DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR 2-HEXANONE

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 2-Hexanone were:

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Comments Provided by Peer Reviewer #1

ATSDR Charge Questions and Responses

SECTION 1 – PUBLIC HEALTH STATEMENT

QUESTION: Does the chapter present the important information in a non-technical style suitable for the average citizen? If not, suggest alternate wording.

COMMENT: Yes, I agree that the chapter is presented in an appropriate style for the average citizen. I noted specifically in the text of the master review document, via the "comments" feature in MSWord, the few instances where I felt some clarification would be helpful.

RESPONSE: *Please see the responses to the marked up document at the end of the Reviewer #1 section.*

QUESTION: In your opinion, do the answers to the questions adequately address the concerns of the lay public?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text? Please note sections that are weak and suggest ways to improve them.

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Are scientific terms used that are too technical or that require additional explanation? Please note such terms and suggest alternate wording.

COMMENT: I believe the text is well designed for the lay public and is not too technical. In a few instances I have made some minor suggestions directly in the text of the master review document.

RESPONSE: *Please see the responses to the marked up document at the end of the Reviewer #1 section.*

SECTION 2 – RELEVANCE TO PUBLIC HEALTH

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

COMMENT: Yes, I agree with the report's characterization of effects reported in humans.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes, I agree with the report's characterization of effects reported in animal studies. Animal studies are an accepted tool to predict potential effects in humans. In this case, some of the neurological effects reported in animal studies were observed in human populations.

RESPONSE: *No response is necessary.*

QUESTION: Have exposure conditions been adequately described? If you do not agree, please explain.

COMMENT: On page 27 Line 21, there is discussion that this chemical has been completely discontinued in both use and manufacture in the United States.

RESPONSE: *No response necessary.*

QUESTION: Comment on any potential for this chemical to be in imported products or if it is not known then perhaps you can just state that while you are not aware of use overseas or in imported products that it is possible even if unlikely.

RESPONSE: *Please see the responses to the marked up document at the end of the Reviewer #1 section.*

QUESTION: Can you provide some information on how many or what proportion of NPL or other hazardous waste sites have detected this compound? Is it commonly found on old waste sites? Are there still drums of waste laying around on waste sites that may contain this chemical and potentially result in releases from leaking drums? It would help clarify in the minds of public health professionals the likelihood of those living near legacy waste sites or facilities as to the potential for historic uses of 2-hexanone to contribute to current exposures and for these professionals to make a judgement about the likelihood of such exposures.

RESPONSE: *Please see the responses to the marked up document at the end of the Reviewer #1 section.*

QUESTION: Similarly, it is possible, although probably not likely since last remaining stocks were sold in the early 80's, that someone could find an old product (lacquer or varnish removers) that might contain this solvent. It could be mentioned that old legacy containers of products could be found and used or disposed of currently. If this were to happen, likely it would be a very short term or one-time use resulting in exposure.

RESPONSE: *A statement was added to the profile regarding the possible exposure via old stock of products containing 2-hexanone as suggested by the Reviewer.*

SECTION 3 – HEALTH EFFECTS

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)? If not, were the major limitations of the studies sufficiently described in the text without providing detailed discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: The studies cited were appropriate.

RESPONSE: *No response is necessary.*

QUESTION: Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)? Please suggest appropriate changes.

COMMENT: Yes, they were appropriate and accurately reflected in the profile.

RESPONSE: *No response is necessary.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT: Yes, however, I have some editorial comments and suggestions listed in the text. See the attached document. In a few instances, the authors of the profile used the terms in an unclear manner and also did not clearly communicate that the NOAEL or LOAEL values are further adjusted with uncertainty factors to develop the MRL level. I suggested a few areas in the text on the attached and marked up document where I thought some additional clarity would be helpful.

RESPONSE: *Responses to editorial and other comments are at the end of Reviewer #1 section where the Reviewer specified the page and line number in the profile.*

QUESTION: Were the appropriate statistical tests used in the studies? Would other statistical tests have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

COMMENT: Yes, however, as noted in the marked up copy, some of the studies combined their control groups with low exposure groups, making an assessment of effect in the low exposure group not possible. Regardless of the statistical tests used, this combination in two of the studies cited limits the interpretation of the data for the low exposure groups.

RESPONSE: *Responses to specific comments are at the end of Reviewer #1 section where the Reviewer specified the page and line number in the profile.*

QUESTION: Are you aware of other studies which 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: Yes, for developmental toxicity, a recent study cited below may be useful to consider because it sheds light on one of the important metabolites of 2-hexanone. I have also attached the paper to my peer review submission.

Cheng, X.; Luo, R.; Wang, G.; Xu, C.; Feng, X.; Yang, R.; Ding, E.; He, Y.; Chuai, M.; Lee, K.; Yang, X. (2015). Effects of 2,5-hexanedione on angiogenesis and vasculogenesis in chick embryos. Reproductive Toxicology, 51: 79–89.

RESPONSE: *ATSDR thanks the Reviewer for providing the article by Cheng et al. (2015); however, the study does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated directly with 2,5-hexanedione relates to environmental or occupational exposures to 2-hexanone. If some evidence existed indicating that 2-hexanone is a developmental toxicant, it would make sense to search for possible mechanisms; in that case, the Cheng et al. (2015) study would have some relevance.*

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

COMMENT: Yes, I believe the cited studies were adequate.

RESPONSE: *No response is necessary.*

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: Yes, however, in several cases the profile authors noted that studies in hens were not appropriate but that studies in rats and cats were appropriate. As I suggested in the marked up copy attached with this summary report, the profile authors should give some rationale for this decision as it is not clear in the text why this decision was made.

RESPONSE: *In various places throughout the profile, the text states the following: "While hens have proved to be a good sensitive model for 2-hexanone-induced neuropathy and studies in this species are useful for hazard identification, they are not useful for risk assessment. Because the digestive and respiratory systems are different from mammals, it is not known whether the dose-response in hens is applicable to humans." The differences in anatomy and physiology between birds and humans are far greater than between most mammals used in research and humans.*

QUESTION: Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the text? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?

COMMENT: Yes, however, it is important to note that many of these studies were done many years ago, when the sensitivity of many measurements and toxicology endpoints were less refined. In addition, I believe that many years ago toxicologists were more inclined to interpret data that more heavily valued frank health effects. I have noted a few places in the marked up text where I think it might be good to highlight this as contributing to some uncertainty.

RESPONSE: *This issue was brought up by the Reviewer in Section 2.3 where studies are discussed in relation to derivation of possible inhalation MRLs for 2-hexanone. The Reviewer stated in a later comment that the discussion regarding categorization of a "LOAEL" vs. a "serious LOAEL" could be further clarified in the text. The Reviewer questioned how an exposure level could be defined as a LOAEL if only one exposure concentration was tested. It should be noted that MRLs are not derived directly from exposure concentrations, but after applying uncertainty factors to the exposure concentration to account for various uncertainties. All this is explained below in the responses to the specific comments made by the Reviewer on the profile.*

QUESTION: Were all appropriate NOAELs and LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies? If not, please explain.

COMMENT: Yes, for what was identified by the authors of the cited papers. However, in a few of the studies the authors combined the control and low exposure groups or had only one exposure level, making the clear identification of a NOAEL or LOAEL not possible. I have noted a few places in the text of the marked up copy where I believe this could be clarified.

RESPONSE: *Responses to these comments are provided below in the responses to the specific comments made by the Reviewer on the profile. The possibility that, in some cases, combining controls and low-exposure groups made it difficult to determine whether effects were treatment-related was included in the revised text. The issue of identifying NOAELs or LOAELs in studies that tested only one exposure level is also explained below.*

QUESTION: If appropriate, is there a discussion of the toxicities of the various forms of the substance? If not, please give examples of toxicological effects that might be important for forms of the substance.

COMMENT: Yes, it was summarized adequately.

RESPONSE: *No response is necessary.*

QUESTION: Were the appropriate statistical tests used in the interpretation of the studies? If not, which statistical tests would have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

COMMENT: See comment above about statistical tests.

RESPONSE: *The Reviewer's comment relates to the reporting of combined control and low-exposure groups in the chronic inhalation studies in cats and rats. As indicated above, the possibility that, in some cases, combining controls and low-exposure groups made it difficult to determine whether effects were treatment-related was included in the revised text.*

QUESTION: Are you aware of other studies that may be important in evaluating the toxicity of the substance? If you are citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT: Yes, for developmental toxicity, a recent study cited below may be useful to consider because it sheds light on one of the important metabolites of 2-hexanone. I have also attached the paper to my peer review submission.

Cheng, X.; Luo, R.; Wang, G.; Xu, C.; Feng, X.; Yang, R.; Ding, E.; He, Y.; Chuai, M.; Lee, K.; Yang, X. (2015). Effects of 2,5-hexanedione on angiogenesis and vasculogenesis in chick embryos. Reproductive Toxicology, 51: 79–89.

RESPONSE: *This response was provided to the same comment above: The Cheng et al. (2015)* study *does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated with 2,5-hexanedione relate to environmental or occupational exposures to 2-hexanone. If some evidence existed indicating that 2-hexanone is a developmental toxicant, it would make sense to search for possible mechanisms; in that case, the Cheng et al. (2015) study would have some relevance.*

QUESTION: Are the LSE tables and figures complete and self-explanatory? Does the "Users Guide" explain clearly how to use them? Are exposure levels (units, dose) accurately presented for the route of exposure? Please offer suggestions to improve the effectiveness of the LSE tables and figures and the "User's Guide."

COMMENT: Yes, I believe the effectively summarize the information and the explanation is good. I have no further suggestions.

RESPONSE: *No response is necessary.*

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

COMMENT: I noted in the attached markup that I felt this was unclear in the document. It very well may be clearly explained and perhaps it just didn't resonate with me, but I still felt unclear about how exactly you categorized the less serious and more serious effects. I believe some more clear and concise discussion would be helpful.

RESPONSE: *This is addressed in detail below in the responses to comments in the marked-up document.*

QUESTION: If MRLs have been derived, are the values justifiable? If no MRLs have been derived, do you agree that the data do not support such a derivation?

COMMENT: Yes, I believe the derived MRL listed in the document is justifiable. I think some more discussion in the text rather than discussing the details only in the appendix would be helpful, as I noted in the marked up copy.

RESPONSE: *The Reviewer specifically requested more details in Section 2.3 regarding how MRLs are derived. The response below to the comments in the marked-up states that the level of detail in Section 2.3 is in accordance with ATSDR guidance and a detailed explanation on how MRLs are derived is provided in Appendix B.*

QUESTION: Have the major limitations of the studies been adequately and accurately discussed? How might discussions be changed to improve or more accurately reflect the proper interpretation of the studies?

COMMENT: Yes, however, as noted in the marked up copy, the discussion of the Krasavage and O'Donoghue (1977) and O'Donoghue and Krasavage (1979) studies the authors only say "However, poor reporting of the results made it difficult to define a no-observed-adverse-effect level (NOAEL) ….." (for example, page 34 of profile). Details are not provided, I would help with the clarity if the authors noted that in these studies they combined the controls with the low exposure groups so the reader can understand more clearly what the limitation of those important studies were.

RESPONSE: *The issue regarding reporting combining results and low-exposure groups contributing to uncertain results was included in the revised text.*

QUESTION: Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Have "bottom-line" statements been made regarding the relevance of the endpoint for human health?

COMMENT: No, I think this report summarizes so much information that you can get lost and miss any "bottom line" statement. I also don't think that Section 3 is the proper place for a bottom line statement either. It is summarized in the public health statement up front, perhaps just a bit more of an explicit statement might be helpful to the lay reader, but this really should be in Section 1. Also, the report at times clearly stated that this chemical is no longer used in the United States but then at other times talked about industries (e.g. wood pulping) that made it sound as if they were still using the chemical. As note in the marked up copy, this was confusing and should be clarified.

RESPONSE: *Bottom line statements are generally presented in Chapter 3. For 2-hexanone, a chemical with a small database compared with other chemicals, it is difficult to make bottom line statements after each end point if there is only one or two studies per end point and no data in humans. For 2-hexanone, the bottom line is that it is a nervous system toxicant in humans and animals and hardly any additional information is available regarding other effects in humans. The following statement was added in Chapter 1, section HOW CAN 2-HEXANONE AFFECT MY HEALTH: Based on the limited number of studies of humans exposed to 2-hexanone and on studies of subjects exposed to the industrial chemical n-hexane, which also produces the breakdown product 2,5-hexanedione, it is clear that the nervous system is a primary target. However, there is no reliable information to determine whether other organs or biological systems in humans could also be targets for 2-hexanone. In addition, statements have been*

added in Section 3.2 at the beginning or at the end of most end points indicating what the available studies tell and what the relevance of the animal data is for human health. While this chemical is no longer used in the United States, it may still be inadvertently produced as a waste byproduct during processing at certain industries. 2-Hexanone as a waste product is not captured for use. As suggested, this has been clarified throughout the profile.

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT: Yes.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes, the authors were able to utilize several studies that had dose response relationships. There were several studies where the study utilized only one exposure value, thereby making it impossible to develop a dose-response curve. The authors should be clear that studies with only one exposure cannot be used for an assessment of dose-response assessments.

RESPONSE: *This is clear in Section 2.3, MINIMAL RISK LEVELS (MRLs). Below, in a more detailed response to comments in the marked-up document, it is explained that single-dose studies that defined LOAELs (but not serious LOAELs) can be used for risk assessment using appropriate uncertainty factors even though no dose-response can be established.*

QUESTION: Has the animal data been used to draw support for any known human effects? If so, critique the validity of the support.

COMMENT: Yes.

