acute-duration oral MRL for α HCH, as the single acute-duration study identified was inadequate for deriving an MRL.

For β-HCH, while chronic studies are available, I agree that the Fitzhugh et al. (1950) study identified a LOAEL that was higher than the LOAEL identified and used to derive an intermediate MRL, thus, I agree that a chronic MRL could not be derived. Similarly, the effect levels for γ-HCH in chronic studies were much higher that that observed for the derivation of an intermediate-duration MRL. I agree with the decision to not derive a chronic MRL for γ-HCH.

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? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 5: α-HCH – intermediate oral MRL was based on a rat study (Sumida et al., 2007) that identified a NOAEL of 2 mg/kg/day for liver effects. Significant elevation in AST, ALT and relative liver weights were seen at the 2 mg/kg dose, however, histopathological changes were observed only at the 20 mg/kg dose. Further, this was the only study that identified a NOAEL and is preferable to studies that established only LOAELs. I agree with the UFs and modifying factor that were applied to derive the MRL.

α-HCH – chronic oral MRL was based on liver effects observed in Wistar rats after lifetime (107 weeks) exposure to α-HCH. This study identified a NOAEL of 0.9 mg/kg/day. I agree with the selection of this study and the UFs and modifying factors that we applied.

β-HCH – acute oral MRL was based on the observation of ataxia in the first 2 weeks of a 13-week study in Wistar rats. 8 mg/kg/day was identified as the NOAEL. I agree with the selection of this study and the UFs and modifying factors that we applied. The same 13-week study (Van Velsen et al., 1986) identified liver hyalinization (0.18 mg/kk/day LOAEL) as a critical effect and was selected to derive an intermediate-duration MRL. In this case a UF of 3 was applied for the use of a LOAEL in addition to UFs of 10 for human variation and species extrapolation – I agree with the application of these UFs

γ-HCH - acute oral MRL was based on a minimal LOAEL of 1 mg/kg/day for developmental effects in rats (Dalsenter et al. 1997b). I agree with the critical effect selected and the use of this study to derive an acute MRL. – I agree with the application of the UFs applied for the MRL derivation.

An intermediate-duration oral MRL was derived for γ-HCH based on a NOAEL of 0.000076 mg/kg/day for cardiac effects in rat pups (Sauviat et al. 2005). While the NOAEL and LOAEL identified in this study were considerably lower that observed in many other studies, I agree with the study and critical effect selection as well as the UFs that were applied.

RESPONSE: *No response needed.*

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

COMMENT 6: Nothing noted.

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 7: I agree that the conclusion made concerning the health effects of HCH isomers adequately reflect the published literature.

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 8: Yes, I agree that the available human studies were presented in the text. Study limitations and confounding were sufficiently presented, yet the descriptions were not excessive in length.

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 9: Yes, I agree that the adequately designed animal studies were presented in the text. In cases where the studies had limitations (small group size, limited dosing, meeting abstract), they were adequately described in this section. Studies that were selected to derive MRLs represented well designed studies.

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 10: Yes, the animal species were appropriate to detect the toxicological endpoints in each study.

RESPONSE: *No response needed.*

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

COMMENT 11: I agree that attention was given to dose response relationships for HCH isomers for both animal and human data. For most human studies, dose-response data was not available, and this was adequately noted in the document.

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 12: I am unaware of any additional studies that are related to the toxicity of HCH isomers that should be included in the profile. The literature review and search strategy was very thorough. The summary figures and tables provided aided greatly in enhancing the readability of this document.

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 13: I am unaware of any additional studies that would be relevant to the derivation of an MRL for any of the HCH isomers that should be included in the profile.

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 14: The appropriate NOAELs and LOAELs were identified for each study and were clearly shown I the text, and LSE tables and figures.

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 15: As described in this chapter, "Serious" effects were listed for LOAELs that evoke failure in a biological system and can lead to morbidity or mortality whereas, "Less serious" effects were ascribed in instances where a LOAEL was for effects that were not expected to cause significant dysfunction or death, or whose significance to the organism was not clear. I agree with the designations 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 16: Limited mechanistic data is available for HCH isomers. To my knowledge, all possible mechanisms of action for HCH isomers were included in this chapter of the profile.

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 17: I agree that the conclusions presented for were appropriate.

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 18: I agree that there was adequate discussion of the available data for absorption, distribution, metabolism and elimination of HCH isomers.

RESPONSE: *No response needed.*

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

COMMENT 19: A five-compartment PBPK model for γ-HCH in rats has been developed. A human PBPK model for β-HCH, and a human dermal PBPK model for γ-HCH have also been developed. I am not aware of other PBPK models that have been developed.

