Disposition of Peer Review Comments for Toxicological Profile for Bromodichloromethane

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 Bromodichloromethane were:

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Comments provided by Peer Reviewer #1

COMMENT: The Reviewer states "The authors of this profile have done a very effective job in summarizing a large, diverse range of relevant studies concerning potential health effects of bromodichloromethane (BDCM). The human studies (my area of expertise) are characterized accurately in regard to methods, results, and interpretation. As noted, most of the health endpoints addressed in the toxicologic studies have had no corresponding human studies whatsoever, some have had a single, cursory study, and it is only in the realm of developmental endpoints that a meaningful body of human studies are available on the toxicity or BDCM. As noted below, that larger literature is also covered well, but I do have some suggestions for ways it could be enhanced.

The integrated consideration of the more extensive animal studies and more limited (in number and quality) human studies is balanced appropriately, with the balance tipped in favor of the animal studies despite the need to extrapolate findings to be applied to humans. As the authors note, the nature of BDCM exposure through drinking water disinfection by-products creates a situation in which it is difficult or may even be impossible to separate BDCM from other disinfection by-products, both other trihalomethanes and other brominated compounds. The small number of studies other than for developmental endpoints are appropriately downplayed as a major source of relevant information, so that the inferences on page 11 are guided by toxicology studies. The authors appropriately note as a research priority the need for epidemiologic studies that address the health endpoints most strongly implicated in toxicology studies (page 113, lines 3-6).

In the case of the developmental effects, the authors briefly summarize the literature and accurately characterize the studies as providing mixed evidence on fetal growth reduction in particular. The literature as a whole is not indicative of an adverse effect being present at typically encountered levels, but there are some suggestions that such effects may occur. The tone of the interpretation seems right, neither excessively dismissive nor credulous. Noting that the major methodologic limitations are due to non-differential misclassification of BDCM exposure is appropriate. This mixed evidence from epidemiologic studies is simply taken into account and integrated from the toxicology research evidence.

A strategic question in trying to make the best use of the human studies is whether there is benefit in considering studies of aggregations of disinfection by-products with some attempt to draw inferences specifically for BDCM. This comes up in the human developmental studies in which the bulk of the evidence pertains to aggregations of disinfection by-products with some secondary analysis of BDCM in particular, often without controlling for other by-products. Some indication of how these types of studies were handled should be noted, i.e., whether those that had some results for BDCM as a secondary exposure (after looking at total trihalomethanes, etc.) were selectively or comprehensively included. For example, the Hoffman et al. (2008) paper attached does provide some results specific to BDCM. If the intention is to be comprehensive, more searching of the literature on disinfection by-products in general seems necessary to find those that include subsets of results for BDCM, while recognizing that it would not change the conclusion."

RESPONSE: *The intention of the discussion of epidemiology studies was to focus on studies that provide analysis for individual trihalomethanes. The Hoffman et al. (2008) study was added to the profile.*

COMMENT: The Reviewer states "It is correctly reported that the dominant source of BDCM exposure is through drinking water treatment by-products, but it is not explained why some water supply areas have higher concentrations of brominated compounds than others. It is noted in Section 5.1, Overview (of potential for human exposure) that the bromide is present in the source water but without comment on why bromide levels vary across supplies. I believe that this reflects proximity to seawater (perhaps in the

past) but is worth noting given the predominance of this source and its uneven geographic distribution in the US and presumably around the world."

RESPONSE: *As noted in Section 5.2.2, the concentration of bromodichloromethane in chlorinated water depends on reaction conditions during the chlorination process, such as temperature, pH, bromide ion concentration in the source water, and chlorination treatment practices.*

COMMENT (page 4, line 19): The Reviewer notes "It should be noted that a shorter time to pregnancy suggests a beneficial effect, i.e., an increased in fertility among those with higher exposure to BDCM."

RESPONSE: *The text was revised to indicate that a positive association was an indicator of a shorter time to pregnancy. However, a judgement that this was a beneficial effect of bromodichloromethane exposure was not discussed by the investigators and was not added to the profile.*

COMMENT (Table 2-1): The Reviewer states "There seem to be some studies that included a look at BDCM in relation to developmental endpoints beyond those cited, e.g., Hoffman et al., 2008. While this paper and others have focused on aggregations of disinfection by-products, like a number of the other studies, it includes results specific to DBCM."