RESPONSE: *No response is necessary.*

SECTION 3.4

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT: Yes, to the extent possible with the currently available research. See comments in text.

RESPONSE: *Responses to comments in the marked-up document can be found below.*

QUESTION: Have the major organs, tissues, etc. in which the substance is stored been identified? If not, suggest ways to improve the text.

COMMENT: Yes, to the extent possible with the currently available research. See comments in text.

RESPONSE: *Responses to comments in the marked-up document can be found below.*

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

COMMENT: Yes, to the extent possible with the currently available research. See comments in text. As noted in the marked up copy, the study by Duguay and Plaa, 1995; I think the most interesting thing about this article and what should be pointed out is that inhalation route resulted in much higher concentrations of 2,5-hexanedione in the plasma and lung than when administered by the oral route. I think this is significant and should be mentioned.

RESPONSE: *This was added to Section 3.4.1.1.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? What other observations should be made?

COMMENT: Yes, to the extent possible with the currently available research. See comments in text.

RESPONSE: *Responses to comments in the marked-up document can be found below*

QUESTION: Is there an adequate discussion of the relevance of animal toxicokinetic information for humans? If not, please explain.

COMMENT: Yes, to the extent possible with the currently available research. See comments in text.

RESPONSE: *Responses to comments in the marked-up document can be found below.*

QUESTION: If applicable, is there a discussion of the toxicokinetics of different forms of the substance (e.g., inorganic vs. organic mercury)?

COMMENT: Not applicable

RESPONSE: *No response is necessary.*

QUESTION: Are the biomarkers of exposure specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

COMMENT: As the authors clearly defined in the text, there are similar metabolites for n-hexane. 2,5-hexanedione is the principle metabolite of concern. However, it should be noted that in the Krasavage 1979 study another short lived metabolite, 5—hydroxy-2-hexanone was found which was likely intermediate but still detectable, especially several days after exposure.

RESPONSE: *5-Hydroxy-2-hexanone is mentioned as a metabolite in Section 3.8.1 and reference to O'Donoghue and Krasavage (1979) was included.*

QUESTION: Are there valid tests to measure the biomarker of exposure? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.

COMMENT: The authors accurately discuss this in the text.

RESPONSE: *No response is necessary.*

SECTION 3.9 INTERACTIONS WITH OTHER CHEMICALS

QUESTION: Discuss the influence of other substances on the toxicity of the substance. Is there adequate discussion of the interactive effects with other substances?

COMMENT: Yes, the authors adequately discuss this.

RESPONSE: *No response is necessary.*

QUESTION: Does the discussion concentrate on those effects that might occur at hazardous waste sites? If not, please clarify and add additional references.

COMMENT: Not specifically, but what the authors discuss is relevant.

RESPONSE: *No response is necessary.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? If not, please clarify and provide any appropriate references

COMMENT: I do not believe the mechanisms are really well understood.

RESPONSE: *No response is necessary.*

QUESTION: Is there a discussion of populations at higher risk because of biological differences which make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?

COMMENT: There is discussion, but I don't believe it is well known. The authors point out their assumptions for children. It could be more clearly high-lighted that populations exposed to multiple chemicals, including ethanol, that this chemical can potentiate the effects.

RESPONSE: *A study of the interaction of 2-hexanone with ethanol is briefly mentioned in Section 3.9, INTERACTIONS WITH OTHER CHEMICALS. ATSDR is not aware of additional studies of interactions between 2-hexanone and ethanol.*

QUESTION: Are treatments available to prevent the specific substance from reaching the target organ(s), or are the actions general for a class of substances?

COMMENT: I believe this question is best answered by a clinical toxicologist. I will defer to them.

RESPONSE: *No response is necessary.*

COMMENT: Is there any controversy associated with the treatment? Is it a "well-accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?

COMMENT: I believe this question is best answered by a clinical toxicologist. I will defer to them.

RESPONSE: *No response is necessary.*

QUESTION: Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

COMMENT: I believe this question is best answered by a clinical toxicologist. I will defer to them.

RESPONSE: *No response is necessary.*

QUESTION: Are there treatments to prevent adverse effects as the substance is being eliminated from the major organs/tissues where it has been stored (e.g., as a substance is eliminated from adipose tissue, can we prevent adverse effects from occurring in the target organ[s])?

COMMENT: I believe this question is best answered by a clinical toxicologist. I will defer to them.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the identified data needs? If not, please explain your response and support your conclusions with appropriate references.

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Does the text indicate whether any information on the data need exists?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Does the text adequately justify why further development of the data need would be desirable; or, conversely, justify the "inappropriateness" of developing the data need at present? If not, how can this justification be improved.

COMMENT: As this chemical is not used or produced in the United States at this time, honestly, it probably should be a low priority. True it does exist on some hazardous waste sites but its environmental persistence is fairly low relatively speaking.

RESPONSE: *No response is necessary.*

SECTION 4 – CHEMICAL and PHYSICAL INFORMATION

QUESTION: Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables? Please provide appropriate references for your additions or changes

COMMENT: No.

RESPONSE: *No response is necessary.*

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL
The level of detail in this chapter should be appropriate to an overview.

QUESTION: Are you aware of any information that is wrong or missing? If so, please provide copies of the references and indicate where (in the text) the references should be included.

COMMENT: No.

RESPONSE: *No response is necessary.*

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT: Yes, it is clear and the authors do a nice job of summarizing the information.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes, to the extent possible.

RESPONSE: *No response is necessary.*

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: Yes, to the extent possible.

RESPONSE: *No response is necessary.*

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: Yes, however, there is a discussion several times throughout the text regarding "wood pulping, coal-gasification, or oil-shale processing operations" and it is unclear in many cases whether this is in reference to current use of this chemical or whether this a natural byproduct of processing (I presume this is the case). The authors should clarify because in many places when this is discussed it sounds like the authors are saying 2-hexanone is currently deliberately used in these operations. I noted several places in the marked up copy where I felt this was the case.

RESPONSE: *While this chemical is no longer used in the United States, it may still be inadvertently produced as a waste byproduct during processing at certain industries. 2-Hexanone as a waste product is not captured for use. As suggested, this has been clarified throughout the profile.*

CHAPTER 7. ANALYTICAL METHODS

QUESTION: Are you aware of additional methods that can be added to the tables? If so, please provide copies of appropriate references.

COMMENT: No.

RESPONSE: *No response is necessary.*

QUESTION: Have methods been included for measuring key metabolites mentioned previously in the text?

COMMENT: No. I don't believe the discuss the measurement of 2,5-hexanedione, a key metabolite.

RESPONSE: *Table 7-1, Analytical Methods for Determining 2-Hexanone in Biological Samples, contains footnotes specifying that the detection limit and percent recovery are for 2,5-hexanedione, the*

metabolite of 2-hexanone. The text was revised in Chapter 7.1 BIOLOGICAL MATERIALS to clarify that the metabolite is what is being detected.

QUESTION: If unique issues related to sampling for the substance exist, have they been adequately addressed in the text? What other discussion should be provided?

COMMENT: Not applicable.

RESPONSE: *No response is necessary.*

CHAPTER 8. REGULATIONS AND ADVISORIES

QUESTION: Are you aware of other regulations or guidelines that may be appropriate for the table? If so, please provide a copy of the reference.

COMMENT: No.

RESPONSE: *No response is necessary.*

CHAPTER 9. REFERENCES

QUESTION: Are there additional references that provide new data or are there better studies than those already in the text? If so, please provide a copy of each additional reference.

COMMENT: Yes, for developmental toxicity, a recent study cited below may be useful to consider because it sheds light on one of the important metabolites of 2-hexanone. I have also attached the paper to my peer review submission.

Cheng, X.; Luo, R.; Wang, G.; Xu, C.; Feng, X.; Yang, R.; Ding, E.; He, Y.; Chuai, M.; Lee, K.; Yang, X. (2015). Effects of 2,5-hexanedione on angiogenesis and vasculogenesis in chick embryos. Reproductive Toxicology, 51: 79–89.

RESPONSE: *As stated before, the study by Cheng et al. (2015) does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated with 2,5-hexanedione relate to environmental or occupational exposures to 2-hexanone. If some evidence existed indicating that 2-hexanone is a developmental toxicant, it would make sense to search for possible mechanisms; in that case, the Cheng et al. (2015) study would have some relevance.*

Review of Unpublished Studies

QUESTION: For each of the unpublished studies included with the profile, prepare a brief evaluation that includes your assessment:

COMMENT: I am presuming you would like me to comment on the following three studies send with the review package:

1) Union Carbide, 1977. Comparative Toxicity to Rats of methoxyacetone and five other aliphatic ketones in their drinking water. Union Carbide TSCA section 8 Submission.

2) Krasavage WJ, O'Donoghue JL. 1977. Chronic inhalation exposure of rats to methyl n-butyl ketone 14 (MnBK). In: Initial Submission: Letter from Eastman Kodak Co to USEPA regarding toxicity studies of 15 2-hexanone and metabolites with cover letter dated 09/28/92. Eastman Kodak Co. Submitted under 16 TSCA Section 8E to U.S. Environmental Protection Agency. OTS0555051.

3) O'Donoghue JL, Krasavage WJ. 1979. Chronic inhalation exposure of cats to methyl n-butyl ketone 16 (MnBK). In: Initial Submission: Letter from Eastman Kodak Co to USEPA regarding toxicity studies of 17 2-hexanone and metabolites with cover letter dated 09/28/92. Eastman Kodak Co. Submitted under 18 TSCA Section 8E to U.S. Environmental Protection Agency. OTS0555051. [Unpublished study to be 19 peer reviewed]

Adequacy of design, methodology, and reporting; all three studies followed acceptable protocols at the time they were conducted and therefore were adequate. The reporting of data was problematic for the Krasavage WJ, O'Donoghue JL. (1977) and O'Donoghue JL, Krasavage WJ. (1979) because the authors combined the control groups with the data from their low exposure groups, making distinctions about treatment-related effects in the low exposure group impossible to discern. Some effects were noted in the lower exposure groups but it cannot be know if this was a treatment related effect. As such, this is a serious flaw in the data handling for these two studies.

Validity of results and author's conclusions; and in general I find the authors' statements to be reasonable based on the data and reports in the documents. As described above there is a limitation in the use of the data from two of the studies because of the way the data was handled. In addition, I have some hesitation about the conclusion in the O'Donoghue JL, Krasavage WJ. (1979) study that degeneration of the axons did not occur in the 100 ppm treated group. It was reported that degenerated axons or myelin ovids were "rare" and therefore the authors assumed this meant that the effect did not occur at these levels. I am unclear if they were "rare" just because the dose was lower and therefore occurred less frequently. The authors did not present a comparison to their control group, therefore I assume that it is possible that these "rare" events (which might not be so rare when you are testing only a small number of animals) may have been treatment related.

RESPONSE: *The problem of combining results from the control and low-exposure groups in the chronic inhalation studies in cats and rats by Krasavage and O'Donoghue (1977) and O'Donoghue and Krasavage (1979) is mentioned in Section 2.3 in the revised draft. Neither study was used for risk assessment, but constitute supporting evidence for considering the nervous system the primary target for 2-hexanone.*

QUESTION: Provide comments on study inadequacies or confounding factors.

COMMENT: See above comments.

RESPONSE: *Please see response to previous comment.*

QUESTION: Provide a summary of your conclusions? Do you agree or disagree with those of the author? If not please explain why.

COMMENT: Please see my comments above. In general I feel that these are useful articles and I agree in a general sense with the authors' summaries of their tests. Any concerns I have a detailed in my above comments, but overall these studies are useful for your evaluation with the limitations I have suggested above.

RESPONSE: *No response is necessary.*

Annotated Comments on the Profile

CHAPTER 1: PUBLIC HEALTH STATEMENT

COMMENT: page 22, line 10: Perhaps consider adding a reference to ground water too, as if you had a spill or improper disposal it could be in groundwater and then enter the water supply if the person has a well or possibly from vapor intrusion in their home. I have dealt with some cases of BTEX compounds being detected in residential indoor air due to vapor intrusion that would be conceivable here too since it is so volatile. If the chemical is no longer widely used, perhaps it is still possible to be at NPL sites if it were stored in drums, etc. and therefore theoretically could still have a current release. If industries listed still use then definitely could have a release, especially with oil shale operations you could get groundwater contamination. Also, perhaps outside the scope of this document, but someone could also be exposed if they used this solvent at work too as part of their job.

I did not find any products listed in the NLM household products database at:
https://householdproducts.nlm.nih.gov/index.htm

But it did contain a lot of products that had MIBK and a few with hexanoic acid, although different I wonder if there is any relevance. Are there any products that still may have MBK in them?

RESPONSE: *The industries listed do not currently use 2-hexanone; however, it may be indirectly produced as a waste product during processing. It is therefore possible that a spill of waste water could result in groundwater contamination. Also, there are currently no commercial products that contain 2-hexanone. It is possible that exposure may occur through products, such as lacquers and solvents, manufactured prior to 1982. This information was added as suggested by the Reviewer.*

COMMENT: page 23, line 9: Very high levels? Might want to qualify so the lay reader understands that this was not the result of exposure to trace levels, to put it in perspective.

RESPONSE: *Some workers may have been exposed to up to 36 ppm 2-hexanone; this was added to the text.*

COMMENT: page 24, line 2: Since there is no information, this is a bit of an overstatement, the reality is that your really don't know and there are some chemicals that behave differently in children than adults, it might be better to replace "would probably" with "may" or "might" to make it more tentative.

