DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR CHLOROBENZENE

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 Chlorobenzene were:

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Comments provided by Reviewer #1

ATSDR Charge Questions and Responses

Chapter 1

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. Pertinent information is included.

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. Such possibilities can exist until proven otherwise.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes. Exposure conditions are adequately described.

RESPONSE: *No response is necessary.*

QUESTION: Do you believe the derived intermediate oral MRL value is justifiable? If you disagree, please explain. (see also Appendix A)

COMMENT: Yes, and justified based upon very well conducted study on dogs which is the basis of revised MRL.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree that the data do not support derivation of acute, intermediate, or chronic inhalation MRLs, or acute and chronic oral MRLs?

COMMENT: Yes. Based upon current guidelines only derivation of intermediate-duration oral MRL was possible. Suggestion: Page 10, line 17: 'that contain' should be changed to 'contaminated with' to indicate that chlorobenzene is not a natural constituent of food.

RESPONSE: *The suggested wording change was made.*

Chapter 2

QUESTION: Does the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature for Chlorobenzene?

COMMENT: Yes. And also unpublished reports provided by industry are included.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes. No changes are suggested.

RESPONSE: *No response is necessary.*

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. Studies are adequately described including the unpublished report used for revised intermediate-duration oral derivation of MRL.

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. No other suggestion.

RESPONSE: *No response is necessary.*

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

COMMENT: No.

RESPONSE: *No response is necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the chlorobenzene isomers?

COMMENT: Chlorobenzene cannot exist in the isomeric form. Therefore, this issue is moot.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes. No change is needed.

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: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: Yes. Literature is properly and adequately utilized.

RESPONSE: *No response is necessary.*

Chapter 3

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

COMMENT: No.

RESPONSE: *No response is necessary.*

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

COMMENT: No.

RESPONSE: *No response is necessary.*

Appendix A – Minimal Risk Levels (MRLs)

QUESTION: Do you agree or disagree with the revised intermediate oral MRL value? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: Agree. Suggestion: Typo in first paragraph last line: proposed intermediate–duration oral MRL should be 0.1 and not 0.2 mg/kg/day as stated.

RESPONSE: *The typographical error in the MRL Rationale Statement was corrected.*

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

COMMENT: Agree.

RESPONSE: *No response is necessary.*

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

COMMENT: None. Current guidelines are followed and relevant softwares are used.

RESPONSE: *No response is necessary.*

Appendix B – Literature Search Framework

QUESTION: Does Appendix B provide a sufficiently clear documentation of ATSDR's health effects literature search strategy and inclusion/exclusion criteria?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Does it provide enough transparency regarding ATSDR's implementation of its inclusion and exclusion criteria (e.g. how ATSDR chose the studies it included in the health effects chapter)?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

Overall Usability of the Profile:

QUESTION: Does the new chapter organization make it easier for you to find the information you need? For example, are you satisfied with the organization of the health effects chapter by organ system rather than exposure route?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Does the profile contain all of the information you need? Is there information you would like to see that is not currently included?

COMMENT: Relevant information is included.

RESPONSE: *No response is necessary.*

QUESTION: If you have used the Toxicological Profiles before, which chapter(s) have you used the most and for what purpose?

COMMENT: Chapter 2 for relevant doses and target organ toxicity.

RESPONSE: *No response is necessary.*

QUESTION: Are the new tables and figures clear and useful? Do they make the toxicological profile easier to read?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

Unpublished Studies (presented in alphabetical order):

QUESTION: The updated chlorobenzene profile includes unpublished studies (see list of nine provided previously) that were unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:

Bioassay Systems Corp. 1982. Nine reports regarding the effects of various chlorinated benzenes with cover letter dated 051183. Submitted to the U.S. Environmental Protection Agency under TSCA Section 4. OTS0511274.

E.I. Dupont. 1977. Mutagenic activity of monochlorobenzene in the Salmonella/microsome assay with cover letter dated 5/10/94 (Sanitized). Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557146.

Miles Inc. 1984. Study for skin-sensitizing effect on guinea pigs of monochlorobenzene, with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557415.

Monsanto. 1967b. 13-week oral administration - dogs: Chlorobenzene: Final report. Prepared by Hazleton Laboratories, Project No. 241-105, February 24.

Monsanto Co. 1976a. Mutagenicity evaluation of BIO-76-88 CP-5535 (LOX): Final report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557063.

Monsanto Co. 1976b. Mutagenicity evaluation of BIO-76-87 CP-5535 (LOX): Report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557065.

Monsanto Co. 1976c. Mutagenicity evaluation of BIO-76-88 CP-5535 (WGK): Final report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557064.

Shell Oil Company. 1991. A Utah Biomedical Test Laboratory report on NIOSH-sponsored inhalation study for IDHL values. HSE-78-0317. Prepared by Utah Biomedical Test Laboratory for Shell Oil Company.

Zeneca Specialties. 1982. Eye irritation classification on monochlorobenzene in rabbits with cover letter dated 05/06/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557374.

COMMENT: All these studies were conducted by well reputed organizations and reports submitted provide detailed description of their findings (Note: this is a general statement applicable to all unpublished studies provided the Reviewer).

RESPONSE: *No response is necessary.*

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes (Monsanto 1967b).

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: Yes. At higher dose (10x) some dogs died before thirteen weeks; probable causes of death are discussed.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes. 1-10x (three different doses).

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: All these studies are well designed and pertinent details are provided.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes.

RESPONSE: *No response is necessary.*

Comments provided by Reviewer #2

ATSDR Charge Questions and Responses

Chapter 1

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: I agree with the effects as reported with no substantial or significant exceptions.

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, that is why animal experiments are done and animal species should be selected and interpreted based on reasonable knowledge of how they respond relative to humans. Additionally, positive findings in animals provide "fail safe" attitude in assessing likely results with humans.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes, exposure conditions for the cited studies were adequately described (not completely in all reports, but adequately).

RESPONSE: *No response is necessary.*

QUESTION: Do you believe the derived intermediate oral MRL value is justifiable? If you disagree, please explain. (see also Appendix A)

COMMENT: Yes, the MRL is justified, after review of Appendix.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree that the data do not support derivation of acute, intermediate, or chronic inhalation MRLs, or acute and chronic oral MRLs?

COMMENT: Yes, after review of the information including Appendix A, it appears that these MRLs cannot be derived.