RESPONSE: *The Hoffman et al. (2008) study was added to the profile; additional studies that examined possible associations with bromodichloromethane specifically were not identified.*

COMMENT (page 61, lines 27-29): The Reviewer states "Provide relevant citation in text."

RESPONSE: *The citation (Kogevinas et al. 2010) was added.*

COMMENT (pages 73-73, Section 3.3.1): The Reviewer states "It would be useful to give a rough estimate at least of the half-life of BDCM in blood and in urine, as you have for alveolar air. They are likely quite brief as well, but perhaps urine has a bit more promise based on its half-life to be of some utility in future studies."

RESPONSE: *An estimated half-time in urine was added to the profile. An estimated blood half-time based on alveolar elimination in swimmers (Pleil and Lindstrom 1997) was added to the profile.*

Comments provided by Peer Reviewer #2:

ATSDR Charge Questions and Responses

COMMENT: The Reviewer states **"**This is an excellent profile for bromodichloromethane. The graphics are great."

RESPONSE: *ATSDR thanks the Reviewer for the compliment.*

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: The Reviewer states "Yes."

RESPONSE: *No suggested revisions.*

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 Reviewer states "Yes – yes agree that hepatic and developmental toxic effects likely to be of concern for humans; when there are multiple experiments, and multiple species showing an effect (e.g. hepatic toxicity) this is strong evidence that this is a target organ."

RESPONSE: *No suggested revisions.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain. Table 1 needs more detail as noted:

COMMENT: The Reviewer states "Table 1 needs more detail as noted: Add more details on what model the BMDL10 is derived from for both the Narotsky and Aida studies;

- note that the Aida 1992 study used microencapsulated bromodichloromethane
- the Narotsky et al 1997 study used oral gavage in corn oil; the Narotsky study used Fisher-344 rats and the Aida study used Wistar rats.

RESPONSE: *The intent of this table is to provide a list of the MRLs for bromodichloromethane. A note was added to the table referring the reader to Appendix A, which provides an in-depth discussion of the MRLs.*

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: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

QUESTION: Regarding the Health Effects in Humans Exposed Tables, Are the study details and author conclusions presented accurately?

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "yes."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "Yes - more description on doses for the Aida 1992 study should be given."

RESPONSE: *A noted was added to the LSE table that the bromodichloromethane was microencapsulated and added to the diet.*

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: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "OK - usually present in drinking water"

RESPONSE: *No suggested revisions.*

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: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "Cantor bladder study."

RESPONSE: *The Cantor et al. (1998) study evaluating the possible association between bladder cancer and total trihalomethane concentration in water was added to the profile.*

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

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "There should be a separate section on Mechanism of action. One possible mechanism of action to discuss is the formation of bromine radicals and oxidative damage. It would be expected that the C-Br would be cleaved more readily than, for example, the C-Cl bond because of lower bond energy. Bond strengths [Kcal]: C-Cl, 95; C-Br – 67). Formation of radicals can be formed and can undergo chemical reactions via Sn2 reactions.
http://www.chemguide.co.uk/mechanisms/nucsub/whatis.html

RESPONSE: *In the revised profile format, the mechanisms of action are discussed along with the health effects and/or toxicokinetics data. Mechanisms associated with bromodichloromethane metabolism are discussed in Section 3.1.3.*

QUESTION: Are the hazard identifications clear and justifiable based on ATSDR's systematic review (SR) process? (In other words, if you follow ATSDR's SR protocol from start to finish, would you come to the same hazard identification conclusions?) If not, discuss where in the process there was a deviation from the protocol.

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

QUESTION: Do you agree with the selection of endpoints that was carried forward through the SR process? If not, please indicate which endpoints you think should or should not have been included and why.

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

QUESTION: Do you agree with the SR framework as presented in Appendix B? Are there any steps that need to be revised? Please offer any suggestions to improve the utility, effectiveness, or clarity of the SR Framework

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "No."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "No."

RESPONSE: *No suggested revisions.*

QUESTION: Is there a discussion of populations at higher risk because of biological differences that make them more susceptible? Do you agree with the choices of populations?

COMMENT: The Reviewer states "Yes."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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 Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "Would suggest that a short-term toxicity studies followed by liver microarray analysis would indicate upregulation of the antioxidant pathway NrF2 - marker for oxidative damage."

RESPONSE: *A note was added to this section that measurements of biomarkers of oxidative stress such as glutathione or Nrf2 could be biomarkers of hepatotoxicity; however, they would not be specific to bromodichloromethane.*

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? If not, please clarify and add additional references.