RESPONSE: *The words "would probably" were replaced with "may."*

COMMENT: page 24, line 14: It is unlikely that your typical family physician will have any idea or know how to find someone was exposed to a chemical like this, therefore this is very unlikely and

suggesting that people ask their family physician may result in more confusion. Maybe you should more strongly suggest that if you suspect you are exposed, in addition to checking with your doctor please check with your state or local environmental agency or health department. I think that public health professionals and environmental professionals should be emphasized more here in addition to the physician.

RESPONSE: *A sentence was added as suggested by the Reviewer.*

COMMENT: page 24, line 19: Is it still used and produced overseas? Are products imported that may contain the chemical?

RESPONSE: *2-Hexanone is still produced overseas, and it is possible that imported products containing, such as foods, may contain this chemical. This information is mentioned throughout the profile and has been added to this section as suggested by the Reviewer.*

COMMENT: page 24, line 24: Perhaps it would be better for them to contact their state environmental agency, state health department, or local health department to obtain information, as the label from old containers may not be informative, might be outdated, and may not give good advice. In addition, they should know that many municipalities hold "house-hold hazardous waste" collection days that might be an appropriate place for disposal.

RESPONSE: *A sentence was added as suggested by the Reviewer.*

COMMENT: page 25, line 21: Design standards to protect different groups of people (e.g healthy adult worker vs. child in a community).

RESPONSE: *The comment refers to standardized text in the section regarding recommendations of the federal government to protect human health. ATSDR appreciates the suggestion and will consider it for future profiles.*

COMMENT: page 25, line 24: Not quite true, I would say that Agencies attempt to update regulations periodically, however, it is not uncommon for standards to be outdated, for example, most OSHA PELs are the same as they have been for over 40 years.

Perhaps say, "Federal Agencies attempt to update regulations periodically as more information becomes available. Sometimes it takes Federal Agencies a long time to go through the process of updating standards and regulations and as such a standard or regulation may change over time."

RESPONSE: *The comment refers to standardized text in the section regarding recommendations of the federal government to protect human health. ATSDR appreciates the suggestion and will consider for it for future profiles.*

CHAPTER 2: RELEVANCE TO PUBLIC HEALTH

SECTION 2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO 2-HEXANONE IN THE UNITED STATES

COMMENT: page 27, line 18: Is there any potential of products containing this solvent being imported? Or old products laying around people's homes?

RESPONSE: *A statement was added to the profile to clarify possible exposure from imported goods and old products.*

COMMENT: page 27, line 21: How many NPL sites or other waste sites have detected 10-hexanone? Are there drums of this located on hazardous waste sites somewhere? Have there been any recent detections on waste sites from legacy operations or waste storage? It would be helpful to the public health professional to list any data you might have.

RESPONSE: *Section 2.1 is intended to provide a summary of exposures to 2-hexanone. It is stated in this section that 2-hexanone has been detected at and near hazardous waste sites. More specific information, including the amount and frequency detected through NPL and other sources, is contained in Chapter 6. No studies were located regarding the presence of 2-hexanone in drums on hazardous waste sites, legacy operations, or waste storage.*

COMMENT: page 27, line 30: Because this is in present tense, it implies that this is still currently used and creates confusion because the previous paragraph says that it is completely discontinued. Clarify and perhaps consider simply saying "included" in the past tense.

RESPONSE: *While this chemical is no longer used in the United States, it may still be indirectly produced as a waste byproduct during processing at certain industries. As suggested by the Reviewer, this has been clarified.*

COMMENT: page 27, line 31: Regarding the statement "These industries include coal gasification plants, oil shale operations, and wood pulping mills," the Reviewer commented that many people in the public and public health professionals may hear about oil shale production or hydraulic fracturing (fracking) in the media and may wonder if this is the same thing and may wonder with the significant increase in shale gas production in the US if you are talking about these operations. You should clarify that point.

RESPONSE: *The text in the profile was revised. In Chapters 2, 3, and 6, oil shale processing was revised to* "in situ *oil shale processing" and the following footnote was added to Section 6.2 to define in situ shale oil production* "In situ *shale oil processing involves drilling into oil shale strata and heating rocks to release crude shale oil, shale gas, and water (referred to as termed retorting)." Additionally, the profile was revised to note that no quantitative data were located on the amount of 2-hexanone released to the atmosphere through shale oil processing or hydrofracturing, although it is expected to be low (ppb).*

SECTION 2.2 SUMMARY OF HEALTH EFFECTS

COMMENT: page 28, line 31: The 1979 O'Donoghue&Krasavage article also did detect 5-hydroxy-2-hexanone with a short half-life and it did likely go to 2,5-hexadione but none-the-less after some time of exposure they were able to detect this metabolite for short periods.

RESPONSE: *The Reviewer is correct regarding what was mentioned in the O'Donoghue and Krasavage (1979) study. However, there is no discussion about 2,5-hexanedione being the chemical responsible for the neurotoxic effects of 2-hexanone. The studies cited in the text have specific discussions regarding this issue. A reference to DiVincenzo et al. (1978) was deleted and Krasavage et al. (1980) was added. The fact the O'Donoghue and Krasavage (1979) detected 5-hydroxy-2-hexanone in the blood from exposed cats was added to the toxicokinetics section.*

COMMENT: page 30, line 26: There is a more recent study that found the metabolite 2,5-hexadione did show cardiac effects and affects on angiogenesis, this 2015 Cheng et al in Reproductive Toxicology reference and article provided in summary document, see attached documents

RESPONSE: *ATSDR thanks the Reviewer for providing the article by Cheng et al. (2015); however, it does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated with 2,5-hexanedione relate to environmental or occupational exposures to 2-hexanone. If some evidence existed indicating that 2-hexanone is a developmental toxicant, it would make sense to search for possible mechanisms; in this case, the Cheng et al. (2015) study would have some relevance.*

SECTION 2.3 MINIMAL RISK LEVELS (MRLS)

COMMENT: page 31, line 28: This wording is awkward. You have a LOAEL value, but the observed adverse effect is serious. It sounds awkward to me that you have a "serious LOAEL". LOAEL refers to an observed value, whereas "serious" refers to the observed effect.

RESPONSE: *The wording is standard for profiles. The Reviewer is correct in that LOAEL refers to a value and "serious" to an effect; therefore, "serious LOAEL" refers to a value (dose or exposure concentration) that induced a serious effect. In this particular case, it happened that the value that induced the serious effect was the lowest dose or exposure concentration tested in the study. If a study tests only one dose or exposure concentration and this exposure level induces an effect, that level is the study LOAEL for that particular effect, but the true LOAEL may be lower.*

COMMENT: page 31, line 33: It sounds very awkward to refer to an observational survey in humans that was not an experimental design as having a LOAEL value. How do you generate a LOAEL value without a dose-response curve. In my opinion, you can only have a LOAEL value when you have a dose response curve in an experimental study, such as in the in-vivo animal study approach. When taking environmental measurements, especially measurements that may not be representative of each individual's dose, it would be hard to construct and determine a meaningful LOAEL value. Since workers had exposure via other routes (dermal) then the air measurements are not indicative of their actual dose.

Also, might be helpful to briefly provide a few more details. What clinical measures did they have and did they have measures on everyone, this is not an experimental design but rather an observational study

RESPONSE: *In general, ATSDR agrees with the Reviewer's comments. It is very difficult to establish true, reliable LOAELs from epidemiological studies. All the text is indicating is that adverse neurological effects were reported and that an exposure concentration was measured (9.2 ppm 2-hexanone); however, as discussed in the text, because of simultaneous exposure to other substances, poor work practices, and lack of protective respirators and gloves, the health effects reported could not be attributed to exposure to 9.2 ppm 2-hexanone, so that exposure level is not a "reliable LOAEL."*

Text was added indicating that other clinical tests conducted to assess liver and kidney function yielded results within normal values. As the text mentions, a total of 1,157 workers were screened. The study does not clearly indicate how many workers were subjected to the various tests.

COMMENT: page 32, line 4: Therefore the air sampling results are not a good indicator of their internal dose received.

RESPONSE: *ATSDR agrees with the Reviewer's assertion.*

COMMENT: page 32, line 5: May not "many"

RESPONSE: *The typographical error was corrected.*

COMMENT: page 32, line 13: Why? I suggest adding some concise explanation.

RESPONSE: *The text states that it is not known whether the dose-response in hens is applicable to humans. Toxicokinetic and pharmacodynamic differences between humans and traditional experimental animals (i.e., mice, rats, dogs, monkeys) to dispose of chemicals are generally well known; that is not the case for dose extrapolation from hens to humans. The digestive process and respiratory system in hens are very different from mammals. The differences in anatomy and physiology between birds and humans are far greater than between most mammals used in research and humans.*

COMMENT: page 32, line 27: You need to clear this language up, you are basically saying that since you had serious effects at the lowest dose tested, you don't know if you actually determined a LOAEL because there are lower untested levels that may have likely induced less serious adverse effects. The studies also do not inform on a NOAEL value, therefore you have a lot of uncertainty.

RESPONSE: *This was partially answered in response to a previous comment. There are two separate issues here. As discussed above, if a study tests a single dose or exposure concentration and that exposure level induces an effect, that dose or exposure level is the study LOAEL for that particular effect, but the true LOAEL may be lower. Obviously, no dose-response can be established. That LOAEL may be used as point of departure for MRL derivation after applying standard uncertainty factors to account for the inability to establish a NOAEL. A separate issue is that if that LOAEL is an exposure level that induces an effect that ATSDR considers a serious effect, it cannot be used a point of departure for MRL derivation, in accordance with ATSDR's guidance.*

COMMENT: page 32, line 30: If they only tested one concentration level, than you do not have a dose-response curve and thus cannot determine any LOAEL or NOAEL value

It is also important to note that uncertainty factors are typically used to justify the acceptable human levels, the old date of some of this literature doesn't seem to reflect that the level used in the animal experiments must be adjusted by the uncertainty factors.

RESPONSE: *As already mentioned, if only one concentration level was tested, that level will be a NOAEL or LOAEL for a specific health outcome in that particular study. However, the true NOAEL or LOAEL would be unknown. The text in the profile presents doses or exposure levels, which are also listed in the LSE tables and plotted in the LSE figures. NOAELs or LOAELs from old or new studies will be adjusted with appropriate uncertainty factors if they are used for MRL derivation.*

COMMENT: page 33, line 4: If they only did one dose, then this isn't a LOAEL, it is only an adverse effect observed at that dose. You need a dose response curve to develop an acceptable level of exposure.

RESPONSE: *As explained previously, because only one dose level was tested and it caused adverse effects, it is a LOAEL for the end point in the study. The true LOAEL may be lower.*

COMMENT: page 34, line 5: This is also subject to the customary experimental procedures and sensitivity when these studies were done. Years ago, I believe that many toxicologists looked primarily for frank effects and did not carefully evaluate more subtle health impacts. If you look closely at the data in these reports, there may have been some animals in the lower exposure groups that had subtle effects in the lower exposure groups but because in some cases the control and low exposure groups were combined, you can't tell if it was a treatment related effect, hence the focus on the higher exposure groups.

RESPONSE: *ATSDR agrees with the Reviewer's comment. A sentence was added stating that because in some cases, control and low-exposure group may have been combined, it was difficult to ascertain whether an effect was treatment-related.*

COMMENT: page 34, line 8: A few animals in these studies at these levels may have showed subtle swelling of their axons but no evidence of degeneration, is that significant or not since swelling can lead to degeneration.

RESPONSE: *Yes, it is correct that swelling can lead to degeneration. However, the point that the text is making is that even if no effects had been observed in any animals in the chronic inhalation studies in rats and cats, the lowest exposure level, 100 ppm, could not have been used to derive a chronic-duration inhalation MRL because that same exposure concentration, 100 ppm, caused severe neurological effects in rats in two intermediate-duration inhalation studies (Egan et al. 1980; Johnson et al. 1977).*

COMMENT: page 35, line 19: This appendix is very helpful. I am wondering if you should clearly state that you used uncertainty factors when calculating your MRL just so risk assessors know what you did and don't have to refer to the appendix. As currently written, you might think you used the actual animal tox test values without any adjustments unless you look up the appendix.

RESPONSE: *The general methods for MRL derivation are described in Appendix B, User's Guide. Risk assessors are usually familiar with the methods used to derive guideline values, whether ATSDR's MRLs, EPA's reference doses (RfDs) or reference concentrations (RfCs), or other agency's guidelines. The text in the profile uses standard language for Section 2.3.*

SECTION 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

COMMENT: page 37, line 33: Provide citation

RESPONSE: *The citation was added (Chou et al. 1998).*

COMMENT: page 38, line 3: Effects likely vary with dose, so I am unclear of the point of this sentence

RESPONSE: *The comment refers to standardized text in Section 3.2, specifically to the following sentence: "LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health***.**" *The sentence tells the reader that a dose that may be a NOAEL in an acute exposure study may or may not be a NOAEL in a longer-term study. ATSDR sees no compelling reason to modify the sentence.*

COMMENT: page 38, line 10: Don't want to imply that these are the levels we'd use, they are adjusted by Uncertainty Factors

RESPONSE: *The comment refers to the following standardized text: "Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike." The text does not imply that exposure levels are directly used to derive MRLs. Lines 14-15 on page 38 refers the reader to Appendix B for details regarding the LSE tables and figures and the methodology for MRL derivation.*

SECTION 3.2.1.3 IMMUNOLOGICAL AND LYMPHORETICULAR EFFECTS

COMMENT: page 43, line 8: Didn't they find that one had spleen effects, what was unclear about the report is it attributed the spleen effect observed to pento-barbitol injection. I was unclear how this injection would give that effect.