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 20: The profile does contain some information on toxicokinetic differences between animals and humans, and the relevance of animal toxicokinetic data to humans, however, it is not extensively discussed in the document.

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 21: I am not aware of any other relevant data concerning children's health or developmental effects that should be included in the profile.

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 22: I agree that the discussion of potentially susceptible populations is adequate and includes scientific rationale for selection of those populations being at potentially higher risk. I am not aware of any publications concerning other populations that might be considered at higher risk for toxicities to HCH isomers.

RESPONSE: *No response needed.*

Biomarkers of Exposure and Effect

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

COMMENT 23: Quantitation of HCH isomers in human serum is a reliable and specific biomarker of exposure to HCH isomers. As discussed in the profile, measurement of some of the metabolites of HCH isomers (phenolic urinary metabolites) are not specific biomarkers of exposure as these metabolites can be identified following exposure to other chlorinated benzenes or phenols.

RESPONSE: *No response needed.*

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

COMMENT 24: As written in the profile, no biomarkers of effect, specific for HCH isomers, have been identified in the literature.

RESPONSE: *No response needed.*

Interactions with Other Chemicals

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

COMMENT 25: The profile does include information concerning the interaction of HCH isomers with other chemicals. The discussion is not limited to effects that might occur at hazardous waste sites. I am not aware of additional information that should be included in this section.

RESPONSE: *No response needed.*

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

COMMENT 26: This section of the document does provide examples of interactive effects between HCH isomers and other substances. Some discussion of the mechanism(s) of these interactions is included. I am not aware of other studies that should be included in this section.

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 27: To my knowledge, the information provided for the chemical and physical properties of HCH isomers are correct.

RESPONSE: *No response needed.*

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

COMMENT 28: Yes, information of the various HCH isomers (α, β, γ, and δ HCH) were included in this section. The range of HCH isomers typically identified in technical grade HCH was also presented.

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 29: Yes, the information in this section appears complete, and I am not aware of any additional information related to the production, import/export, use or disposal of HCH isomers that should be included in this profile.

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 30: Yes, adequate information on release into the environment and potential routes of human contact is included in chapter 5 of the profile.

This section describes the number of hazardous waste sites in which HCH isomers has been identified, but also states that the number of sites in which HCH isomers have been evaluated is not known.

I am not aware of other information that could be included in this section of the profile.

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 31: Yes, this chapter of the profile contained an adequate summary of the available information on the transport, partitioning, transformation and degradation of HCH isomers in all media. I am not aware of any additional data that can be added to this section of the profile.

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 32: Yes, monitoring data for HCH isomers and technical grade HCH has been collected and a thorough summary of the monitoring for HCH in air, water and sediment/soil, as well as other media is presented in Chapter 5 of the profile. Comprehensive summary tables are also provided in this section. It appears that proper units were used for each medium, and the form of the chemical measured was also included. Further, there appears to be an adequate discussion of the quality of the data. I am not aware of any other studies or information that should be included in this section.

RESPONSE: *No response needed.*

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

COMMENT 33: Yes, chapter 5 of the profile adequately describes sources and pathways of exposure for the general population, those who were occupationally exposed, and other populations with potentially high exposures (through medicinal use for lice and scabies). In the latter category, workers who work at facilities that produce, process, or use γ HCH were considered populations with potentially high exposures. In addition, exposure from use of shampoos containing γ-HCH (for lice and scabies treatment) and individuals living near hazardous waste sites were considered populations that might have high exposures. I agree with the selection of these populations.

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 34: I am not aware of any additional studies that have been performed and would fill in a data gap.

RESPONSE: *No response needed.*

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

COMMENT 35: The toxicological profile for HCH isomers was very thorough and included the identification is studies that are missing that preclude the development of acute-, intermediate- and chronic-duration MRLs for HCH isomers. Concerning health effects, the profile identified that inhalation exposure were lacking for all endpoints in which adverse health outcomes have been observed following other routes of exposure. In general, I agree that these are data gaps however, since use of HCH isomers has largely been eliminated, I would not consider these types of studies of "high" priority. Concerning human epidemiological studies, since current exposures to HCH isomers is limited, I also agree that follow-up of occupational cohorts established previously may be the most useful approach to obtaining additional human data. The profile states that it would be useful to design studies to identify specific biomarkers of effect for health outcomes of concern for HCH isomers. Given that biomarkers of effect that would be considered specific to HCH isomers were not defining, it is unclear what studies would be able to satisfy this datagap. As written, the sections on ADME and toxicokinetics are vague and the utility of performing studies such as these was not described. Concerning environmental fate and bioavailability from environmental media, I agree that additional information on the transport, transformation, and persistence of HCH isomers and the bioavailability of HCH isomers from soils and groundwater, particularly at hazardous waste sites, are needed to identify the most important routes of human exposure to HCH. As bioconcentration values in zebra fish for α and β HCH have been reported, I agree that additional information on the potential bioaccumulation of α , β , and δ HCH isomers in terrestrial and aquatic food chains is a data gap. Information concerning current exposure levels in environmental media and human residing near hazardous waste sites, would be useful, but given the reduction in current use and disposal patterns, it is questioned whether this would represent a "high" priority data gap.