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

QUESTION: Is information provided in Chapter 4 on the various forms of the substance? If not, please explain.

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

QUESTION: Are you aware of any information in Section 5.2 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: The Reviewer states "OK"

RESPONSE: *No suggested revisions.*

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: The Reviewer states “OK.”

RESPONSE: *No suggested revisions.*

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: The Reviewer states “OK.”

RESPONSE: *No suggested revisions.*

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: The Reviewer states “OK.”

RESPONSE: *No suggested revisions.*

QUESTION: Do you know of other studies that may fill a data gap? If so, please provide the reference.

COMMENT: The Reviewer states “No.”

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states “Yes.”

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states “Yes.”

RESPONSE: *No suggested revisions.*

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

COMMENT: The Reviewer states "OK."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "Good summary."

RESPONSE: *No suggested revisions.*

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: The Reviewer states "See above."

RESPONSE: *The Cantor et al. (1989) study suggested by the Reviewer has been added to the profile.*

Annotated Comments

COMMENT: The Reviewer suggested the addition of this reference for bladder effects: Cantor KP, Lynch CF, Hildesheim ME, et al. 1998. Drinking water source and chlorination byproducts. I. Risk of bladder cancer. Epidemiol 9:21-28.

RESPONSE: *The Cantor et al. (1998) study evaluating the possible association between bladder cancer and total trihalomethane concentration in water was added to the profile.*

COMMENT (Table 1-1): The Reviewer states "Add more details on what model the BMDL10 is derived from for both the Narotsky and Aida studies;

- note that the Aida 1992 study used microencapsulated bromodichloromethane
- the Narotsky et al 1997 study used oral gavage in corn oil; The Narotsky study used Fisher -344 rats and the Aida study used Wistar rats. "

RESPONSE: *Table 1-1 is intended to be a list of MRLs derived for bromodichloromethane. A footnote was added to the table to refer the reader to Appendix A for an in-depth discussion of MRL derivations.*

COMMENT (page 11, line 4): The Reviewer states "page 2 notes the following:

- Hepatic effects are a presumed health effect for humans
- Renal effects are a suspected health effect for humans
- Immunological effects are a suspected health effect for humans
- The data are inadequate to conclude whether reproductive effects will occur in humans
- Developmental effects are a presumed health effect for humans

This section should correspond in some way to what is said on page 2 of the report."

RESPONSE: *The introduction for Chapter 2 was revised and now includes the results of the systematic review (categorization of potential human health effects), which are presented in the Relevance to Public Health section.*

COMMENT (Table 2-1): The Reviewer states "indicate that none of the human studies were used to establish MRL values and why not—this could be put in a footnote"

RESPONSE: *Given that the basis of the MRLs is discussed at the end of the previous section, ATSDR does believe that it is necessary to include a footnote that none of these studies were used to as the basis of the MRL in health effect summary tables in Chapter 2.*

COMMENT (Table 2-2): The Reviewer states "For Table 2.2 indicate that none of these studies were used to establish MRL values – this could be put in a footnote"

RESPONSE: *See RESPONSE to previous comment.*

COMMENT (page 52, line 6): The Reviewer states "Add more details on the Aida 1992 dosing scheme"

RESPONSE: *A note was added to the LSE table that bromodichloromethane was microencapsulated and added to the diet.*

Comments provided by Peer Reviewer #3:

COMMENT 1 (Chapter 1): The Reviewer states "Agreeing with the text: The prevailing data indicate that primary source of trihalomethanes are as the byproducts of the process of chlorinating drinking water which reaches over 80% of the U.S. population. This is an inadvertent exposure as most municipalities have accepted this as a necessary safety measure to insure the removal of water-borne disease vectors. Ingestion of water or the inhalation of water vapor are the most likely routes of exposure for humans. Significant exposure by inhalation are most likely to occur only with close contact with the source of water (shower or bathing) or water vapor such as in a shower or swimming. Exposure risks to all of these categories vary according to activity, gender and duration."