RESPONSE: *No response is necessary.*

Chapter 2

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

COMMENT: In my opinion, Yes.

RESPONSE: *No response is necessary.*

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

COMMENT: The intent was to locate adequately described human studies. Study limitations were appropriately described and the intent to avoid lengthy descriptions was observed.

RESPONSE: *No response is necessary.*

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: It appears that study designs were adequate and adequately described; I detected no significant omissions that would prohibit the use of the data.

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: within the limits posed by the fact that one is forced to operate with negative evidence, the animal species were appropriate (we truly are faced with data limitations for this compound).

RESPONSE: *No response is necessary.*

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

COMMENT: No, I am not aware of other studies that should be considered.

RESPONSE: *No response is necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the chlorobenzene isomers?

COMMENT: No, I am not aware of other studies that should be considered.

RESPONSE: *No response is necessary.*

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

COMMENT: I did not find any omissions for NOAELS and/or LOAELs in the texts or tables. I do not have any appropriate changes to suggest.

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: Yes, I agree.

RESPONSE: *No response is necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: I did not identify any mechanisms of action that should have been added to the discussion.

RESPONSE: *No response is necessary.*

Chapter 7

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

COMMENT: I am not aware of any regulations or guidelines that should be added.

RESPONSE: *No response is necessary.*

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

COMMENT: No listed guidelines should be removed; all are relevant.

RESPONSE: *No response is necessary.*

Appendix A – Minimal Risk Levels (MRLs)

QUESTION: Do you agree or disagree with the revised intermediate oral MRL value? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: I agree with the revised MRL; the added study (basis of the revision) was appropriately done and provides a reliable lower value for creating the MRL.

RESPONSE: *No response is necessary.*

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

COMMENT: I agree with 10 for uncertainty in translating from animal to human and 10 for human variability as consistent with usual procedures.

RESPONSE: *No response is necessary.*

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

COMMENT: It would be helpful to clarify (as much as possible) the use of the word "effects" (in the Monsanto Co. 1967b, see above) because the previous MRL was based on "increased liver weight and serum enzymes".

RESPONSE: *The specific hepatic effects are identified in all pertinent sections of the Toxicological Profile for Chlorobenzene.*

Appendix B – Literature Search Framework

QUESTION: Does Appendix B provide a sufficiently clear documentation of ATSDR's health effects literature search strategy and inclusion/exclusion criteria?

COMMENT: I am not expert in designing literature searches of the complexity described; however, the description appeared to fully disclose the methodology and resultant search terms which appeared to be appropriate and adequate.

RESPONSE: *No response is necessary.*

QUESTION: Does it provide enough transparency regarding ATSDR's implementation of its inclusion and exclusion criteria (e.g. how ATSDR chose the studies it included in the health effects chapter)?

COMMENT: Yes it was transparent (please also see answer to 1. immediately above).

RESPONSE: *No response is necessary.*

Overall Usability of the Profile:

QUESTION: Does the new chapter organization make it easier for you to find the information you need? For example, are you satisfied with the organization of the health effects chapter by organ system rather than exposure route?

COMMENT: Yes the changes make it easier for me to find the information provided, and it will likely make it easier for others. I do not have strong feelings for the use of organizing effects by organ systems or routes. I like the organization and formatting of the Figures (minor suggestions were made in the annotated document I have provided).

RESPONSE: *See responses to the comments on annotated pages.*

QUESTION: Does the profile contain all of the information you need? Is there information you would like to see that is not currently included?

COMMENT: Yes. I did not identify any information that I believe is available that should have been included.

RESPONSE: *No response is necessary.*

QUESTION: If you have used the Toxicological Profiles before, which chapter(s) have you used the most and for what purpose?

COMMENT: Yes, I have used (as well as reviewed) Toxicology Profiles previously on many occasions, primarily as an expert witness in court where I have found them to be generally helpful and accepted as authoritative by attorneys, judges, and juries.

RESPONSE: *No response is necessary.*

QUESTION: Are the new tables and figures clear and useful? Do they make the toxicological profile easier to read?

COMMENT: Definitely, yes. The new tables and figures are clear and much appreciated. I have made minor suggestions for changes in the annotated copy of the document which I have provided.

RESPONSE: *See responses to the comments on annotated pages.*

Reviewer #2 comments on annotated pages

COMMENT: In this figure there are several spacing errors: example: Clinicals signs of neurotoxicity**.**

RESPONSE: *The spacing issues in Figure 1-1 were resolved.*

COMMENT: oral studies is jargon and not clear. Suggest rephrasing to make clearer.

RESPONSE: *Under "Immunological Effects," the statement "Results from 13-week oral studies of …", was revised to state "Results from 13-week studies of orally exposed …".*

COMMENT: This paragraph is a very good, and needed, explanation!

RESPONSE: *Regarding the text in Section 2.1 that discusses Levels of significant exposure (LSEs), ATSDR thanks the Reviewer for the complement.*

COMMENT: "the Number of Studies" is somewhat clumsy and could be reworded for clarity.

RESPONSE: *In Figure 2-1, ATSDR considers the figure title to adequately describe the content of the figure.*

COMMENT: "counts" is not as clear as it could be.

RESPONSE: *In Figure 2-1, the statement "counts represent studies examining endpoint" was revised to "counts represent the number of studies examining endpoint."*

COMMENT: Just my opinion, but I would prefer a footnote (b) to lead the reader to definitions.

RESPONSE: *In Table 2-1, regarding abbreviations in the "Parameters monitored" column, the last row of the table is devoted to footnotes and acronym definitions. This is standard ATSDR table format. It is not considered necessary to assign footnotes to acronyms in the body of a table as this would require additional footnotes for each set of related acronyms.*

COMMENT: I assume that the numbers associated with the letter designating the species indicate the number of animals in the test? Perhaps this could be clarified in the box identifying the species.

RESPONSE: *Regarding the comment on the numbers that are presented in LSE figures (e.g., Figure 2-2), the number associated with each symbol refers to the Figure key (number) in the preceding LSE Table, as stated in the LSE Table.*

COMMENT: I suggest a footnote (c) in context in this Table to alert the reader to the definitions at the end of the Tables.

RESPONSE: *Regarding the comment on Table 2-2, ATSDR LSE tables consistently follow the same format as that shown in LSE tables for chlorobenzene. It does not appear necessary to provide additional information to direct the reader to the end of a table for definitions of acronyms.*

COMMENT: As previously noted, I suggest that the numbers associated with the species designation (in the box) be defined.