RESPONSE: *O'Donoghue and Krasavage (1979) stated that one cat showed an enlarged spleen due to sodium pentobarbital anesthesia, but there was no further discussion of this finding. They also stated that no gross changes that could be related to exposure to 2-hexanone were detected. There is no mention of the spleen in the section describing the results of the histopathological examination. Intravenous pentobarbital was used to collect biopsy specimens from two randomly selected cats. Euthanasia with sodium pentobarbital has been reported to cause postmortem artifacts in dogs, including splenomegaly due to smooth muscle relaxation. In this study, the cats were deeply anesthetized with sodium pentobarbital before being perfused. However, no splenomegaly was reported in other exposed cats or in cats in the control group, which were also euthanized with sodium pentobarbital.*

SECTION 3.2.1.4 NEUROLOGICAL EFFECTS

COMMENT: page 43, line 17: In studies of humans this is what was found. However, I wouldn't want to imply that other effects observed in animal studies couldn't occur in humans

RESPONSE: *ATSDR agrees with Reviewer. Nowhere is it implied that other effects observed in animals could not occur in humans.*

COMMENT: page 43, line 18: In human studies?

RESPONSE: *Yes, "human studies" added to the text.*

COMMENT: page 44, line 14: Strong wording, stylistically I wonder if "most likely" would be a better way to phrase this.

RESPONSE: *Word "definite" changed to "most likely."*

COMMENT: page 44, line 22: More info needed to support this statement. Why are hens not a good model but rats and cats, as used in other studies, are ok?

RESPONSE: *As mentioned in a previous response to this comment, toxicokinetic and pharmacodynamic differences between humans and traditional experimental animals (i.e., mice, rats, dogs, monkeys) to dispose of chemicals are generally well known; that is not the case for dose extrapolation from hens to humans. The digestive process and respiratory system in hens are very different from mammals. The differences in anatomy and physiology between birds and humans are far greater than between most mammals used in research and humans.*

COMMENT: page 45, line 16: It might be useful to suggest that this could lead to nerve degeneration.

RESPONSE: *A sentence was added as suggested by the Reviewer.*

SECTION 3.2.1.6 DEVELOPMENTAL EFFECTS

COMMENT: page 47, line 9: Suggest also including the Chen et al 2015 article on cardiac and angiogenic effects in this summary. Article and citation provided in attached document.

RESPONSE: *The Reviewer probably refers to the article by Cheng et al. (2015). As indicated earlier, the study by Cheng et al. (2015) does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated with 2,5-hexanedione relate to environmental or occupational exposures to 2-hexanone.*

SECTION 3.2.2.2 SYSTEMIC EFFECTS

RENAL EFFECTS

COMMENT: page 49, line 18: This sentence is too confident and sounds too definitive to me, perhaps change "sensitive" to "primary" so it reads …. Kidney is not a primary target……

RESPONSE: *The word "sensitive" was changed to "primary."*

SECTION 3.2.2.4 NEUROLOGICAL EFFECTS

COMMENT: page 51, line 8: Same comment before, why are studies that use rats and cats ok but those using hens are not appropriate, additional info would be helpful.

RESPONSE: *To establish dose-response relationships, mammals are preferred over hens/chickens for the reasons already mentioned above. Please see response to comment on page 44, line 22.*

SECTION 3.2.2.6 DEVELOPMENTAL EFFECTS

COMMENT: page 52, line 29: Did you want to include information from the Peters et al. 1981 study here and the Cheng et al. 2015 study? Feels like it was accidentally left blank.

RESPONSE: *As indicated earlier, the study by Cheng et al. (2015) does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated with 2,5-hexanedione relate to environmental or occupational exposures to 2-hexanone. Peters et al. (1981) is an inhalation study, so it is mentioned in Section 3.2.1.6.*

SECTION 3.4 TOXICOKINETICS

COMMENT: page 53, line 18: Provide citation as to where this was found, which article?

RESPONSE: *This paragraph is an overview of Section 3.4; traditionally, no references are included in this section.*

SECTION 3.4.1 ABSORPTION

3.4.1.1 INHALATION EXPOSURE

COMMENT: page 53, line 33: I think the most interesting thing about this article and what should be pointed out is that inhalation route resulted in much higher concentrations of 2,5-hexanedione in the plasma and lung than when administered by the oral route. I think this is significant and should be mentioned.

RESPONSE: *A sentence was added in Section 3.4.1.1 indicating that the concentration of 2,5-hexanedione in plasma was higher following inhalation exposure than after oral exposure. Section 3.4.2.2 already stated that no 2,5-hexanedione was detected in the lungs following oral exposure to 2-hexanone.*

3.4.2 DISTRIBUTION

3.4.2.1 INHALATION EXPOSURE

COMMENT: page 54, line 32: They also detected in the Krasavage 1979 study another short lived metabolite, 5-hydroxy-2-hexanone which was likely intermediate but detectable.

RESPONSE: *Text was added to Section 3.4.2.1 stating the findings from O'Donoghue and Krasavage (1979).*

3.5.3 ANIMAL-TO-HUMAN EXTRAPOLATIONS

COMMENT: page 65, line 15: Same comment as above for this statement, provide some rationale as to why this is a reasonable assumption.

RESPONSE: *The comment refers to hens not being a good model for human dose-response assessments. As previously stated, because the digestive and respiratory system from hens is different from humans, it is not known whether the dose-response in hens is applicable to humans. Please see previous responses to this issue.*

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

COMMENT: page 65, line 23: Is this still the case today? A lot has changed since the early 90's.

RESPONSE: *The comment refers to the sentence: "However, appropriate terminology to describe such effects remains controversial." The sentence appears in a boilerplate paragraph in Section 3.6. TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS." The Reviewer asks whether the word "controversial" is still appropriate. ATSDR appreciates the comment and will consider pertinent revisions for future profiles.*

COMMENT: page 66, line 6: Is this still the case today?

RESPONSE: *The comment asks whether the endocrine system still remains controversial, as stated in standardized text in Section 3.6. ATSDR appreciates the comment and will consider pertinent revisions for future profiles.*

3.7 CHILDREN'S SUSCEPTIBILITY

COMMENT: page 66, line 26: There is a lot of speculation that some systems are not "fully developed" at age 18, in particular many neuro-behavioral elements and development is thought to still occur in the early 20's, so maybe you want to reword and just remove the word "fully"

RESPONSE: *The sentence was revised to state that "most" biological systems will have fully developed. The word "most" replaced the word "all."*

3.8.2 BIOMARKERS USED TO CHARACTERIZE EFFECTS CAUSED BY 2-HEXANONE

COMMENT: page 70, line 22: Regarding the sentence "There are no biomarkers specific for exposure to 2-hexanone," the Reviewer asked "Is the metabolite 5-hydroxy-2-hexanone specific to this exposure?"

RESPONSE: *No, it is also a metabolite of n-hexane.*

3.12.2 IDENTIFICATION OF DATA NEEDS

CHRONIC-DURATION EXPOSURE AND CANCER

COMMENT: page 77, line 29: This combined heading makes the reader think that this is about chronic exposure and cancer resulting from that chronic exposure, however the first paragraph here is not really about cancer effects whereas the second paragraph is about cancer. The result is that this is a little unclear the way it is worded and perhaps these two paragraphs should be broken up into two separate headings.

RESPONSE: *ATSDR will consider the suggestion for future profiles.*

DEVELOPMENTAL TOXICITY

COMMENT: page 78, line 3: May also want to cite the Cheng et al 2015 study already mentioned and attached.

RESPONSE: *As previously stated, the article by Cheng et al. (2015) does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated with 2,5-hexanedione relate to environmental or occupational exposures to 2-hexanone. If some evidence existed indicating that 2-hexanone is a developmental toxicant, it would make sense to search for possible mechanisms; in that case, the Cheng et al. (2015) study would have some relevance.*

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

COMMENT: page 87, line 17: I think you should clarify if you are referring to old sites no longer in use or do you mean currently operating facilities? I presume you mean old sites that have contamination from past operations, but this should be clarified.

RESPONSE: *2-Hexanone may be indirectly generated as a waste product during processing at currently operating coal gasification plants, oil shale operations, and wood pulping mills. This information is clarified in Chapter 6 as suggested by the reviewer; however, Section 6.1 Overview is a brief summary of the potential for human exposure and this level of detail was not included in this section.*

6.2 RELEASES TO THE ENVIRONMENT

COMMENT: page 87, line 27: Some concise details would be helpful. If you mean released from current operations, can you explain why/how if the chemical is not made in the US. Is it a release of trace quantities from materials processed? Env professionals will want some more detail to better understand.

RESPONSE: *2-Hexanone may be released in very low levels from currently operating industries via liquid waste water containing 2-hexanone or as a volatilized gas from waste water into the surrounding air. As suggested by the Reviewer, these details were added to the profile.*

6.2.1 AIR

COMMENT: page 89, line 3: Do you mean transfer stations? Incinerators?

RESPONSE: *This refers to a composting disposal facility. The text was revised to provide clarification.*

COMMENT: page 89, line 3: From what, where did it come from? If not used or sold in the US anymore, where did it come from, past disposal?

RESPONSE: *The paper cited refers to air emissions from municipal solid waste composting facilities. It is not stated in the reference what specific materials were being disposed of at the facility; however, it is mentioned that ketones of possible metabolic origin may be produced from the microbial digestion of large bioorganic compounds. Kumar et al. (2011) reported that 2-hexanone has been found as a volatile emission of composted material. This paper was added to the profile for clarification of this issue.*

COMMENT: page 89, line 7: The previously mentioned value of 1700 ug/m3 is quite high, but here you say it is small. This is confusing and should be clarified, where did it come from in the municipal solid waste facilities.

RESPONSE: *It is stated here that atmospheric emissions from industrial sources are likely to be small. The value of 1,700 µg/m^3 refers to a municipal solid waste facility, which is not considered an industrial source since this waste comes from non-industrial sources, such as residential homes, restaurants, retail centers, and office buildings. Also, this value, while seemingly high, is considered below workplace air guidelines. A sentence was added as to what is processed at a municipal solid waste facility.*

6.3 ENVIRONMENTAL FATE

6.3.1 TRANSPORT and PARTITIONING

COMMENT: page 90, line 28: For those scientists not familiar with the use of Log Kow values, I think some additional concise comment stating what values would partition to the fatty tissues would be good to reference. A log Kow of 1 means that 10 times the amount will partition to octanol versus water, however, I have heard that unless the log Kow is over 2 (or the octanol partitioning is 100 times the conc in the water or more) you don't expect it to significantly partition into the fat. Some additional statement to put the value into perspective would be helpful.

RESPONSE: *The Reviewer is correct in stating that, generally speaking, substances with a log K_{ow} ranging from 2 to 7 have the most potential to partition to fat. A statement regarding this information was added to the text as suggested by the Reviewer.*

COMMENT: page 90, line 28: Is "not" the best word, this is a pretty absolute statement.

RESPONSE: *The sentence was revised, replacing the word "not" with "not likely to."*

COMMENT: page 90, line 30: Similarly, referencing a value that would be of concern to put the value of "4" into context would be useful to the reader who is not familiar with these terms.

RESPONSE: *Substances with bioconcentration factor (BCF) values <30 are considered to have low bioconcentration potential. A statement regarding this information was added to the text to put the BCF value of 2-hexanone into context, as suggested by the Reviewer.*

6.3.2 TRANSFORMATION AND DEGRADATION

6.3.2.2 WATER

COMMENT: page 91, line 21: Does this imply it will not degrade in groundwater?

RESPONSE: *The text was revised to clarify that possible photolysis would occur in surface waters, and that potential biodegradation with microorganisms may occur in both surface water and groundwater.*

Comments Provided by Reviewer #2:

COMMENT: Public Health Statement Regarding 2-Hexanone (2-HXN). This section was very well written with respect to clarity, minimal use of scientific terms and tone. Key information was provided regarding the chemical identity of 2-HXN, environmental exposure conditions, metabolic fate and human health risks. Particularly important, the potential effects on children's health were indicated, as were steps taken by the government to limit 2-HXN intoxication.

RESPONSE: *No response is necessary.*

COMMENT: Relevance to Public Health. Section 2.1 provides an appropriately generalized overview of 2-HXN environmental disposition and potential routes of exposure. It is important to recognize that active exposure in the US is unlikely since 2-HXN is no longer manufactured or used. Nonetheless, as pointed out in this section, a remaining exposure scenario is through proximity to an industry-associated hazardous waste site (e.g., coal gasification plants, oil shale operations) where 2-HXN storage might be compromised. It was also pointed out that children were exposed to 2-HXN via the same routes as adults, although the primary routes were likely to be dermal and oral. In this regard, no studies were cited concerning the presence of 2-HXN in breast milk.

RESPONSE: *No studies regarding the presence of 2-hexanone in breast milk were located in a literature search; therefore, the importance of this route of exposure to children has not been determined.*

COMMENT: Section 2.2 provides a summary of health effects. Whereas detailed epidemiological data are limited, the available evidence indicates that 2-HXN exposure primarily affects the nervous system. Hence, the authors describe the neurotoxicity as a peripheral axonopathy characterized by axonal swellings and degeneration. Axon damage was accompanied by typical neurological deficits (e.g., ataxia, skeletal muscle weakness). The conclusion that 2-HXN causes a peripheral neuropathy is however antiquated since more recent research involving the active metabolite 2,5-hexanedione (HD) and quantitative morphometric analyses of PNS/CNS regions demonstrated significant involvement of the CNS. In this section, the authors should de-emphasize the importance of axon swellings and acknowledge the more recent classification of central-peripheral distal axonopathy (LoPachin et al., 2003, 2005; reviewed in LoPachin and Lehning, 1997; LoPachin and DeCaprio, 2004; LoPachin and Gavin, 2015). The fact that workers might have been exposed to other toxic chemicals (e.g., Allen et al., 1975; Davenport et al., 1976) is a critical issue when considering epidemiological outcomes and the potential consequences of interaction among neurotoxicants (see ahead). The observation that 2-HXN intoxication was exclusively manifest as neurotoxicity is not uncommon (e.g., regardless of dose-rate, acrylamide (ACR) produces selective neurotoxicity; See LoPachin et al., 2002). This phenomenon is due to the relatively slow turnover of proteins in distal axons. As a consequence, toxicant-adducted and inactivated proteins are not replaced but rather accumulate over time, which produces selective cumulative expression of neurological deficits (reviewed in LoPachin and Gavin, 2012, 2015).