RESPONSE: *No suggested revisions.*

COMMENT 2 (Chapter 1): The Reviewer states "Common adverse effects in animals and humans are expected in most cases: Both humans and non-human vertebrates are likely to have the same adverse effects to toxic exposures. For example, it would be expected that adverse effects on sperm for most mammals as sperm motility is generally universal. Non-vertebrates could have different routes of exposure, target of toxicity and sensitivity to exposures due to differences in physiology. All adverse effects observed in animals should be considered as possible hazard risks for human unless there is data to indicate otherwise. On the other hand humans have targets of toxicity that are not present on non-primate models so the reverse is not true.

RESPONSE: *No suggested revisions.*

COMMENT (Chapter 1): The Reviewer states "Exposure conditions and targets vary: As a relatively small molecule the trichloromethanes are likely removed from circulation quickly and immediate direct measures of blood concentration or the accumulation in urine are currently the best indicators of effective exposures. Mean blood levels resulting from most exposures are relatively low although the levels obtained by transdermal exposure alone have not been determined. The animal studies attribute several adverse effects of bromodichloromethane exposure, these demonstrate clear toxic effects in several organs. Similar results from ambient exposures have not confirmed the potential for similar adverse effects in humans. Based on animal studies the suspected targets of toxicity in humans are primarily the liver and the kidney. Adverse effects on the immune system is suspected and some data indicates an adverse effect on reproduction in both humans and animal models. This report cites modest associations between exposures and adverse effects in human but it fails to mention a direct effect of bromodichloromethane on human trophoblast cells. One study (Chen et al, 2004) demonstrated that the secretion of chorionic gonadotrophin (CG) was reduced in primary cultures of human term syncytiotrophoblasts exposed to bromodichloromethane. In that study the authors extended this observation by evaluating the effects of bromodichloromethane on the morphological differentiation of mononucleated cytotrophoblast cells to multinucleated syncytiotrophoblast-like colonies. Addition of BDCM to cytotrophoblast cultures inhibited the subsequent formation of multinucleated colonies in a dose-dependent manner, as determined by immunocytochemical staining for desmosomes and nuclei. The effect was seen at BDCM concentrations between 0.02 and 2 mM and was confirmed by quantitative image analysis. Secretion of bioactive and immunoreactive chorionic gonadotropin was also significantly inhibited in a dose-dependent manner under these culture conditions, and cellular levels of CG were also reduced. Trophoblast viability was not compromised by exposure. It was concluded that bromodichloromethane disrupts syncytiotrophoblast formation and inhibits CG secretion in vitro. Although other tissue targets are not ruled out, these data substantiate a placenta target and could have implications for understanding how trichloromethanes contribute to adverse pregnancy outcomes in

humans as recently documented by Cao et al., 2016) which is in your review as well as Page-Larivier et al 2016 which is not included. These additional data may indicate a mechanism that is also associated with reduced luteinizing hormone production in the rat as indicated in this report (Beilmeier et al., 2001, 2004, 2007). "

RESPONSE: *The Chen et al. (2004) and Page-Laivier et al. (2016) studies cited by the Reviewer, as well as the Chen et al. (2003) study which also examined the effect of bromodichloromethane on chorionic gonadotrophin, were added to the profile.*

COMMENT (MRLs): The Reviewer states "MRLS as presented are generally justified: In general the MRLs reported here are adequate except for the potential risk of adverse effects to the syncytotrophoblast in the higher primate pregnancy. Since only one study suggests a mechanism of adverse effect on reproduction in the human targets of the syncytotrophoblast (Chen et al., 2004) then nonhuman primate studies would be required to address this possibility. Rodent animal models do not have a syncytotrophoblast therefore there is no relevant exposure data upon which to estimate a MRL for human pregnancies. Specifically, in the absence of an accidental high exposure to humans….particularly reproductive age and pregnant women, It is not possible to make direct comparisons between experimental high dose exposures in animal models and humans. In additional, a lack of studies using nonhuman primates, specifically on reproductive functions and development, prevents drawing firm conclusions regarding potential risks to human reproduction in general.
suggests including a brief definition of the uncertainty factor"

RESPONSE: *A data need was identified for* in vivo *studies in nonhuman primates to support the findings in the Chen et al. (2003, 2004)* in vitro *studies; these data would be useful for interpreting the results of increases in spontaneous abortions found in epidemiology studies.*

COMMENT: The Reviewer states "Additional relevant citations:

Chen J, Thirkill TL, Lohstroh PN, Bielmeier SR, Narotsky MG, Best DS, Harrison RA, Natarajan K, Pegram RA, Overstreet JW, Lasley BL**,** Douglas GC. Bromodichloromethane inhibits human placental trophoblast differentiation. Toxicol Sci. 2004 Mar; 78(1):166-74.