RESPONSE: *Regarding the comment on the numbers that are presented in LSE figures (e.g., Figure 2-3), the number associated with each symbol refers to the Figure key (number) in the preceding LSE Table, as stated in the LSE Table.*

COMMENT: This statement is very unhelpful and could be misinterpreted. A more useful interpretation would be appreciated.

RESPONSE: *Regarding the comment on the sentence in Section 2.4 that states "The authors of these studies suggested that low levels of chlorobenzene exposure might initiate inflammatory reactions in the lung," the statement was deleted.*

COMMENT: This is very helpful and needed because of the uncertainties in the immediately previous comments.

RESPONSE: *Regarding the statement "However, limited details in the study report and lack of supportive evidence from other animal studies preclude meaningful evaluation of chlorobenzene-induced hematological effects following inhalation exposure," ATSDR thanks the Reviewer for the comment.*

COMMENT: It would be helpful to say if these results were statistically significantly different.

RESPONSE: *Regarding the statement in Section 2.9 "In a 2-generation inhalation study of rats, repeated inhalation exposure to chlorobenzene vapor at 150 ppm resulted in increased mean relative liver weight and increased incidence of hepatocellular hypertrophy of parental males," the statement was revised to "In a 2-generation inhalation study of rats, repeated inhalation exposure to chlorobenzene vapor at 150 ppm resulted in statistically significantly increased mean relative liver weight and increased incidence of hepatocellular hypertrophy of parental males (incidence data not included in the study report)."*

COMMENT: Without being to lengthy, this could be made clearer regarding when toxicity was observed.

RESPONSE: *Regarding the statement in Section 2.10 "Nair et al. (1987) reported tubular dilatation with eosinophilic material, interstitial nephritis, and foci of regenerative epithelium in 2 generations of parental male rats exposed to chlorobenzene vapor at concentrations ≥150 ppm," the effects were noted at necropsy. It does not appear necessary to provide additional information regarding timing of the effect.*

COMMENT: Could this be simplified by eliminating the repetition… to laboratory animal studies where…

RESPONSE: *Regarding the statement in Section 2.12 "Available information regarding chlorobenzene-induced ocular effects is limited to observations of chlorobenzene-induced ocular irritation in laboratory animals," the statement was replaced with "Limited information was located regarding chlorobenzene-induced ocular effects."*

COMMENT: Would be clearer to say… since no human data were found

RESPONSE: *Regarding the statement in Section 2.14 "Since there are no human data on immunotoxic effects …," the statement was revised to "Since no human data were located regarding immunotoxic effects…".*

COMMENT: It would be helpful to cite the value and give a reference.

RESPONSE: *Regarding the statement in Section 2.15 "Humans occupationally exposed to chlorobenzene intermittently for up to 2 years at levels above current federal limits displayed signs of neurotoxicity including numbness, cyanosis (from depression of respiratory center), hyperesthesia, and muscle spasms," the statement was deleted because specifics of exposure levels were not included in the study report.*

COMMENT: jargon; reword, please.

RESPONSE: *Regarding the statement in Section 2.15 "Ataxia and narcosis were observed in rats exposed to chlorobenzene vapor for 30 minutes at concentrations ≥5,850 ppm; most rats were narcotic by 25 minutes," the statement was replaced with "Ataxia and narcosis were observed in rats exposed to chlorobenzene vapor for 30 minutes at concentrations ≥5,850 ppm; most rats displayed these effects within 25 minutes following initiation of exposure."*

COMMENT: Picky, but omit : "levels" and change dose to doses.

RESPONSE: *Regarding the statement in Section 2.19 "Male rats showed a significant ($p<0.05$) increase in the incidence of neoplastic nodules of the liver in the 120 mg/kg/day dose group, but no increases were found at lower dose levels," the phrase "dose levels" was replaced with "doses."*

COMMENT: suggest adding: by referring to

RESPONSE: *Regarding the introductory statement in Chapter 7 "This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency," the statement was revised to "This table is not an exhaustive list, and current regulations should be verified by referring to the appropriate regulatory agency."*

COMMENT: When I printed this none of these references (only those which appear to be hyperlinked?) printed; is this intentional?

RESPONSE: *Regarding the references in Table 7-1, the table from the file containing the Peer Reviewer's comments was printed. All references in the table printed properly.*

COMMENT: Is it possible to describe the effect?

RESPONSE: *Regarding the body weight endpoint for the rat in the study of Monsanto Co. (1967a), there was no effect to describe (i.e., the highest dose level of 250 mg/kg/day did not affect body weight).*

COMMENT: Is it possible to describe the effect?

RESPONSE: *Regarding the hematological endpoint for the rat in the study of Monsanto Co. (1967a), there was no effect to describe (i.e., the highest dose level of 250 mg/kg/day did not affect hematological parameters).*

Unpublished Studies (presented in alphabetical order):

QUESTION: The updated chlorobenzene profile includes unpublished studies (see list of nine provided previously) that were unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:

Bioassay Systems Corp. 1982. Nine reports regarding the effects of various chlorinated benzenes with cover letter dated 051183. Submitted to the U.S. Environmental Protection Agency under TSCA Section 4. OTS0511274.

E.I. Dupont. 1977. Mutagenic activity of monochlorobenzene in the Salmonella/microsome assay with cover letter dated 5/10/94 (Sanitized). Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557146.

Miles Inc. 1984. Study for skin-sensitizing effect on guinea pigs of monochlorobenzene, with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557415.

Monsanto. 1967b. 13-week oral administration - dogs: Chlorobenzene: Final report. Prepared by Hazleton Laboratories, Project No. 241-105, February 24.

Monsanto Co. 1976a. Mutagenicity evaluation of BIO-76-88 CP-5535 (LOX): Final report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557063.

Monsanto Co. 1976b. Mutagenicity evaluation of BIO-76-87 CP-5535 (LOX): Report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557065.

Monsanto Co. 1976c. Mutagenicity evaluation of BIO-76-88 CP-5535 (WGK): Final report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557064.

Shell Oil Company. 1991. A Utah Biomedical Test Laboratory report on NIOSH-sponsored inhalation study for IDHL values. HSE-78-0317. Prepared by Utah Biomedical Test Laboratory for Shell Oil Company.

Zeneca Specialties. 1982. Eye irritation classification on monochlorobenzene in rabbits with cover letter dated 05/06/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557374.