RESPONSE: *The term "peripheral neuropathy" is used in relation to the reports of Allen et al. (1975) and Billmaier et al. (1974) describing neurological effects in workers exposed to 2-hexanone in a fabric finishing plant. This is how these investigators described their observations. Allen et al. (1975) stated "Ultimately, 86 cases of a toxic peripheral neuropathy were identified." The title of the Billmaier et al. (1974) report is "Peripheral Neuropathy in a Coated Fabrics Plant." ATSDR agrees with the Reviewer in that studies in animals have also shown involvement of the central nervous system. This is mentioned in Section 2.2, and references to LoPachin et al. (2003, 2005) and Zhang et al. (2010) have been added.*

Axon swellings are mentioned because these features were observed in animal studies and were reported by the investigators.

COMMENT: In Section 2.2, the authors also indicated that 2-HXN neurotoxicity in animals recapitulates that in humans. This is important since it indicated the validity of animal models of human exposure. The ATSDR toxicological profile also identified possible design deficiencies in early 2-HXN toxicity studies; e.g., MiBK contamination, lack of dose-response design. Such information is critically helpful when interpreting corresponding data. The review indicated the lack of studies regarding 2-HXN induced cancer and, reproductive and developmental toxicity.

RESPONSE: *No response is necessary.*

COMMENT: Section 2.3 introduces the concept of Minimal Risk Levels (MRLs). The ATSDR overview indicated that an acute-duration inhalational MRL for human exposure to 2-HXN could not be determined due to inadequacies of the epidemiological database. In a discussion related to calculation of an inhalational MRL, the reviewers state that the main reason for not calculating the intermediate-duration MRL was that lower 2-HXN dose-rates (50 – 100 ppm) produced neurological effects (swollen axon, demyelination) that were classified as "serious" LOAELs (see definition of "serious" in Section 3.2). ATSDR cannot use a serious LOAEL to calculate an MRL (Duckette et al., 1979; Egan et al., 1980). However, it is not clear why ATSDR would classify swollen axons or any neuropathological alteration as "serious" without evidence that the effect and corresponding frequency are a necessary component of the neurotoxicological process; i.e., research indicates that swollen axons are an epiphenomenon related to low dose, long-term 2,5-HD intoxication (reviewed in LoPachin and Lehning, 1997; LoPachin and DeCaprio, 2004). In this particular case, since swollen axons are not necessary for the expression of 2,5-HD (and therefore 2-HXN) neurotoxicity, an intermediate-duration inhalational MRL could be calculated. This section indicated that a chronic-duration inhalational MRL for 2-HXN could not be determined due to inadequate data. The ATSDR review indicated that acute- and intermediate-duration oral MRLs could not be calculated due to an inconsistent database. In contrast, a chronic-duration oral exposure MRL has been derived. Finally, the utility of Table 2-1 is uncertain given the focus on swollen axons in different nervous tissue regions. This table could be a source of confusion, since Section 3.5.2. (Mechanism of Toxicity) shifts to the neurotoxicological relevance of axon atrophy as per the studies of LoPachin and colleagues.

RESPONSE: *Axonal swelling itself was not characterized as a serious effect, but it is mentioned because it was reported in several animal studies. However, effects such as widespread demyelination in the sciatic nerve from rats exposed to 50 ppm 2-hexanone (Duckett et al. 1979) or advanced degeneration in teased fibers in calf muscle branches and fiber degeneration in the spinal cord from rats exposed to 100 ppm 2-hexanone (Egan et al. 1980) are considered serious neurological effects. ATSDR has stated the following: "In general, a dose that evokes failure in a biological system and can lead to morbidity or mortality is referred to as serious LOAEL" (Chou et al. 1998). ATSDR recognizes that a considerable amount of judgment is required in classifying an adverse outcome and, in some cases, the data are insufficient to determine whether an effect will lead to significant dysfunction. Extensive demyelination in peripheral nerve and degeneration in spinal cord tracts can lead to significant dysfunction; therefore, they were classified as serious effects.*

Table 2-1 is derived from O'Donoghue et al. (1978). These investigators mention axon atrophy occurring in rats treated with 2-hexanone; however, they did not quantify axon atrophy as they did with axonal swelling and myofibrillar atrophy. Although axonal atrophy is the more important lesion that correlates with nerve dysfunction and behavioral alterations, swelling was considered a valid surrogate for MRL

derivation. LoPachin et al. (2000) (Toxicol Appl Pharmacol 167:75-86) stated that "Development of swelling presumably initiates axonal degeneration and subsequent behavioral deficits (e.g., gait abnormalities, hindlimb weakness)." EPA (2009) also considered peripheral nerve swelling, as reported by O'Donoghue et al. (1978,) toxicologically significant and derived an RfD for 2-hexanone based on the incident data.

COMMENT: 3. Health Effects. Section 3.1 described the overall purpose of this section and the intended audience. Section 3.2, as the title indicated, was a discussion of health effects by route of exposure. In this section, the authors provided a definition of "Serious" and "Less Serious" as these terms relate to the LOAEL and degree of toxicity. However, this discussion should be moved to Section 2.3 (pg 32), where the application of this concept is introduced. Furthermore, although the rational for developing this classification system is understandable, the design is problematic. Specifically, according to definition, Serious effects relate to toxicity leading to biological system failure and morbidity or mortality. Based on this system, swollen axons were labeled as serious and therefore MRLs could not be calculated. Yet substantial evidence has suggested a lack of pathophysiological relevance. Therefore, such labels must be linked to the knowledge that a cellular/biochemical event is mechanistically-based and neurotoxicologically relevant. This relevance cannot be assumed using inference or judgement. In general, specific neurotoxicological events that develop as a product of molecular mechanism (e.g., protein adduct formation) will progress as a function of both dose-rate and exposure duration (see LoPachin et al., 2002; LoPachin and Gavin, 2012). Sections 3.2.1- 3.2.1.3. provided a very comprehensive, organ-based catalogue of the toxic consequences associated with inhalation exposure to 2-HXN. Section 3.2.1.4 is a detailed discussion regarding the neurological effects of inhalation exposure. This section re-stated the peripheral nature of the neuropathy and presence of swollen axons. These statements, however, are assumptions based primarily on studies conducted during the 1970s. In keeping with a weight-of-evidence approach, a balanced discussion should be presented incorporating recent findings regarding γ-diketone induced distal axon atrophy and the concept of a central-peripheral distal neuropathy. Despite these concerns, this section was very detailed and presented the limitations of related animal studies. Section 3.2.2 described the toxicological effects of oral 2-HXN exposure. Section 3.2.2.4. focused on the neurological consequences of oral 2-HXN. The ATSDR review discussed the important concept that daily oral exposure of animals to 2-HXN caused the same neurological effects induced by inhalation administration. Section 3.2.3. indicated that no information was available regarding the dermal route of intoxication. Generally, the animal species (e.g., rat, mice, Guinea pigs) used in these defining studies were appropriate. However, the majority of research cited was outdated ($\geq$35 years old) and contemporary studies have not been conducted to confirm the original findings. Furthermore, the dose-rates and exposure durations were limited in scope. Given these concerns, the older animal database are unreliable. Regarding the use of animal data to calculate Levels of Significant Exposure (LES), my reservations concerning the "Serious" and "Less Serious" LOAEL concentrations are indicated above. Additionally, grouping LOAEL/NOAEL data according to exposure durations (e.g., Table 3.1; Intermediate Exposure) is misleading since dose-rate dictates the exposure duration (see LoPachin et al., 2002). At the molecular level, the degree of exposure determines the rate of cumulative protein adduct formation. Therefore, low-dose intoxication requires longer exposure durations to achieve toxic adduct levels that mediate neurotoxicity (see LoPachin and Gavin, 2012, 2014, 2015). Technically, the LES tables and figures were well organized and informative.

RESPONSE: *The suggestion to move the discussion regarding the definition of "Serious" and "Less Serious" LOAELs (standardized text in Section 3.2) to Section 2.3 will be considered for future profiles.*

As indicated in the response to the previous comment, swollen axons were not considered serious effects. What was labelled as serious was the widespread demyelination in rats exposed to 50 ppm (Duckett et al.

1979). Because 50 ppm was the lowest exposure concentration tested in intermediate-duration studies, an intermediate-duration inhalation MRL was not derived.

Regarding Section 3.2.1.4, the term "peripheral neuropathy" was used by the investigators who described the effects of 2-hexanone in humans (e.g., Allen et al. 1975, Billmaier et al. 1974; Mallov 1976). The investigators used the term "peripheral" likely because they conducted electrophysiological tests of peripheral nerve function and, in some cases, took biopsies of peripheral nerves and, for the most part, the central nervous system was not evaluated. The presence of swollen axons was also mentioned in studies of humans, so the text in the profile is just repeating what the investigators reported. More recent studies have been conducted in rats administered 2,5-hexanedione directly rather than 2-hexanone. As indicated in response to comments from Reviewer #3, Section 3.2 is devoted to summarizing information regarding the chemical subject of the profile, in this case, 2-hexanone. It would be contrary to standard practice to summarize studies conducted with the metabolite, 2,5-hexanedione. However, Section 3.5.2, Mechanisms of Toxicity, include information regarding 2,5-hexanedione.

The Reviewer is correct in noting that the majority of the animal studies cited in Sections 3.2.1 (inhalation), 3.2.2 (oral), and 3.2.3 (dermal) were conducted several decades ago and no recent studies have been conducted with 2-hexanone to confirm the original findings. This could be a data need; however, given that 2-hexanone is no longer made in the United States and its uses have been restricted, significant exposure of the general population to this chemical is not likely at present, so it is unlikely that new studies will be conducted.

The Reviewer noted that grouping LOAEL/NOAEL data according to exposure durations (e.g., Table 3.1; Intermediate Exposure) is misleading since dose-rate dictates the exposure duration. The purpose of the LSE tables is to identify safe dose levels (NOAELs) and the lowest dose levels that induce adverse effects (LOAELs) for different periods of exposure (acute, 1–14 days; intermediate, 15–364 days; chronic, ≥365 days) in order to develop guidance values (MRLs) that are likely to be without appreciable risk to human health. In general, the higher the dose, the shorter the duration needed to induce an adverse effect, so an acute-duration MRL is usually not protective for chronic exposure, but a chronic duration MRL is protective for acute exposure. It is unclear to ATSDR why this is misleading.

COMMENT: Section 3.4 was a detailed discussion of the toxicokinetics of 2-HXN. This is a very well done section that offers critical information to anyone who might want to understand the risks of 2-HXN exposure. Each potential route of exposure was well described. In particular, Section 3.4.3 presented the proposed metabolic pathway of 2-HXN which included evidence that the parent compound undergoes metabolic activation to the neurotoxic γ-diketone metabolite, 2,5-HD (Fig. 3-3). The finding that the neurotoxicity induced by oral 2-HXN and analogues was strongly correlated to urinary concentrations of 2,5-HD is critically important to defining the causative role of this metabolite. Although it might be protocol, it is not clear why the lengthy discussion of PBPK/PD modeling in Section 3.4.5 (bolded) was included, since this type of model has not been developed for 2-HXN. This and other bolded, explanatory sections (Section 3.6) are distracting.

RESPONSE: *The Reviewer refers to Section 3.4.5 (standardized text in the PBPK section) and Section 3.6 (standardized text in the section on effects mediated through the neuroendocrine axis). ATSDR understands that these sections may be distracting, particularly when there are no or very limited data on the subject chemical. Standardized text will be reduced to a minimum in future versions of toxicological profiles.*

COMMENT: Section 3.5 is entitled "Mechanisms of Action" and is followed by Section 3.5.1 labeled "Pharmacokinetic Mechanisms". Pharmacokinetic studies determine the processes (route of exposure, absorption) and corresponding rate by which a given toxicant gets to the respective site of action. In addition, pharmacokinetic studies show how a toxicant distributes to different tissues and how it is removed through metabolism and excretion. Mechanisms of action are the initiating molecular events that that lead to secondary cellular changes and subsequent toxicity. Therefore, Section 3.5 might be labeled "Pharmacokinetic Processes and Tissue Distribution", whereas section 3.5.2 should be labeled "Mechanisms of Toxicity". Regardless, Section 3.5.2 provides a synopsis of presumed molecular mechanisms that mediate 2-HXN/2,5-HD neurotoxicity. The accompanying reference list (pg 62, line 10) should include several related publications (see list provided at the end of this review). Although atrophy is now the presumed pathognomonic feature, the neuropathy is still classified as a central-peripheral distal axonopathy. As the authors of this section indicate, several studies suggested that swollen axons were an epiphenomenon related to low-level 2,5-HD dose-rates. Therefore, the discussion regarding the mechanism of these swollen axons (pg 62, lines 30-34) is unnecessary and should be deleted. The last paragraph of this section (pg 63, lines 29-pg 64 lines 1-4) should be significantly revised. The reviewers should consult research by Zhang et al. (2010), which provided evidence that 2,5-HD impaired binding of microtubule associated proteins (e.g., MAP1A, tau) to cognitive sites on microtubules. Presumably, this disruption was caused by 2,5-HD adduct formation with ε-amino groups on lysine residues that mediate such protein-protein interactions. Based on the critical role in cytoskeletal physiology, MAPs could represent a relevant target of γ-diketone axonopathy. The research by Zhang et al. (2010) also suggested that higher molecular weight neurofilament (HMW NF) derivatives were not a consequence of 2,5-HD cross-linking of these proteins, since they also appeared in control nervous tissues. Rather, these derivatized NFs likely represented baseline levels of proteins that have been cross-linked by normal activities of axon transglutaminases that increase cytoskeletal stability. The elevated content of HMW NF complexes in 2,5-HD intoxicated rats might represent excess fragmentation of the stationary cytoskeleton possibly as a result of 2,5-HD impaired polymer maintenance.