Pagé-Larivière F[1], Tremblay A[1], Campagna C[2], Rodriguez MJ[3], Sirard MA[4].Low concentrations of bromodichloromethane induce a toxicogenomic response in porcine embryos in vitro. Reprod Toxicol. 2016 Dec; 66:44-55. doi: 10.1016/j.reprotox.2016.09.010. Epub 2016 Sep 23.

RESPONSE: *The Chen et al. (2004) and Pagé-Lariviere et al. (2016) studies have been added to the profile.*

COMMENT (Chapter 2): The Reviewer states "This chapter provides an overall lay explanation of the real and potential health effects of bromodichloromethane exposures. This overview provides working definitions of exposure routes, durations and settings. Ample data are presented and presented as both real-world and experimental results. Animal (experimental) and human (observation) data are presented but insufficient explanations are given to explain the potential difference of these two kinds of investigations in terms of the quality of data presented and strengths of the conclusions reached. More importantly a more detailed description of levels of observed adverse effects is particularly important. Lacking early in this discussion is any explanation how these levels in rodent models are translated into potential health effects in humans by extrapolation although it does appear later. More importantly, no

explanation is currently provided to explain why controlled animal experiments may show clearer adverse effects compared to observational human studies that are prone to be less consistent or convincing. In short, the bullets on page 11 are cryptic and incomplete and require some amplification or editing in order for the lay reader to fully appreciate the difference between controlled, well-designed and randomized experimental results compared to population-based study results. The use of the category of "no human studies available" on page 12 (Figure 2-1) indicates a complete lack of information and that any study in humans would have been acceptable. A more appropriate terms would be "no comparable human data exist"."

RESPONSE: *It is noted that the intended reader of this chapter of the profile is the scientific community rather than the lay public. ATSDR believes that it is beyond the scope of the profile to include a discussion of differences between experimental and observational studies. However, Appendix B includes an evaluation of limitations for the human and animal studies in the form of risk of bias scores and confidence ratings. The bulleted text in the chapter introduction has been revised to provide better support for the basis of identifying potential sensitive health effects in humans. Figure 2-1 has been revised and no longer includes the statement "no human studies available."*

COMMENT (Table 2-1): The Reviewer states "Table 2-1 (pages 13-14) provides excellent summaries of most studies involving humans but the lay reader may be in conflict with the category of "no human studies available" in the previous table on page 12. This potential confusion could be reduced by inserting a short paragraph explaining the difference between observational data that demonstrate potential associations versus experimental studies that can be used to deduce an answer by showing a potential causal pathway. The studies cited are adequately described as all of them were essentially observational studies with little experimental design."

RESPONSE: *See the RESPONSE to the previous comment.*

COMMENT (Table 2-1): The Reviewer states "Study conclusions, limitation and limits: All of the studies listed in this table have multiple and serious limitations….as do all population-based studies. Furthermore the source and route of exposure vary greatly. These categorical differences in study design need to be identified and highlighted. NOAELS and LOAELS are not given for the human studies because actual dose levels were only approximated. This needs to be clarified. Study limitations for the human observational studies are usually found at the end of the discussion section of each publication are just as important as the conclusions and should be condensed and included in the table. Once again, the study by Chen et al, 2004, (see above in Chapter 1) which was one of the few human studies that indicated causality was omitted."

RESPONSE: *Table 2-1 is designed to provide a high-level, descriptive overview of the epidemiology studies—design, relevant results, and citations. It is not intended as the sole source of epidemiological information for the chapter. A discussion of the limitations of these studies is included in the sections with epidemiology data (e.g., developmental toxicity). The Chen et al. (2004) study was added to the profile.*

COMMENT (Tables 2-2 and 2-3): The Reviewer states "Appropriateness of species: Tables 2.2 and 2.3 are excellent and present the significant exposure reports for inhalation and oral routes, respective and is generally straight-forward. However, the strain of mouse or rat (1 rabbit study) is included with no explanation as to why this study could be important. In contrast, the figures 2-2 and 2-3 are extremely difficult to interpret and if they are to be included require additional information for interpretation.