Bioassay Systems Corp 1982

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Not relevant to this mutagenicity test with *Drosophila.*

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: Not relevant to this study.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes, the study was designed to detect a mutation frequency increment of 0.2%.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Yes. it was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree that mutagenicity was not induced.

RESPONSE: *No response is necessary.*

E.I. Dupont 1977

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: This is not relevant to this study.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: This is not relevant to this study.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Interpreting for a bacterial mutagenicity test: Yes: doses from 750 to 3000 microgram per plate in 6 dose levels. 5 strains of S. typhimurium with metabolic activation, and appropriate solvent control and positive control (2AA). Duplicate plates. Prior to test, chlorobenzene was tested for toxicity to tester strains.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Yes, was appropriately designed.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree; the agent did not produce a significant increase over spontaneous mutation frequency.

RESPONSE: *No response is necessary.*

Miles Inc. 1984

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes, test group = 20, control group = 20 with a total of 40 male guinea pigs used.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: Not relevant to this skin sensitization test.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes. Doses of 1%, 50%, 25%, and 25% (included intradermal induction, topical induction and 1st and 2nd challenges.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Yes. Study was appropriately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree there was no sensitizing effect.

RESPONSE: *No response is necessary.*

Monsanto Co. 1967b

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes 16 male and 16 female beagles.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: Yes, to the extent I believe this is feasible; there was a dose effect and thorough histology and pathology (gross and microscopic) was done plus observations for appearance and behavior and body weights and organs weights were measured; extensive records.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes: 0, 0.025. 0.050, and 0.250 ml/kg/day with 4 of each male and female.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Yes, the study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree: in the highest dose group there was 4 deaths out of 8; zero deaths in the other dose groups.

RESPONSE: *No response is necessary.*

Monsanto Co. 1976a

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Not relevant to this mouse lymphoma cell assay for mutagenicity.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: Not relevant to this mouse lymphoma cell assay for mutagenicity.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Four doses selected after toxic dose established.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Yes, was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree that the agent was not mutagenic under the test conditions.

RESPONSE: *No response is necessary.*

Monsanto Co. 1976b (identified by Reviewer #2 as Monsanto 1996b)

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Not relevant to this microbial mutagenicity assay using S. cerevisiae and S. typhimurium.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: Not relevant.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes; 4 dose levels (0.01 microliter to 5 microliter per plate).

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Yes, study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes. I agree that mutagenicity was not demonstrated in these tests.

RESPONSE: *No response is necessary.*

Monsanto Co. 1976c

COMMENT: The Reviewer identified this study as Monsanto 1996b (actually Monsanto Co. 1976b) and stated that the study was supplied as a duplicate (presumably meaning Monsanto Co. 1976b).

RESPONSE: *The identity of the pdf to which the Reviewer referred is not known.*

Shell Oil Co. 1991

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes: 20 test and 20 control both sexes (10 rats, 10 guinea pigs each sex). Adequate (good) animal care practices.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: Yes, as I reasonably interpret this question. Adequate controls were run, there was a dose response, and the effects were those reasonably expected. In a technical sense, it is practically impossible to rule out every other possible cause of death.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes and yes. There were three doses (2990 ppm, 5850 ppm, and 7970 ppm).

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Yes. It was adequately designed and reported. Source and care of animals provided; calibration of doses was described in detail including problems detected and overcome; they found "possible neurotoxicity" (ataxia plus narcosis progressing with dose to narcosis and twitching (convulsions and tremors) at highest of three doses. Documented pathology was deemed to be irreversible which designation was predetermined as adequate to establish IDLH (see p. 35)

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree with conclusions.

RESPONSE: *No response is necessary.*

Zeneca Specialties 1982

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: No, only one test with 9 animals; Yes animal care was appropriate.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: This is not relevant to this eye irritant test.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: No. One dose only with eye washed within 20 to 30 sec. or not; other eye was control; scored through 7 days.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: No, the study was useful and showed mild to moderate eye irritation that was reversed by 7 days. They happened to use a useful exposure dose and the results are valuable.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree.

RESPONSE: *No response is necessary.*

Comments provided by Reviewer #3

ATSDR Charge Questions and Responses

Chapter 1

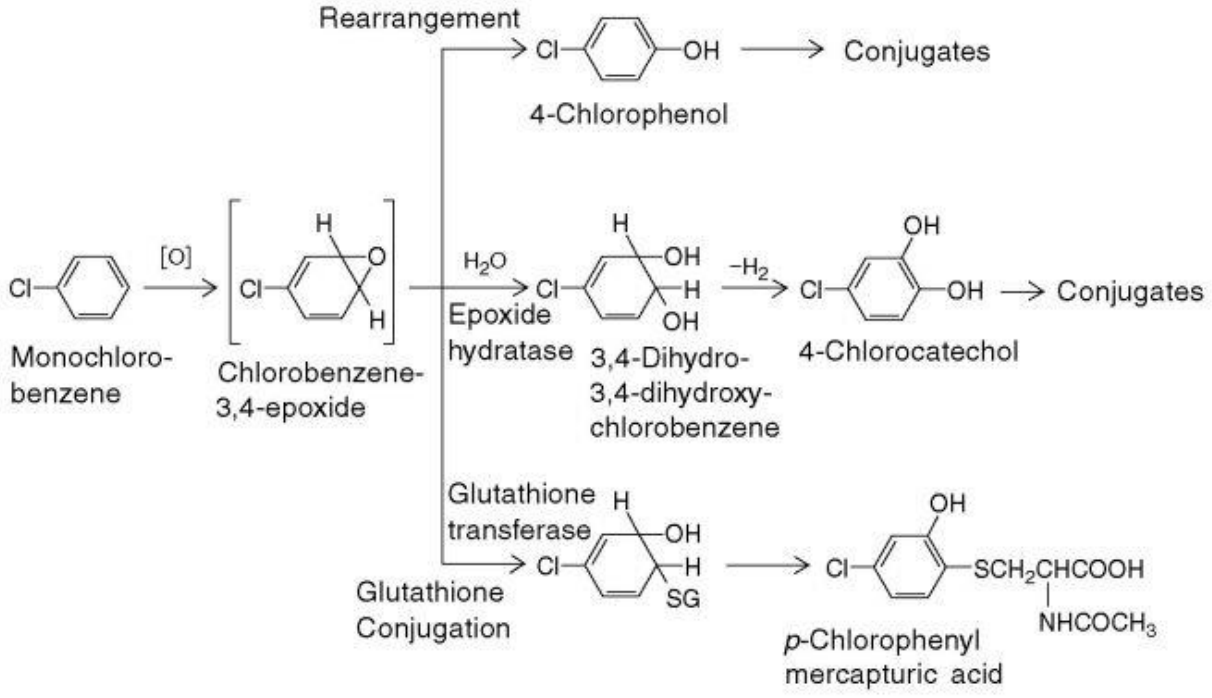
QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT: Essentially no direct observations of effects are documented in humans for this compound.