RESPONSE: *The Reviewer stated that pharmacokinetic studies determine the processes (route of exposure, absorption) and corresponding rate by which a given toxicant gets to the respective site of action. In addition, pharmacokinetic studies show how a toxicant distributes to different tissues and how it is removed through metabolism and excretion. Mechanisms of action are the initiating molecular events that that lead to secondary cellular changes and subsequent toxicity. Therefore, Section 3.5 might be labeled "Pharmacokinetic Processes and Tissue Distribution," whereas Section 3.5.2 should be labeled "Mechanisms of Toxicity." The titles of the sections are standard in toxicological profiles; however, ATSDR will consider the Reviewer's suggestion for future profiles.*

The Reviewer suggested adding some references that he provided to a list of references that appears at the beginning of Section 3.5.2, Mechanism of Action (page 62, line 10). As indicated in the paragraph alluded to by the Reviewer, Section 3.5.2 was extracted from review articles because of the extensive nature of the literature that covers 2-hexanone and n-hexane, as well as 2,5-hexanedione itself. Of the list of articles provided by the Reviewer, one article is a review article (LoPachin et al. 2000) and was added to the list on page 62, line 10. The rest of the articles provided by the Reviewer are cited in the review articles. Three of these articles (LoPachin et al. 2003, 2005; Zhang et al. 2010) were included in Section 2.2, SUMMARY OF HEALTH EFFECTS, as evidence of involvement of the central nervous system in animals exposed to 2,5-hexanedione.

The Reviewer recommended that a discussion on the mechanism of swollen axons is unnecessary and should be deleted. The following text was deleted: "The precise mechanism by which 2,5-hexanedione induces axonal swelling has not been elucidated. However, a generalized disruption of neurofilament structure and function has been proposed as the molecular basis for 2,5-hexanedione-induced axonal swelling. Results from some studies suggest that axonal swellings are composed of chemically cross-

linked neurofilaments that could not travel through constricted nodes of Ranvier and therefore accumulated."

The Reviewer stated that the last paragraph in Section 3.5.2, Mechanisms of Action, should be significantly revised and provided summary data from Zhang et al. (2010) that should replace the existing text. The last two sentences of the existing paragraph were deleted and replaced with text provided by the Reviewer.

COMMENT: Sections 3.6, 3.7 and 3.8 pertain to toxicities mediated by the neuroendrocrine axis, the susceptibility of children and biomarkers of exposure, respectively. Specific studies related to these topics were not located by the ATSDR reviewers. However, Section 3.9 discusses the possible interactions with other chemicals. Although relatively nonspecific drug/chemical interactions were identified, this issue is important, especially for those living near waste sites. 2,5-HD is a hard electrophile that produces neurotoxicity by reacting with hard nucleophiles; e.g., nitrogen groups on lysine and histidine residues. Thus, on a theoretical basis, 2-HXN (2,5-HD) might cause joint toxic effects by interacting in a synergistic/additive fashion with other hard environmental electrophiles (e.g., chloroethyline oxide, vinyl chloride) derived from toxic waste sites (e.g., see Zhang et al., 2016). Section 3.11 discusses methods for reducing toxic effects. One possible noted method was interference with the 2,5-HD mechanism. In this regard, hard nucleophiles such as carnosine or hydralazine might be useful as chemical scavengers of 2,5-HD, the hard electrophilic metabolite of 2-HXN. Section 3.12 discusses the adequacy of the database and presents a very informative figure (3-5) regarding the existing health effects information. The figure demonstrates that, although neurotoxicity is the principle observed effect in humans, animal studies suggest additional toxicities; e.g., reproductive, developmental. This is not surprising and likely reflects differences in species and corresponding dose-rate/exposure durations. Defining the toxicological relevance of these differences will require additional studies; e.g., does the animal data portend toxicities other than neurotoxicity in humans (see next section)? Section 3.12.2 suggests studies to address data needs. Acute- and intermediate-duration exposure studies are proposed to provide more complete and therefore definitive dose-rate data. However, a 90 day study might be inappropriate, since lower, environmentally relevant dose-rates might not express neurotoxicity within this time frame (see more detailed discussions in Lehning et al., 2000; LoPachin et al., 2002, 2003). Chronic-duration (lower dose-rate) studies were not supported. Genotoxicity, immunotoxicity, reproductive and developmental toxicity studies were proposed in this section. However, these are relatively low-priority studies based on the presumed protein adduct mechanism for 2,5-HD/2-HXN toxicity and the lack of a rational protein target in the respective tissues (slow turnover). The existing animal-based research regarding neurotoxicity is deficient in many respects and therefore the development of a more comprehensive/corroborative database is important. Improvement of the human epidemiology database is also critical. However, as the ATSDR reviewers point out, acquisition of data will be difficult since 2-HSN is not used or manufactured in the US. Research to develop biomarkers for 2-HXN exposure are proposed. However, accurate determination of exposure requires a mechanism-based understanding of the target protein; i.e., there is evidence that axonal proteins other than NF are primary targets. Therefore, before viable exposure biomarkers can be developed, a better understanding of the 2HXN target proteome is necessary. Due to numerous identified deficiencies, the proposed pharmacokinetic and tissue distribution studies might fill important data gaps.

RESPONSE: *Regarding Section 3.9, INTERACTIONS WITH OTHER CHEMICALS, the Reviewer noted that, on a theoretical basis, based on its hard electrophile properties, 2,5-hexanedione might cause joint toxic effects by interacting with other environmental electrophiles (e.g., vinyl chloride, chloroethylene oxide) derived from waste sites. While this might be theoretically possible, without any experimental evidence, it would be inappropriate to include this in Section 3.9.*

Regarding Section 3.11.3, Interfering with the Mechanism of Action for Toxic Effects, the Reviewer stated that strong nucleophiles such as carnosine and hydralazine might be useful as chemical scavengers of 2,5-hexanedione (an electrophile) and possibly mitigate the toxicity of 2-hexanone. Again, while theoretically possible, without any studies available in animals or in vitro examining this possibility, it would be inappropriate to include this in Section 3.11.3.

Regarding Section 3.12.2, Identification of Data Needs, the Reviewer disagreed with the profile's statement that a 90-day study with pure 2-hexanone would be valuable for establishing dose-response relationships. The Reviewer states that a 90-day study might be inappropriate, since lower, environmentally relevant dose-rates might not express neurotoxicity within this time frame. If a 90-day inhalation or oral study that tests multiple doses establishes a NOAEL and LOAEL, then it would be possible to derive an intermediate-duration inhalation or oral MRL for 2-hexanone.

The Reviewer noted that a better understanding of the 2-hexanone target proteome is necessary. A sentence was added in the Neurotoxicity section of the data needs stating that continued research aimed at determining the mode of action of 2,5-hexanedione, the active neurotoxic substance, would be valuable.

COMMENT: 4. Chemical and Physical Information. Tables 4-1 and 4-2 provide an appropriate and detailed physicochemical background of 2-HXN.

RESPONSE: *No response is necessary.*

COMMENT: 5. Production, Import/Export, Use and Disposal. To the best of my knowledge, this section provides appropriate information.

RESPONSE: *No response is necessary.*

COMMENT: 6. Potential for Human Exposure. Based on the guidelines provided for ATSDR toxicological profiles, this section offers accurate and appropriate information regarding the environmental disposition and fate of 2-HXN.

RESPONSE: *No response is necessary.*

COMMENT: 7. Analytical Methods. This section provided a comprehensive overview of applicable methods.

RESPONSE: *No response is necessary.*

COMMENT: 8. Regulations, advisories and Guidelines. Most of the regulatory benchmarks (e.g., MRL, LOAEL) were based on peripheral neuropathy in rodent models. It is not clear how the different regulatory agencies define "peripheral neuropathy". However, it is likely that the definition includes axonal swellings and degeneration. If so, the accuracy and reliability of the regulatory parameters is uncertain since neurofilamentous swellings and distal degeneration are epiphenomena linked to lower-levels exposure rates. Therefore, this uncertainty, even if mathematically accounted for, impacts the validity of these measures.

RESPONSE: *Chapter 8 only presents relevant regulations, advisories, and guidelines from various agencies, but does not discuss their rationale for the derivation. Because neurofilamentous swelling and distal degeneration are phenomena linked to low levels of exposure, regulations, advisories, and guidelines based on these phenomena may be overly conservative and will probably stand until results from new studies suggest they need to be changed.*

COMMENT: The following references should be added to the profile (references will be emailed separately).

- LoPachin, R.M., Lehning, E.J., Stack, E.C. and Saubermann, A.J.: 2,5-Hexanedione-Alters Elemental Composition and Water Content of Peripheral Nerve Myelinated Axons. *J. Neurochem.* 63: 2266-2278, 1994.

- Lehning, E.J., Dyer, K.S. Jortner, B.S. and LoPachin, R.M.: Axonal Atrophy is a Specific Component of 2,5-Hexanedione Peripheral Neuropathy. *Toxicol Appl Pharmacol*, 135: 58-66, 1995.

- Lehning, E.J., Jortner, B.S., Fox, J.H., Arezzo, J.C., Kitano, T. and LoPachin, R.M. γ-Diketone Peripheral Neuropathy: I. Quantitative Morphometric Analysis of Axonal Atrophy and Swelling. *Toxicol Appl Pharmacol*, 165: 127-140, 2000.

- LoPachin, R.M. Lehning, E.J., Ross, J.F., Reid, M., Das, S. and Mansukhani, S., Neurological Evaluation of Toxic Axonopathies in Rats; Acrylamide and 2,5-Hexanedione. *NeuroToxicology* 23: 95-110, 2002.

- LoPachin, R.M., Jortner, B.S., Reid, M.L., Das, S. γ-Diketone Central Neuropathy: Quantitative Morphometric Analysis of Axon Changes in Rat Nerve Roots and Spinal Cord White matter Regions. *Toxicol Appl Pharmacol* 193: 29-46, 2003.

- LoPachin, R.M., Jortner, B.S., Reid, M.L. and Monir, A. γ-Diketone Central Neuropathy: Quantitative Analysis of Cytoskeletal Components in Myelinated Axons of the Rat Rubrospinal Tract. *NeuroToxicology* 26: 1021-1030, 2005.

- Zhang, L., Gavin, T., DeCaprio, A.P. and LoPachin, R.M. γ-Diketone Neuropathy: Analysis of Cytoskeletal Motors and Highways in CNS Axons. *Toxicol. Sci.* 117: 180-189, 2010.

- LoPachin, R.M. and Lehning, E.J.: Forum Position Paper: Relevance of Axonal Swellings and Atrophy to γ-Diketone Neuropathy. *NeuroToxicology* 18(1): 7-22, 1997.

- LoPachin, R.M., Lehning, E.J. and Opanashuk, L.A. Rate of Neurotoxicant Exposure Determines Morphologic Manifestations of Distal Axonopathy. *Toxicol. Appl. Pharmacol.*, 167: 75-86, 2000.

- LoPachin, R.M. and Gavin, T. Molecular Mechanism of Acrylamide Neurotoxicity: Lessons Learned from Organic Chemistry. *Environ. Health Persp.* 120: 1650-1657, 2012.

RESPONSE: *Studies in animals directly administered 2,5-hexanedione are relevant to the profile on 2-hexanone only in relation to a mode of action, and relevant information should be mentioned in*

Section 3.5.2, Mechanism of Action. However, as indicated at the beginning of Section 3.5.2, the information in this section was extracted from review articles because of the extensive nature of the literature that covers 2-hexanone and n-hexane, as well as 2,5-hexanedione itself. Of the list of articles provided by the Reviewer, one article is a review article (LoPachin et al. 2000) and was added to the list on page 62, line 10. The rest of the articles provided by the Reviewer are cited in the review articles. Three of these articles (LoPachin et al. 2003, 2005; Zhang et al. 2010) were included in Section 2.2, SUMMARY OF HEALTH EFFECTS, as evidence of involvement of the central nervous system in animals exposed to 2,5-hexanedione.