Apparently Appendix C is intended to be used as a user guide. This needs to be footnoted here and perhaps detailed paragraph might be considered to explain that strain-specific responses can sometime explain the observed differences in result. In fact, species-specific differences are presented later in the renal and immunological toxicity sections later in the text and at least one case of a strain difference is discussed. The lay reader should be prepared for these possibilities before they are confronted with them. While one rabbit study is presented there is an absence of nonhuman primate animal models in over 60 total studies reviewed. This seems inappropriate and short-sighted. Are there none…and if so, why not? This is clearly a case where the appropriate animal model has not been not used."

RESPONSE: *The animal strains are reported as part of the study description. If strain-specific differences are found, this is discussed in the text. The intent of the LSE tables is to provide the reader with relevant details of a study's designs and results. A reference to the User's Guide for the LSE tables and figures is made in the introductory boilerplate to Chapter 2. ATSDR disagrees with the Reviewer that the lay reader should be prepared for possible species differences before they are discussed. The intended audience for Chapter 2 is the scientific community who are likely aware of the possibility of species and strain differences. The Reviewer notes that it seems inappropriate and short-sighted that there are no nonhuman primate studies discussed in the profile. ATSDR did not identify nonhuman primate studies for bromodichloromethane; the Agency is unable to address the Reviewer's comment on why there are no nonhuman primate studies. It is noted that the need for nonhuman primate studies was added to the data needs discussion. The Reviewer has not supplied support for the note that this is a case where the appropriate animal model has not been used. In the absence of data to the contrary, it is ATSDR's practice to assume that the most sensitive experimental animal is an appropriate model for humans; this approach is consistent with numerous agencies assessing human health risk. No toxicity, toxicokinetic, or mechanistic data were identified to suggest that rodents are not a good model of bromodichloromethane toxicity in humans.*

COMMENT (Tables 2-2 and 2-3): The Reviewer states "Adequacy of the presentation: While dose is listed for each study in table 2-2, whether or not a dose-response was observed is not in that table. This could be added as a yes/no column. A short statement indicating why a dose-response is essential for considering exposure experimental results as positive. NOAELS and LOAELS are listed appropriately when available. It is not possible to judge why some are missing but an indication as to why they are missing could be placed in the table. Statistical methods for the animal studies are not included anywhere I the text or tables and would probably not be of value to the lay reader. In a quick review of Pub Med there seems to be subsequent publications that may be relevant, but that will always be the case."

RESPONSE: *Discussions of the dose-response relationships are provided in the text. The LSE tables include the highest NOAEL and lowest LOAEL values for a particular endpoint. If the lowest dose is a LOAEL, then NOAELs are not listed; similarly, if the highest dose is a NOAEL, then LOAEL values are not listed.*

COMMENT (Tables 2-2 and 2-3): The Reviewer states "Levels of Significance: The summaries of categorical health outcomes are presented as short summaries in the tables and are difficult to digest in one reading. For example, since death is an absolute binary end point and the exposures are categorical it seems that a table could simplify these portions of the presentation. This, in fact was done for genotoxicity (Table 2-4) and that presentation is concise and easily digested by lay readers. It can only be assumed that the text accurately summarizes the results of each individual study unless each report was reviewed separately and cross checked with table 2.2. Upon inspection they seems to conform to that criterion. Perhaps cross-referencing between these two sections would be appropriate. For convenience to the lay reader for each category of toxicity. In addition, a similar short and similarly composed

summary might be added to those sections. These summaries should include a concise listing of adverse the effects that were identified. The statistical approaches when listed seem appropriate for that particular study."

RESPONSE: *The LSE tables present a summary of the design and results for available toxicity studies discussed in Chapter 2. They are intended to support the discussion of individual health effects presented in the text of the profile. It is noted that the lay public is not the intended audience for Chapter 2; the discussion of the health effects associated with bromodichloromethane is prepared for the scientific community. Given the intended audience, ATSDR disagrees with the Reviewer that the text should be a concise listing of identified health effects. Rather, it should be a high-level discussion of available data for a given endpoint.*

COMMENT (Chapter 2): The Reviewer states "Evaluation of the text: With only a few exceptions the categorical toxicity sections are excellent and referencing back to table 2-1 as is done in the renal section should be employed as often as possible. The endocrine section on toxicity leads off with a statement that is inconsistent with the findings. There is evidence of endocrine disruption if only for luteinizing hormone and chorionic gonadotropin. Perhaps the authors do not recognize the reproductive system as part of the endocrine system. The section on developmental toxicity is perhaps the least clear. There seems to be many observational indications of adverse effects on human pregnancies with very little hard data to support the fetus as a target. However, the fetus is highly vulnerable for a number of reasons (which should be pointed out) and placental transfer could make in utero exposures higher than predicted from the maternal compartment. This section seems to be written with a wishful bias that there is no reason to suspect developmental adverse effects in humans despite there being many studies demonstrating this as fact in rodent models. This section should be rewritten."