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: The main concern does not arise from observed effects in animals. The concern is from metabolism to an epoxide compound:



(metabolism figure is from Greim, H The MAK-Collection for Occupational Health and Safety: Annual Thresholds and Classifications for the Workplace, 2011 Wiley-VCH Verlag GmbH & Co. KGaA)

Epoxides, such as the Chlorobenzene-3,4-epoxice shown above, react with DNA and other macromolecules, leading to risks of genetic changes and cancers. The negative evidence in several in vitro assays provided in several documents by the makers/sponsors of this compound are not sufficient to allay this concern. In general the experiments documented use metabolic enzyme systems derived from rat liver. While this is standard practice for routine testing, it would have been better to test preparations derived from human microsomes, or otherwise possible differences between the metabolic activities of human and rat liver enzyme systems. Another useful step would be to compile estimates of cancer potency for a series of other epoxides from the scientific literature. That could help form a Bayesian "prior" distribution for the epoxide metabolites derived from chlorobenzene. This would at least help characterize the quantitative uncertainty in likely cancer potency for chlorobenzene.

RESPONSE: *ATSDR acknowledges the lack of available information regarding the carcinogenicity of chlorobenzene and its reactive metabolites. The following statement was added to Section 2.20 (Genotoxicity):*

> …chlorobenzene undergoes CYP450 catalyzed oxidation to form the 3,4- and 2,3-epoxides of chlorobenzene. Both epoxides can be formed in liver and lung (and other tissues such as kidney and adrenal cortex) and are capable of covalently binding to DNA, RNA, and proteins.

Chapter 6 points out the need for additional information on interspecies differences in chlorobenzene metabolism and the need for human data and additional animal data to further evaluate the carcinogenicity of chlorobenzene. The following statement was added to the cancer section of Chapter 6:

> Although available human and animal data have not provided convincing evidence regarding the carcinogenicity of chlorobenzene, additional mechanistic studies should be designed to evaluate possible genotoxic mechanisms of carcinogenicity because chlorobenzene metabolism results in the formation of epoxides that can react with DNA, RNA, and proteins. Any *in vitro* assays should be performed using human microsomes due to interspecies differences in chlorobenzene metabolism.

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

COMMENT: This question does not seem to apply to the facts in this case.

RESPONSE: *No response is necessary.*

QUESTION: Do you believe the derived intermediate oral MRL value is justifiable? If you disagree, please explain. (see also Appendix A)

COMMENT: I don't believe that the MRL derived in Appendix A is well justified. The derivation uses as its point of departure an effective LOAEL (a BMD10) rather than a NOAEL without further adjustment or discussion. In my opinion the authors should have applied an additional LOAEL/NOAEL adjustment factor of at least 3, yielding a revised candidate MRL of 0.03 mg/kg-day.

RESPONSE: *The BMD_{10} is a prediction of the threshold of a response (in this case a 10% change in incidence from that of controls. It is intended to reduce uncertainty in the dose (or exposure) range between an identified LOAEL and NOAEL. ATSDR does not consider it necessary to apply an additional adjustment factor.*

QUESTION: Do you agree that the data do not support derivation of acute, intermediate, or chronic inhalation MRLs, or acute and chronic oral MRLs?

COMMENT: I think these MRLs could have been derived by using data for other routes and exposure times with suitable quantitative adjustments derived from data for other compounds.

RESPONSE: *It is standard procedure for ATSDR to derive MRLs based on the most appropriate data from studies of the chemical in question. ATSDR does not typically perform route-to-route extrapolation to derive MRLs for a particular exposure route based on data for a different exposure route. ATSDR would not attempt to derive an MRL for a particular substance based on data for other compounds.*

Chapter 2

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

COMMENT: Yes.

RESPONSE: *No response is necessary.*

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

COMMENT: No usable human studies of chlorobenzene were identified in the text.

RESPONSE: *No response is necessary.*

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: The dog studies identified in the text and used for MRL derivation were marginal, but usable.

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: I think the primary concern for this compound is carcinogenesis/mutagenesis because of the metabolism to an epoxide. Much more could have been done to derive an uncertainty distribution for the likely cancer potency of chlorobenzene based on available data for the potency of other epoxide compounds.

RESPONSE: *ATSDR does not conduct cancer potency evaluations. However, ATSDR will include any information regarding the carcinogenicity of chlorobenzene or relative potency that may be published in the future. The following text was added to the cancer section of Chapter 6:*

> Although available human and animal data have not provided convincing evidence regarding the carcinogenicity of chlorobenzene, additional mechanistic studies should be designed to evaluate possible genotoxic mechanisms of carcinogenicity because chlorobenzene metabolism results in the formation of epoxides that can react with DNA, RNA, and proteins. Any *in vitro* assays should be performed using human microsomes due to interspecies differences in chlorobenzene metabolism.

The following statement was added to Section 2.20 (Genotoxicity):

> … chlorobenzene undergoes CYP450 catalyzed oxidation to form the 3,4- and 2,3-epoxides of chlorobenzene. Both epoxides can be formed in liver and lung (and other tissues such as kidney and adrenal cortex) and are capable of covalently binding to DNA, RNA, and proteins.

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

COMMENT: It is not possible for me, in the time available, to assemble the database of prior observations of carcinogenesis by epoxide compounds that could be analyzed to derive the uncertainty distribution called for in my response to question 4 above.

RESPONSE: *No response is necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the chlorobenzene isomers?

COMMENT: As far as I know there is only one isomer of chlorobenzene.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes, as far as I can tell.

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: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: There has not been as full a discussion of likely mutagenic mechanisms for carcinogenic action for the epoxide metabolites of this compound.