RESPONSE: *Inhalation toxicity data for HCH isomers are needed if inhalation MRLs are to be derived. While levels of HCH isomers in some environmental media have declined over time, there is residual contamination and therefore a need for data to assess health outcomes from exposure. No change was made to the MRL and Health Effects subsections of Section 6.1. The text of the subsection on Absorption, Distribution, Metabolism, and Excretion in Section 6.1 was revised as follows:*

> Information is available to evaluate the toxicokinetics of HCH isomers following oral and dermal exposure in animals and humans. Studies evaluating toxicokinetic properties following inhalation exposure would be helpful. Limited information suggests differences in the metabolism of the HCH isomers. Additional data on metabolism of the α-, β- and δ-HCH isomers would be beneficial, especially if such information was linked to differences in specific health outcomes. *In vitro* studies using rat liver microsomes have demonstrated the formation of a reactive epoxide metabolite; however, investigations have not been conducted to examine the epoxide formation i*n vivo* or its role in inducing mutagenic and carcinogenic effects. Further information on the

possible role of epoxide formation in carcinogenesis *in vivo*, as well as its rate of formation under various conditions, would be useful.

The text of the subsection on Biomarkers of Exposure and Effect in Section 6.1 was revised as follows:

Additional studies designed to assess mechanisms of action and/or adverse outcome pathways may serve to identify specific biomarkers of effect for health outcomes of concern for HCH isomers (e.g., liver, neurological, developmental, and immune system effects).

The text of the subsection on Exposure Levels in Environmental Media in Section 6.1 was substantially abbreviated, as follows:

γ-HCH has been detected in air, surface water and groundwater, sediment, soil, and food. A gradual decrease of α- and γ-HCH air has been seen across the decades (Atlas and Giam 1988; Cortes and Hites 2000; WQP 2021), and there is evidence of decreases of α- and β-HCH in surface water and groundwater although the data have a large range (WQP 2021). Trends for soil, reflecting varying land uses, are not as clear for the isomers. Although the use of γ-HCH as a pesticide was voluntarily canceled in 2006 (EPA 2006b), it is uncertain whether new environmental measurements will show considerably lower levels of HCH since there are remaining impacts from importing and processing HCH, and evidence of persistency of the isomers. For example, a study of a pesticide reformulating and packaging facility reported groundwater contamination at the site (Chartrand et al. 2015). Therefore, additional information on the levels of γ-, α-, β-, and δ-HCH isomers would be beneficial to determine current potential human exposure to the chemicals from environmental media, particularly near hazardous waste sites.

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

COMMENT 36: Yes, I agree that the data needs section is presented in a neutral, non-biased manner.

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 37: I am not aware of any additional regulations or guidelines for HCH isomers that should be included in this section.

RESPONSE: *No response needed.*

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

COMMENT 38: None noted.

RESPONSE: *No response needed.*

Appendices

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

COMMENT 39: Appendices A-G were well organized and concise. The overall presentation style in the document and appendices makes the document easy to follow, readable, and contained pertinent information for the various audiences that may read the profile

Table and Figure in Appendix D are labeled as "Table 2-X" and "Figure 2-X" – are this correctly identified?

RESPONSE: *The table and figure in Appendix D are examples and as such are given generic names. No change was made.*

Unpublished Studies (If Applicable to Review)

COMMENT 40: There are no unpublished studies that need reviewed for HCH isomers.

Thus, these do not apply:
~~For each of the unpublished studies included with the profile, prepare a brief evaluation using the following questions as prompts:~~

- ~~Did the study use an adequate number of animals and practice good animal care?~~
- ~~Did the study account for competing causes of death?~~
- ~~Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?~~
- ~~If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.~~
- ~~Do you agree with the conclusions of the author? If not, please explain.~~