Comments on Unpublished Studies

COMMENT: The Reviewer provided the following comments on the Union Carbide (1977) unpublished study:

Introduction: The purpose of this study was to evaluate neurotoxicity associated with subchronic exposure to methoxyacetone and selected aliphatic ketones. Female rats (n = 5/group) were exposed orally (drinking water) for 120 days to methoxyacetone or one of a structural series of aliphatic ketones: diethyl ketone, ethyl n-butyl ketone, methyl isobutyl ketone, methyl n-butyl ketone and methyl tert-butyl ketone. A preliminary range-finding study was conducted to identify appropriate dose-rates and potential toxic responses. With the exception of methoxyacetone, the ketone concentrations selected were used at or near maximum water solubility. The study design involved two components that: 1) measured indices of relative neurotoxicity (Comparative Toxicity to Rats of Methoxyacetone and Five other Aliphatic Ketones in Their Drinking Water) and 2) determined the type of neuropathy and distribution in the CNS and PNS of intoxicated rats (Comparative Pathology on Rats given Methoxyacetone and Five other Aliphatic Ketones in Drinking Water). In the first component, the investigators measured changes in food/water intake, rates of body weight gain, organ weight, gross pathology and neurological/neuromuscular function as indices of developing toxicity. In the histopathological component, the investigators conducted a comprehensive analysis of gross pathological changes in nervous tissue and systemic organs.

Results: Relative to the other ketones tested, methyl n-butyl ketone (MnBK) administered orally (1.0gm/kg/d) produced significant reductions in weight gain and neurotoxicity as evidenced by posture, incoordination, hind limb skeletal muscle weakness and atrophy. Methoxyacetone, at a significantly higher daily dose-rate (2-4 gm/kg/d), was associated with slight muscle weakness and atrophy. None of the other ketone analogues tested caused discernable gross neurotoxic effects. Regardless of the oral MnBK dose-rate, peripheral neuropathy, characterized by axonal swellings and myelin clumping, was a significant presence in intoxicated rats. Skeletal muscle atrophy was determined to be causally related to the peripheral neuropathy. The MnBK neuropathy did not intensify as a function of proximal to distal sciatic nerve regions. Occasional swollen axons were noted in the CNS of treated rats. Regardless of dose, neither methoxyacetone nor the other ketone analogues were associated with a peripheral neuropathy.

Discussion: The results of this very comprehensive study indicate that MnBK is a significant cumulative neurotoxicant. In contrast, the results suggest that methoxyacetone and a selected series of structurally related aliphatic ketones were not neurotoxic. Subsequent structure-activity relationship (SAR) studies have demonstrated that the neurotoxicity of MnBK and n-hexane is based on the metabolic conversion of these parent compounds to 2,5-hexanedione (2,5-HD), a γ-diketone with neurotoxic capacity. More recent research indicates that, as a hard electrophile, 2,5-HD will form covalent adducts with hard nucleophilic sites such as the nitrogen groups on lysine and histidine residues of axonal proteins (see Zhang et al., 2010).

There is some concern, however, regarding the overall reliability of the data. For example, an n = 5 is insufficient, especially for the toxicity tests that involve animal behavior. The study design also did not

include temporal analyses during the 120 day exposure period. This would provide information regarding the development of neuropathy and corresponding relationship to the onset of neurological deficits. The authors stated that, with the exception of the insoluble ethyl n-butyl ketone (0.03% solubility), ketones were administered at definable adverse effect levels and therefore individual relative and absolute neurotoxic potentials can be estimated. However, despite this disclaimer, the studies would benefit from a higher resolution dose-response analysis. The study outcome is also weakened by the lack of a more quantitative experimental approach to, for example, histological data collection. It is notable that Krasavage and O'Donoghue exposed male rats to MnBK via inhalation and found minimal neurotoxicity associated with two dose rates; i.e., 100ppm and 330ppm. In the present Carnegie-Mellon study, female rats were exposed to MnBK through drinking water and a significant peripheral neuropathy was reported. These differential findings could represent important route, dose and/or gender susceptibilities. Overall, the Carnegie-Mellon study was comprehensive and carefully conducted. However, several concerns regarding design and interpretation suggest that the findings might ultimately be unreliable. The present report could be a basis for recognizing data gaps.

RESPONSE: *ATSDR agrees with the Reviewer that the small number animals of a single sex is a limitation of the Union Carbide (1977) study and behavioral examination of the animals during the exposure period would have provided valuable information. It is noted that the results of the Union Carbide (1977) study are support by the Krasavage et al. (1980) study, which also found peripheral neuropathy.*

COMMENT: The Reviewer provided the following comments on the O'Donoughue and Krasavage (1979) unpublished study:

Introduction: A previous outbreak of peripheral neuropathy (c1974) in a fabric printing plant suggested that exposure to methyl n-butyl ketone (MnBK) was the causative chemical. Therefore, the stated purpose of this research was to determine the effects of MnBK on cats exposed intermittently for two years. Animals (n = 4 per control and experimental groups) were exposed by inhalation at dose-rates of 100ppm or 330ppm and were examined for neurological signs and changes in body weight. Blood sample were acquired for plasma measurements of electrolyte, MnBK and metabolite (5-hydroxy-2-hexanone, 2,5-hexanedione) concentrations. At the end of the MnBK exposure period, nervous tissue samples were collected for examination of neuropathological changes in both CNS and PNS regions.

Results: The authors report no changes in clinical neurological parameters or body weight during MnBK exposure. Regardless, the data presented in Figure 2 suggest that the 330 ppm group exhibited a significant increase in weight gain. No serum changes were evident, whereas measurements of the parent compound and metabolites indicated that 2,5-hexanedione (2,5-HD) increases were dose- and time-related. Based on these and other findings it was eventually suggested that this γ-diketone metabolite mediates MnBK neurotoxicity. No gross pathological changes were noted at any dose-rate. Neuropathological examination revealed typical dose-dependent central-peripheral axonopathic changes. Thus, the 100ppm exposure group exhibited occasional giant axonal swellings and degeneration, whereas the higher dose-rate (330ppm) was associated with significant axonopathic changes in the cerebellum, pons, spinal cord and peripheral nerves; e.g., tibial motor nerve branches. The sensory aspects (e.g., Pacinian corpuscles) of the PNS were not affected. A single teased fiber study was conducted to address the issue of axonopathic frequency. Although this semi-quantitative process is imprecise, approximately 20% of the teased fiber showed some form of axonopathy; e.g., myelin damage and swollen axons.

Discussion: Although the experimental design of this research was thorough and consistent with contemporary (1970s) neurotoxicological research, the approach was nonetheless descriptive. Thus, for example, neuropathological changes were classified as either: Present (P), Absent (A) or Normal (N) with a frequency of: Minimal (1), Minor (2), Moderate (3) or Severe (4). This approach significantly limits data interpretation and reliability. From a statistical standpoint, the observational design is complicated

by the relatively small n (4 per group). Furthermore, as the authors admit, the exposure-range (100ppm and 330ppm) was insufficient to determine either a no-effect level or dose-rate dependency. A high resolution temporal design involving multiple time-point analyses could have been used to address the relationship between the developing axonopathy and the onset of corresponding neurological deficits. In this regard, a close correspondence between the MnBK neuropathy and neurological changes would suggest causality. Finally, interpretation of the data would have benefited from an extended final time-point; i.e., > 2years. This concern is warranted since 2,5-HD produces a cumulative neurotoxicity which might have expressed at the lower dose-rate (100ppm) beyond 2 years (LoPachin et al., 2002). In hindsight, the research by O'Donoghue and Krasavage represents a preliminary investigation and, as such, cannot be used as a sole basis for assessing MnBK neurotoxicity.

RESPONSE: *ATSDR agrees with the Reviewer that there are several limitations to the interpretation of the study results. These data are used to support identifying neurotoxicity as a sensitive target of 2-hexanone toxicity.*

COMMENT: The Reviewer provided the following comments on the Krasavage and O'Donoughue (1977) unpublished study:

Introduction: A previous outbreak of peripheral neuropathy (c1974) in a fabric printing plant suggested that exposure to methyl n-butyl ketone (MnBK) was the causative chemical. Therefore, the stated purpose of this research was to determine the effects of MnBK on male rats exposed for 18 months (72 weeks) for 6 hrs/day x 5 days/week. Animals (n = 18 per control and experimental groups) were exposed by inhalation at dose-rates of 100ppm or 330ppm and were examined for neurological signs and changes in body weight. At the end of the MnBK exposure period, nervous tissue samples were collected for examination of neuropathological changes in both CNS and PNS regions.

Results: The authors found that MnBK produced dose-dependent stasis of weight gain over the 18 month experimental period. However, the investigators found no evidence, either clinical or morphological, of neurotoxicity in the lower dose-rate group. The evidence of neurotoxicity in the higher exposure group was equivocal.

Discussion: The investigators offer no discussion regarding the limited expression of neurotoxicity in rats exposed to MnBK by inhalation. Increases in dose-rate or exposure length might have revealed a response in these studies. The value of the data presented in this report is difficult to assess given the limited findings regarding MnBK.

RESPONSE: *This study is used to support the identification of the nervous system as a critical target of 2-hexanone toxicity. The study is not used for MRL derivation due to the study limitations.*

Comments Provided by Reviewer #3

General Comments

The document is well written, informative and unbiased. Recognizing that the majority of the pertinent studies on the topic at hand have been carried out decades ago, the literature in general, is encompassing and complete. The authors have met the stated goal, covering the health associated with potential exposures from conception to adulthood. Relevant animal and *in vitro* models are generally covered. The tone of the profile is factual and the summaries are consistent with the evidence provided. Where the authors may have missed some pertinent studies, I have annotated those in the text of the document. The criteria and discussion on those studies chosen to emphasize human exposures and health hazards are adequate and well detailed. NOAELs and/or LOAELs have been adequately identified for each study. The Tables and Figures are excellent and informative.

ATSDR Charge Questions and Responses

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?

COMMENT: I could not locate additional data germane to the topic. The Profile reflects current-state knowledge of this topic.

RESPONSE: *No response is necessary.*

QUESTION: Are there any general issues relevant to child health that have not been discussed in the profile and should be?

COMMENT: The issue is well covered and this reviewer has no additional recommendations.

RESPONSE: *No response is necessary.*

QUESTION: If you answer yes to either of the above questions, please provide any relevant references.

RESPONSE: *No response is necessary.*

Additional Comments

I wish to point couple issues for the authors' considerations.

COMMENT: It is stated in the Guidelines for Peer review of ATSDR's Toxicological Profiles that "….the emphasis of the document is on providing succinct interpretations on key literature." This does not appear to be the case. The literature is generally reported in a "dry" manner, with little if any attempt to provide succinct interpretations on the respective findings. It should be incumbent on the authors to synthesize the findings in some fashion and to integrate to various findings into succinct mechanisms of toxicity etc. This is lacking in this document, and it basically reads as a compilation of abstracts.

RESPONSE: *Interpretations of the literature and bottom line statements are generally presented in Chapter 3. For 2-hexanone, a chemical with a small database compared with other chemicals, it is difficult to make bottom line statements after each end point if there is only one or two studies per end point and no data in humans. Nevertheless, statements have been added in section 3.2 at the beginning or at the end of most end points indicating what the available studies tell and what the relevance of the animal data is for human health. For 2-hexanone, the bottom line is that it is a nervous system toxicant in humans and animals and hardly any additional information regarding other effects in humans is available. The following statement was also added in Chapter 1, section HOW CAN 2-HEXANONE AFFECT MY HEALTH: Based on the limited number of studies of humans exposed to 2-hexanone and on studies of subjects exposed to the industrial chemical n-hexane, which also produces the breakdown product 2,5-hexanedione, it is clear that the nervous system is a primary target. However, there is no reliable information to determine whether other organs or biological systems in humans could also be targets for 2-hexanone.*

COMMENT: The most toxic metabolite of 2-hexanone is 2,5-hexanedione. As I stated in the document, the relationship between the parent compound and this metabolite in terms of pharmacokinetics and toxicokinetics is not well developed. One cannot get a sense (if it exists in the first place) on how much of the parent compound is converted to 2,5-hexanedione, tissue specificity etc. If the information is lacking, it needs to be stated.

Nevertheless much seems to be known about the toxicity of 2,5-hexanedione in numerous target organ tissues, and the literature is replete with recent studies on the topic (see below for just a few examples). Given the plethora of data on the toxicity of 2,5-hexanedione, one wonders whether lack of recognition of the direct effects of 2-hexanone toxicity in various target organs and upon various routes of exposure reflects the mere absence of such studies. Some thought should be directed at how the data on 2,5-hexanedione might be useful in providing additional information to the reader on the toxicity of 2-hexanone.

RESPONSE: *It is not ATSDR practice to summarize studies of metabolites of the chemical subject of the toxicological profile. ATSDR is concerned with environmental and occupational exposure of humans to 2-hexanone; therefore, studies in animals directly administered 2,5-hexanedione are not included in the profile. Use of studies of animals directly exposed to 2,5-hexanedione for risk assessment would require knowledge on how much of the parent compound is converted to 2,5-hexandione and, as the reviewer pointed out, that information is not available. A PBPK model would be helpful, but so far, none has been developed. However, it is appropriate to discuss 2,5-hexanedione in Section 3.5.2, Mechanism of Toxicity, as the document does. A statement was added in Section 3.5.1, Pharmacokinetic Mechanisms indicating the lack of information on this issue.*

COMMENT: Ignoring this body of literature seems contrived to this reviewer. Greater attempt should be made to describe the relevance of the animal data to human health outcomes upon exposure to 2-hexanone.

Some examples of recent literature on 2,5-hexanedione that need to be incorporated (there are others, not all the pertinent literature seem to be included in the report):

> Effects of 2,5-hexanedione on angiogenesis and vasculogenesis in chick embryos. Cheng X, Luo R, Wang G, Xu CJ, Feng X, Yang RH, Ding E, He YQ, Chuai M, Lee KK, Yang X. Reprod Toxicol. 2015 Jan;51:79-89. doi: 10.1016/j.reprotox.2014.12.006. Epub 2014 Dec 27.