RESPONSE: *The following was added to the first paragraph of Section 2.20 (Genotoxicity): However, as shown in Figure 3-1, chlorobenzene undergoes CYP450 catalyzed oxidation to form the 3,4- and 2,3-epoxides of chlorobenzene. Both epoxides can be formed in liver and lung (and other tissues such as kidney and adrenal cortex) and are capable of covalently binding to DNA, RNA, and proteins.*

Chapter 7

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

COMMENT: No.

RESPONSE: *No response is necessary.*

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

COMMENT: No.

RESPONSE: *No response is necessary.*

Appendix A – Minimal Risk Levels (MRLs)

QUESTION: Do you agree or disagree with the revised intermediate oral MRL value? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: I disagree. As discussed above in my response to question 4 in the first set of charge questions, I would apply an additional factor of 3 to reach candidate revised MRL of 0.02 mg/kg-day.

RESPONSE: *As stated previously in response to the peer review comment regarding application of an additional uncertainty factor of 3 to the provisional intermediate-duration oral MRL of 0.06 mg/kg/day,*

the BMD_{10} is a prediction of the threshold of a response (in this case, a 10% change in incidence from that of controls). The point of departure ($BMDL_{10}$) is the lower 95% confidence limit on the BMD_{10} and is considered to adequately address uncertainty in estimating a NOAEL. ATSDR does not consider it necessary to apply an additional adjustment factor.

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

COMMENT: See above.

RESPONSE: *See response above.*

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

COMMENT: See above.

RESPONSE: *See response above.*

Appendix B – Literature Search Framework

QUESTION: Does Appendix B provide a sufficiently clear documentation of ATSDR's health effects literature search strategy and inclusion/exclusion criteria?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Does it provide enough transparency regarding ATSDR's implementation of its inclusion and exclusion criteria (e.g. how ATSDR chose the studies it included in the health effects chapter)?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

Overall Usability of the Profile:

QUESTION: Does the new chapter organization make it easier for you to find the information you need? For example, are you satisfied with the organization of the health effects chapter by organ system rather than exposure route?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Does the profile contain all of the information you need? Is there information you would like to see that is not currently included?

COMMENT: As indicated above, I believe an appropriate assessment for this compound must include a detailed discussion and uncertainty analysis of the likely cancer risk from the epoxide metabolites. This would require a review and structure-activity-based analysis of the distribution of cancer potencies observed with other epoxide compounds.

RESPONSE: *As stated in response to a previous comment on these issues, ATSDR does not conduct cancer potency evaluations. However, ATSDR will include any information regarding the carcinogenicity of chlorobenzene or relative potency that may be published in the future. The following text was added to the cancer section of Chapter 6:*

> Although available human and animal data have not provided convincing evidence regarding the carcinogenicity of chlorobenzene, additional mechanistic studies should be designed to evaluate possible genotoxic mechanisms of carcinogenicity because chlorobenzene metabolism results in the formation of epoxides that can react with DNA, RNA, and proteins. Any *in vitro* assays should be performed using human microsomes due to interspecies differences in chlorobenzene metabolism.

The following statement was added to Section 2.20 (Genotoxicity):

> … chlorobenzene undergoes CYP450 catalyzed oxidation to form the 3,4- and 2,3-epoxides of chlorobenzene. Both epoxides can be formed in liver and lung (and other tissues such as kidney and adrenal cortex) and are capable of covalently binding to DNA, RNA, and proteins.

QUESTION: If you have used the Toxicological Profiles before, which chapter(s) have you used the most and for what purpose?

COMMENT: I have not used them.

RESPONSE: *No response is necessary.*

QUESTION: Are the new tables and figures clear and useful? Do they make the toxicological profile easier to read?

COMMENT: The tables and figures provide useful detail.

RESPONSE: *No response is necessary..*

Comments provided by Reviewer #4

ATSDR Charge Questions and Responses and General Comments

Chapter 1

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 human health effects reported in the text. There are virtually no data with reliable exposure measurements for chlorobenzene available.

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: A single case report of a suicidal male alcoholic exposed to chlorobenzene described the finding of severe liver necrosis. As hepatic effects were noted following exposure to chlorobenzene in animals, I agree that the hepatic effects observed in animals are likely to be of concern to humans.

RESPONSE: *No response is necessary.*

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

COMMENT: Yes, I agree that the exposure conditions have been adequately described.

RESPONSE: *No response is necessary.*

QUESTION: Do you believe the derived intermediate oral MRL value is justifiable? If you disagree, please explain. (see also Appendix A)

COMMENT: A provisional intermediate-duration oral MRL of 0.1 mg/kg/day was derived for chlorobenzene based on dose-related hepatic changes (bile duct hyperplasia) in dogs treated orally with chlorobenzene 5 days/week for 13 weeks. I agree with the derived intermediate MRL.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree that the data do not support derivation of acute, intermediate, or chronic inhalation MRLs, or acute and chronic oral MRLs?

COMMENT: Yes, I agree that the data do not support the derivation of acute, intermediate or chronic inhalation MRL's for chlorobenzene. For the oral route of exposure, acute and chronic oral MRL's were

not derived. The Monsanto 1967 subchronic study in dogs was used to derive an intermediate MRL for chlorobenzene. The decision to not derive a chronic oral MRL for chlorobenzene was cited as "No nonlethal and nonneoplastic effects were observed in the rats or mice following chronic-duration oral exposures at doses resulting in adverse nonneoplastic effects in animals following intermediate-duration exposures. Therefore, no chronic-duration oral MRL was derived for chlorobenzene." No discussion was included as to why the 90 day study (Monsanto, 1967) was not used to derive a chronic MRL (adding a UF of 10 for less that lifetime exposure).

RESPONSE: *One 2-year oral toxicity and carcinogenicity study of rats gavaged with chlorobenzene at 60 or 120 mg/kg/day reported decreased survival and increased incidences of neoplastic liver lesions at 120 mg/kg/day in the absence of other signs of exposure-related adverse effects (NTP 1985). There were no signs of adverse effects in mice similarly treated at 30 or 60 mg/kg/day (males) or 60 or 120 mg/kg/day (females) (NTP 1985). No nonlethal and nonneoplastic effects were observed in the rats or mice following chronic-duration oral exposures at doses resulting in adverse nonneoplastic effects in animals following intermediate-duration exposures. Therefore, no chronic-duration oral MRL was derived for chlorobenzene.*

EPA (IRIS 2003) derived a chronic reference dose (RfD) of 0.02 mg/kg/day for chlorobenzene based on histopathologic changes in the liver of dogs administered chlorobenzene in daily capsule for 13 weeks (the same critical effect employed by ATSDR to derive a provisional intermediate-duration oral MRL). EPA included an uncertainty factor of 10 to account for extrapolation from the 13-week exposure to a chronic exposure scenario. It is not standard policy for ATSDR to extrapolate from an intermediate-duration exposure protocol to a chronic-duration exposure protocol in the absence of adequate chronic-duration data.

COMMENT: Figure 1-2, p 12 - I believe there is an error in the figure - Chronic effects (120-125) – neoplastic liver modules – should be "liver nodules".

RESPONSE: *The typographical error in Figure 1-2 was corrected.*

COMMENT: P 14, line 6 "High-dose male rats exhibited" – it would be useful to include what the dose was in this sentence.

RESPONSE: *"High-dose" in Section 1.2 was further identified as the 120 mg/kg/day dose.*

Chapter 2

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

COMMENT: Yes, I agree with the conclusions made in Chapter 2.

RESPONSE: *No response is necessary.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for

confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT: Virtually no human exposure studies are available. Those that were identified (occupational exposure and intentional ingestion) were described in the profile. These studies were not adequately designed, and were not controlled human exposure studies.

RESPONSE: *No response is necessary.*

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, adequately designed animal studies were identified and described in the text. The process used for scientific review of the literature and the outcome of the data analysis (as described in Appendix B) thoroughly evaluated the appropriateness of the existing scientific literature and determined the overall confidence in each study. I agree with those conclusions.

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, I agree with the selection of the principal studies and the species selected.

RESPONSE: *No response is necessary.*

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

COMMENT: I am not aware of additional studies that should be included in the profile that are relevant to evaluating the health effects of chlorobenzene.

RESPONSE: *No response is necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the chlorobenzene isomers?

COMMENT: I am not aware of additional studies that would assist in deriving MRLs for chlorobenzene.

RESPONSE: *No response is necessary.*

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

COMMENT: I agree that appropriate NOAELs (when available) and LOAELs were identified for each study and were accurately written in tables and figures. The process used for scientific review of the literature and the outcome of the data analysis (as described in Appendices B) thoroughly evaluated the appropriateness of the existing scientific literature and determined the overall confidence in each study, a process that included evaluating the appropriateness of NOAELs and/or LOAELs. I have no changes to suggest for this section of the profile.

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: As noted in the profiles document, LOAELs have been categorized into "less serious" or "serious" effects. "Serious" is defined as effects that elicit alteration of a biological system that can lead to morbidity or mortality whereas "Less serious" is defined as effects that are not expected to cause significant dysfunction or death. While it is acknowledged that this is not a quantitative classification, but rather a categorization based on judgement and knowledge of biological processes, I agree with this classification and feel that these descriptors have been appropriately cited in the LSE tables included in this chapter.

RESPONSE: *No response is necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain.

COMMENT: Discussion of possible mechanism(s) of action was not included in chapter 2. In general, no specific mechanism(s) for the observed toxicity has been proposed largely due to a lack of data concerning mechanism(s) of action for chlorobenzene

RESPONSE: *No response is necessary.*

Chapter 7

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

COMMENT: I am not aware of other regulations or guidelines that should be included in this section.

RESPONSE: *No response is necessary.*

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

COMMENT: There is not any information that I am aware of that should be removed from this Chapter.

RESPONSE: *No response is necessary.*

Appendix A – Minimal Risk Levels (MRLs)

QUESTION: Do you agree or disagree with the revised intermediate oral MRL value? Explain. If you disagree, please specify the MRL value that you propose.

COMMENT: Yes, I agree with the revised MRL based on the incidences of bile duct hyperplasia (sexes combined). The 55 mg/kg/day is considered to represent a LOAEL for chlorobenzene-induced liver effects (bile duct hyperplasia) which was observed in a dose-related manner (incidences of 3/8 and 7/8 at 55 and 280 mg/kg/day, respectively). Therefore, the 28 mg/kg/day dose level is considered a NOAEL for liver effects, and data for this endpoint was selected for benchmark dose analysis to derive the MRL. Liver effects were consistently observed in several species lending support to selecting this endpoint to derive a MRL.

RESPONSE: *No response is necessary.*

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

COMMENT: The UF's applied were 10 for extrapolation from animals to humans, and 10 for human variability (total = 100). No explanation as to the adequacy of the database was discussed. It is questioned whether an additional uncertainty (UFD = 3) should be included to account for the relatively limited data that is available for chlorobenzene toxicity.

RESPONSE: *Although the database of information is rather limited, ATSDR considers the derived intermediate-duration oral MRL to be sufficiently protective without the need for an additional uncertainty factor for database deficiencies.*

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

COMMENT: I have no specific comments that I feel need to be addressed concerning the MRL assessment. One correction, in the opening paragraph in this section, the last sentence "a new intermediate-duration oral MRL of 0.2 mg/kg/day is proposed based on the results from the dog study" Shouldn't this be 0.1 mg/kg/day?

RESPONSE: *The typographical error in the intermediate-duration oral MRL worksheet was corrected.*

Appendix B – Literature Search Framework

QUESTION: Does Appendix B provide a sufficiently clear documentation of ATSDR's health effects literature search strategy and inclusion/exclusion criteria?

COMMENT: Yes, the search strategy and inclusion/exclusion criteria were clearly documented.

RESPONSE: *No response is necessary.*

QUESTION: Does it provide enough transparency regarding ATSDR's implementation of its inclusion and exclusion criteria (e.g. how ATSDR chose the studies it included in the health effects chapter)?

COMMENT: Yes, how exclusion/inclusion criteria were used to select the studies described in the profile was made clear to the reader.

RESPONSE: *No response is necessary.*

Overall Usability of the Profile:

QUESTION: Does the new chapter organization make it easier for you to find the information you need? For example, are you satisfied with the organization of the health effects chapter by organ system rather than exposure route?

COMMENT: Yes, I find the chapter organization easier to navigate, and the overall flow of the document is logical and easy to navigate. I do prefer chapter 2 organized by organ systems rather than route of exposure. It makes it easier to read the health effects tables as well.

RESPONSE: *No response is necessary.*

QUESTION: Does the profile contain all of the information you need? Is there information you would like to see that is not currently included?

COMMENT: All information that I am aware of is included in this document. It presents a comprehensive scientific review of chlorobenzene.