Exposure to 2,5-hexanedione can induce neural malformations in chick embryos. Cheng X, Wang G, Ma ZL, Chen YY, Fan JJ, Zhang ZL, Lee KK, Luo HM, Yang X. Neurotoxicology. 2012 Oct;33(5):1239-47. doi: 10.1016/j.neuro.2012.07.005. Epub 2012 Jul 25.

Time-dependent alteration of cytoskeletal proteins in cerebral cortex of rat during 2,5-hexanedione-induced neuropathy. Song F, Zhang C, Yu S, Zhao X, Yu L, Xie K. Neurochem Res. 2007 Aug;32(8):1407-14. Epub 2007 Apr 20.

RESPONSE: *ATSDR thanks the reviewer for providing the articles by Cheng et al. (2015); however, it does not seem relevant for inclusion in the toxicological profile for 2-hexanone. Human and animal studies have clearly shown that 2-hexanone is a neurotoxic chemical and that the toxic entity is the metabolite, 2,5-hexanedione. It is unclear how results obtained in chick embryos treated with 2,5-hexanedione relate to environmental or occupational exposures to 2-hexanone. If some evidence existed indicating that 2-hexanone may be a developmental toxicant, it would make sense to search for possible mechanisms, and the Cheng et al. (2015) study would have had some relevance.*

As mentioned earlier in response to a comment from another reviewer, studies in animals directly administered 2,5-hexanedione are relevant to the profile on 2-hexanone only in relation to a mode of action, so relevant information can be mentioned in Section 3.5.2, Mechanism of Action. Because the information in Section 3.5.2 was taken from review articles, which cover the results of Song et al. (2007), it is unnecessary to specifically cite this individual article in Section 3.5.2.

COMMENT: Dosing within and across chapters should be standardized. Some doses are reported as ppm, others as mg/kg/day, etc. At a minimum there should be some guidance, as how might be able to contrast the various doses. As noted above the tables and Figures are excellent; yet dosing is reported in a variety of units. Perhaps in the Tables this issue can be addressed, using analogous units of exposure.

RESPONSE: *Because Section 3.2 is divided by route of exposure, the units of exposure in the inhalation section are ppm (for some chemicals, mg/m^3 may be preferred). In the oral exposure section, the units of exposure (or doses in this case) are mg/kg/day. The dermal exposure section presents the exposure units that were used in the studies. This is also the case in the LSE tables and figures and is standard practice in toxicological profiles.*

COMMENT: Statistical tests used in the studies are not always mentioned, nor is it possible to ascertain how the soundness of methods in each of the papers was evaluated. The same issue is also pertinent to the animal studies.

RESPONSE: *It is not standard practice in toxicological profiles to mention the specific statistical test that were used in the studies summarized in the text of Section 3.2. However, the term "significant" is used throughout the section to indicate that the p-value for differences between a treated group and the control group is <0.05.*

Annotated Comments on the Profile

FORWARD

COMMENT: page V, line 9: Seems redundant.

RESPONSE: *The comment refers to the following sentence that appears on page v, FOREWORD: "The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein." The Reviewer believes that "toxicologic" and "adverse" seem redundant. This is standard text in toxicological profiles; however, ATSDR will consider appropriate revisions for future profiles.*

CHAPTER 2 RELEVANCE TO PUBLIC HEALTH

2.3 MINIMAL RISK LEVELS MRLs)

INHALATION MRLs

COMMENT: page 33, line 24: Why is this necessary here?

RESPONSE: *The comment refers to the sentence "It should be mentioned that there is no toxicokinetic evidence indicating that rats are not a valid animal model to conduct hazard characterization and risk assessment for exposure to 2-hexanone." Because earlier text stated that hens are not a good animal model for dose-response assessment for 2-hexanone, it seemed appropriate to assure the reader that no inhalation MRL was derived from data on rats because the lowest LOAELs identified in rats were classified as serious LOAELs.*

COMMENT: page 34, line 2: The document reports exposures in ppm and mg/kg/day etc. There needs to be some standardization, as it is difficult to make comparisons.

RESPONSE: *As previously indicated, doses administered to animals orally are expressed in mg/kg/day units. In studies in which animals were exposed to vapors of 2-hexanone, the units are ppm. This is standard in toxicological profiles. No comparisons can be made in the absence of a PBPK model that could be used for route-to-route extrapolation.*

CHAPTER 3 HEALTH EFFECTS

3.2.1.4 NEUROLOGICAL EFFECTS

COMMENT: page 46, line 20: The potential for ethanol to modulate the neurotoxicity of 2-hexanone should be addressed (here or in the oral exposure section)

Pharmacodynamic and metabolic interactions between ethanol and two industrial solvents (methyl n-butyl ketone and methyl isobutyl ketone) and their principal metabolites in mice. Sharkawi M, Granvil C, Faci A, Plaa GL. Toxicology. 1994 Nov-Dec; 94(1-3):187-95.

Pharmacological and metabolic interactions between ethanol and methyl n-butyl ketone, methyl isobutyl ketone, methyl ethyl ketone, or acetone in mice. Cunningham J, Sharkawi M, Plaa GL. Fundam Appl Toxicol. 1989 Jul; we13(1):102-9.

RESPONSE: *Both studies are mentioned in Section 3.10, INTERACTIONS WITH OTHER CHEMICALS; that section seems a more appropriate place for citing the two papers.*

3.4 ABSORPTION

3.4.1.1 INHALATION EXPOSURE

COMMENT: page 53, line 35: The following authors performed both oral and INHALATION analyses

Tissue concentrations of methyl isobutyl ketone, methyl n-butyl ketone and their metabolites after oral or inhalation exposure. Duguay AB, Plaa GL. Toxicol Lett. 1995 Jan;75(1-3):51-8.

RESPONSE: *The Reviewer is correct; that is why the study results are mentioned both in the inhalation (3.4.4.1) and oral (3.4.1.2) sections.*

3.4.3 METABOLISM

COMMENT: page 56, line 6: Are there any estimates on how much of this metabolites is being produced, since this is the major neurotoxic metabolite.

RESPONSE: *No mass balance studies have been conducted with 2-hexanone to determine how much 2,5-hexanedione is produced from exposure to a known amount of 2-hexanone. This was added in Section 3.5.1, Pharmacokinetic Mechanisms.*

CHAPTER 9. REFERENCES

COMMENT: page 122, line 10: Include in the report the findings from this report:
Toxicology and carcinogenesis studies of methyl isobutyl ketone (Cas No. 108-10-1) in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program. Natl Toxicol Program Tech Rep Ser. 2007 Feb;(538):1-236.

RESPONSE: *ATSDR disagrees. It is unclear why the results of a study of a different chemical, methyl isobutyl ketone, should be included in the toxicological profile for 2-hexanone. Metabolism of methyl isobutyl ketone does not produce the active metabolite γ-diketone, 2,5-hexanedione, so the toxicities are different.*

The Reviewer made several editorial suggestions to the profile; unless otherwise noted, the suggested revisions were made.

Comments on Unpublished Studies

COMMENT: The Reviewer provided the following comments on the O'Donoughue and Krasavage (1979) unpublished study:

-Adequacy of design, methodology, and reporting

Issues of concern identified:

- The study is descriptive and lacks statistical analyses.
- The exclusion criteria for some of the cats and their replacements are vague and poorly described.
- Neurotoxic responses were detected only on the terminal examination of the animals, making me wonder if the methods were inadequate to determine earlier changes.
- One wonders how at the termination of the study so many abnormalities are identified; yet, nothing was evident in clinical and biopsy examinations prior to the termination of the study.
- Serum levels of MnBK and two metabolites showed inconsistent data, it is hard to explain the lack of persistence of the effects from week to week, raising serious issues about the methodology.
- Absent statistical evaluation, my confidence in these studies is minimal. One can probably mention them in the CDC/ATSDR report, but I would be cautious in drawing any conclusions about the LOEL given the limited nature of these studies.

-Validity of results and author's conclusions; and

- Based on the above, one should be cautious in accepting the authors' conclusions.

-Study inadequacies or confounding factors.

- See above.

-Provide a summary of your conclusions? Do you agree or disagree with those of the author?

- I disagree with the conclusions. For the rationale, please see all arguments above.

RESPONSE: *The Reviewer raised several issues with this study:*

- *Exclusion of some cats without explanation. ATSDR only identified one instance of a cat being removed from the study. In this case, the investigators noted that one cat in the control group was removed on study day 8 due to chronic dermatitis.*
- *Neurotoxic responses only noted at termination: The cats were examined daily for overt signs of neurotoxicity and complete neurological examinations (no details were provided) were initially conducted monthly and as needed later in the study. The observed lesions in the peripheral nervous system were graded as minimal or minor and may not have resulted in overt signs of neurotoxicity.*
- *Serum levels of 2-hexanone and metabolites are inconsistent: ATSDR agrees with the Reviewer that the values seem to be inconsistent and the investigators do not address this issue. Given the small number of animals per group (n=4), it is difficult to draw any conclusions regarding the inconsistencies.*
- *Minimal confidence in the findings due to lack of statistical evaluation: ATSDR agrees that there is low confidence in the study given the small number of animals tested. This study is used as supporting data for identifying the nervous system as a critical target and is not used for concentration-response determinations or MRL derivations.*

COMMENT: The Reviewer provided the following comments on the Union Carbide (1977) unpublished study:

-Adequacy of design, methodology, and reporting

Issues of concern identified:

- The study utilized an approximate dose of 1 gm/kg/day of methyl n-butyl, which led to reduced food and water consumption. A food deprivation group as a positive control is not included in the study, and one wonders whether the noted significant effects of 2-hexanone are mere reflection of the food deprivation and accompanying stress experienced by the rats. With severe muscle wasting and reductions in kidney weights (etc.) one wonders how the frank effects of this compound can be discerned from the sequalae of the weight loss and muscle wasting.

-Validity of results and author's conclusions; and

- Based on the above, one should be cautious in accepting the authors' conclusions.

-Study inadequacies or confounding factors.

- See above

-Provide a summary of your conclusions? Do you agree or disagree with those of the author?

- I disagree with the conclusions. I am not confident the effects can be specifically attributed to the tested compound absent positive controls for starvation and food deprivation.

RESPONSE: *The Reviewer stated that reduced food intake and water consumption were observed in the 1000 mg/kg/day dose level and that a food deprivation group should have been used as a positive control. According to the data reported in Table 40-4 of the report, there were no significant alterations in food or water consumption. Food and water intakes in the 1,010 mg/kg/day group were 15 and 11% lower than the controls; these nonsignificant decreases in intakes are not likely to have resulted in the marked decrease in body weight gain (69%) that was observed in this dose group; thus, it appears that the decrease in body weight gain is related to toxicity rather than decreases in food or water consumption.*

COMMENT: The Reviewer provided the following comments on the Krasavage and O'Donoughue (1977) unpublished study:

-Adequacy of design, methodology, and reporting

Issues of concern identified:

- The study is descriptive and lacks statistical analyses.
- The high-dose group gained lesser weight after 20 weeks of exposure, suggesting malnutrition. It is hard to distinguish the direct effects of methyl n-butyl ketone on the various endpoints from those associated with essentially food deprivation and the stress associated with it.
- There is mention of spontaneous lesions in various tissues (page 6), which "were not attributed to MnBK exposure". What is the basis for this assertion?
- Each animal seems to be treated as a case control, no evidence is provided for analogous toxicity or damage across the animals in each of the dosing groups. The relevance of these findings is unclear.

- There seems to be no consistency within the treatment groups vis-à-vis any of the specific effects. Specific effects are recognized in some animals, but not in others within the same treatment group (e.g. identical dosing).
- Why are some effects deemed to be directly associated with MnBK treatment while others spontaneous?
- The authors themselves seem to have no confidence in their results, see page 10 – "The results of the clinical and morphological portions of the study are ambiguous in the high dose group. Low dose animals did not develop clinical signs of morphologic lesions and neuropathy."
- The authors also state on the same page "These observations indicate that dose dependent decreased weight gain is toxicologically significant and likely indicates a more general toxic response". My interpretation is that they are unable to conclude with any certainty that the effects (inconsistent at best) are related to the MnBK.

-Validity of results and author's conclusions; and

- Based on the above, one should be cautious in accepting the authors' conclusions.

-Study inadequacies or confounding factors.

- See above

-Provide a summary of your conclusions? Do you agree or disagree with those of the author?

- The study offers inconclusive information, and the study is of limited validity.

RESPONSE: *ATSDR agrees with the Reviewer that there are a number of limitations to the Krasavage and O'Donoghue (1977) study.*

The Reviewer raised the following concerns

- *Lack of statistical analysis: ATSDR agrees that the lack of statistical analysis limits the interpretation of the results.*
- *Body weight: A decrease in body weight gain was observed in the high-concentration group after 20 weeks of exposure; however, the terminal body weight was within 10% of the controls and was not considered adverse.*
- *Spontaneous lesions: The investigators noted that lesions were observed in controls and treated animals; based on the results in Table 4 of the paper, the incidences of lesions in the urogenital, cardiovascular, and endocrine tissues do not appear to be concentration related.*
- *Consistency of effects within treatment groups: The observed lesions and overt signs of neurotoxicity were not consistently observed within a treatment group. We suspect that data were reported for individual animals (the Reviewer referred to this as treating them as treating the animals as case controls) in an attempt to link the overt signs with the histological alterations and because some effects were suspected to be unrelated to 2-hexanone exposure.*
- *Investigators confidence in the results: In several places in the report, the investigators noted that the findings are ambiguous or equivocal due to the low response rates.*

Due to the limitations in interpreting the results of this study, ATSDR used it for hazard identification purposes and dose-response data were not used to establish MRLs. The study limitations are discussed in Sections 2.3 and 3.2.1.4.