RESPONSE: *No response is necessary.*

QUESTION: If you have used the Toxicological Profiles before, which chapter(s) have you used the most and for what purpose?

COMMENT: Yes, I have used Toxicological Profiles previously. As a Toxicologist, I most often use the health effects chapter to gain an overview of various chemicals toxicity profiles, doses, etc. I also use these documents in classroom exercises (undergraduate and graduate toxicology courses) as these are an excellent resource for understanding effects of chemicals.

RESPONSE: *No response is necessary.*

QUESTION: Are the new tables and figures clear and useful? Do they make the toxicological profile easier to read?

COMMENT: Yes.

RESPONSE: *No response is necessary.*

Unpublished Studies (presented in alphabetical order):

QUESTION: The updated chlorobenzene profile includes unpublished studies (see list of nine provided previously) that were unavailable to ATSDR when preparing the original profile. Please comment on the quality of the study, namely:

Bioassay Systems Corp. 1982. Nine reports regarding the effects of various chlorinated benzenes with cover letter dated 051183. Submitted to the U.S. Environmental Protection Agency under TSCA Section 4. OTS0511274.

E.I. Dupont. 1977. Mutagenic activity of monochlorobenzene in the Salmonella/microsome assay with cover letter dated 5/10/94 (Sanitized). Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557146.

Miles Inc. 1984. Study for skin-sensitizing effect on guinea pigs of monochlorobenzene, with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557415.

Monsanto. 1967b. 13-week oral administration - dogs: Chlorobenzene: Final report. Prepared by Hazleton Laboratories, Project No. 241-105, February 24.

Monsanto Co. 1976a. Mutagenicity evaluation of BIO-76-88 CP-5535 (LOX): Final report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557063.

Monsanto Co. 1976b. Mutagenicity evaluation of BIO-76-87 CP-5535 (LOX): Report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557065.

Monsanto Co. 1976c. Mutagenicity evaluation of BIO-76-88 CP-5535 (WGK): Final report/mutagenicity evaluation of chlorobenzene with cover letter dated 05/09/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557064.

Shell Oil Company. 1991. A Utah Biomedical Test Laboratory report on NIOSH-sponsored inhalation study for IDHL values. HSE-78-0317. Prepared by Utah Biomedical Test Laboratory for Shell Oil Company.

Zeneca Specialties. 1982. Eye irritation classification on monochlorobenzene in rabbits with cover letter dated 05/06/94. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8d. OTS0557374.

Bioassay Systems Corp 1982

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: 1) N/A - in vitro chromosomal aberration assay in Chinese hamster ovary cells.
2) N/A - Drosophila sex linked recessive lethal test - although adequate numbers were used.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: 1) N/A - in vitro chromosomal aberration assay in Chinese hamster ovary cells.
2) One endpoint measured in the Drosophila sex linked recessive lethal test is mortality.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: 1) Yes, 5 concentrations were tested, and were chosen based on the water solubility of chlorobenzene.
2) Yes, and the manner in which the concentration of chlorobenzene was selected was adequately described.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: 1) The study was adequately designed and reported.
2) The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: 1) The conclusions of this study were described as ambiguous. I agree with that conclusion as no concentration-related trends were observed, and marginal positive findings in one assay (for both activation and no activation conditions) were not repeatable.
2) I agree with the study conclusions.

RESPONSE: *No response is necessary.*

E.I. Dupont 1977

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: N/A – Mutagenicity assay (Salmonella/microsome assay)

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: N/A – Mutagenicity assay (Salmonella/microsome assay)

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree with the study conclusions.

RESPONSE: *No response is necessary.*

Miles Inc. 1984

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes, group sizes were adequate, standard animal housing conditions were provided to the guinea pigs.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: No deaths occurred in this study.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Multiple dose groups were not used, however, the skin sensitization protocol utilized various concentrations of the test chemical and appear to be a standard approach for assessing this endpoint.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree with the study conclusions.

RESPONSE: *No response is necessary.*

Monsanto Co. 1967b

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes, 8 beagle dogs (4 per sex) per each of 3 dose groups were evaluated in this 90 day study. Standard animal care practices appeared to be followed in this study.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: No, however, animal deaths occurred only in the high dose group between the 3rd and 5th weeks of the study, thus were considered to be treatment related.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes, 3 dose groups (plus control) were evaluated in this study and spanned a fairly wide range (0.025 – 0.25 ml/kg/day).

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: I agree with the study conclusions.

RESPONSE: *No response is necessary.*

Monsanto Co. 1976a

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: N/A – Mutagenicity assay (Mouse lymphoma assay)

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: N/A – Mutagenicity assay (Mouse lymphoma assay)

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree with the study conclusions.

RESPONSE: *No response is necessary.*

Monsanto Co. 1976b

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: N/A – Mutagenicity assay (bacteria and yeast assays)

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: N/A – Mutagenicity assay (bacteria and yeast assays)

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree with the study conclusions.

RESPONSE: *No response is necessary.*

Monsanto Co. 1976c

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: N/A – Mutagenicity assay (bacteria and yeast assays)

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: N/A – Mutagenicity assay (bacteria and yeast assays)

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree with the study conclusions.

RESPONSE: *No response is necessary.*

Shell Oil Co. 1991

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes, 10 animals per group (guinea pigs and SD rats) were used, both sexes were equally included. Standard laboratory animal care was provided.

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: N/A – no deaths occurred in this study.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: Yes, a sufficient number of air concentrations of chlorobenzene were evaluated.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: The study was adequately designed and reported.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: I agree with the study conclusions.

RESPONSE: *No response is necessary.*

Zeneca Specialties 1982

QUESTION: Did the study use an adequate number of animals and practice good animal care?

COMMENT: Yes, 9 albino rabbits were used and evaluated for parameters of eye irritation over a course of 7 days. Appropriate animal care was indicated

RESPONSE: *No response is necessary.*

QUESTION: Did the study account for competing causes of death?

COMMENT: No deaths occurred in this study.

RESPONSE: *No response is necessary.*

QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

COMMENT: No, only a single dose was evaluated.

RESPONSE: *No response is necessary.*

QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

COMMENT: Although only one dose was evaluated, the observation of moderate eye irritation following chlorobenzene administration was valid.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the conclusions of the author? If not, please explain.

COMMENT: Yes, I agree with the study conclusions.

RESPONSE: *No response is necessary.*