RESPONSE: *Alterations in reproductive hormones are discussed in the Reproductive section of the profile (Section 2.16); to avoid confusion, a statement was added to the Endocrine section of the profile (Section 2.13) referring the reader to Section 2.16 for a discussion on alterations in reproductive hormone levels. ATSDR disagrees with the Reviewer's statement that Section 2.17 is written with a wishful bias that there is no reason to suspect developmental effects in humans. It is noted that the first sentence in this section that developmental toxicity is a presumed health effect of bromodichloromethane in humans. ATSDR attempted to present an unbiased discussion of the available epidemiology studies examining developmental endpoints, which needs to include a discussion of inconsistent results and study limitations.*

COMMENT (Chapter 2): The Reviewer states "Mechanisms of action: Mechanisms of toxic effect are not consistently discussed in this presentation and is not a separate topic in this draft. It is mentioned specifically only once (page 51, line 10) and this seems to be an exception compared to other parallel section. This may confuse the lay reader. It may be worth considering excerpting the objective statement (i.e., aims) of the experimental reports as these statements general include the rationale for identifying a mechanism of action."

RESPONSE: *There are limited data on the mechanism of action of bromodichloromethane. If available, mechanistic data are discussed under specific health effect sections in Chapter 2 and in the toxicokinetics section (3.1). A note to this effect was added to the introduction in Section 2.1.*

COMMENT (Chapter 2): The Reviewer states: Completeness of tables: The final tables in chapter 2 are highly informative as presented but could be even more informative if dose/duration was added to each study. The same is not true for the figures.

RESPONSE: *The dose and duration information was added to Table 2-5.*

COMMENT: The Reviewer states "Hazard Identification/Systematic Review: The hazard identifications, when included, are clear and justifiable based on ATSD's SR process. No deviations from protocol were found. I do agree with the selection of endpoints as they seemed to indicate most if not all measured endpoints. Appendix B is comprehensive and no suggested improvements are presented,

RESPONSE: *No revisions were suggested.*

COMMENT (Chapter 3): The Reviewer states "The language is appropriate for the lay reader**."**

RESPONSE: *No revisions were suggested.*

COMMENT (Toxicokinetics): The Reviewer states "The relevant published literature on the toxicokinetics of bromodichloromethane is adequately covered by this draft chapter. With the exception of potential placental transfer and assessments of accumulation of bromodichloromethane in the fetal compartment this draft is adequate to explain fate and transport of this molecule in biological systems. A missing element however is any discussion of the changes in methodology changes over time. It is noteworthy that pharmacokinetics performed in the late 1970s and early 1980s are compared directly to more recent studies. This is an oversight where examples try to show that the continued use of pharmacokinetic data of 40-50 years earlier are still relevant. A good example of this is with trichlocarban in which industry relied on insensitive methods to justify their use of potentially toxic antimicrobials and denied the results of more modern methodology. At a minimum these old, less sensitive methods should be flagged with caveats provided in the text."

RESPONSE: *It is beyond the scope of the profile to include a discussion of methodology advances over the last 30-40 years, particularly if there is no evidence to suggest an issue with older bromodichloromethane data. When available, more recent data were given more weight than older data.*

COMMENT (Section 3.1): The Reviewer states "The first five bullets list seem to capture the known exposure routs, metabolism and disappearance rates. It may be of value to explain why PK/PD cannot be performed with humans."

RESPONSE: *Although there are no human toxicokinetic data available for bromodichloromethane, toxicokinetic data are available for humans for other chemicals, much derived from experimental studies.*

COMMENT (Section 3.1.1): The Reviewer states "This reviewer has some difficult in accepting any data that are over thirty years old without discussing the difference in technology. Specifically the Smith et al. 1985 report is dated and the naive readers will not discern this if it is not brought to their attention."

RESPONSE: *Although the Smith et al. (1985) study is dated, there is no indication from other studies that the findings are invalid.*

COMMENT (Section 3.1.3): The Reviewer states "This section is well-written. As a very small molecule bromodichloromethane is less likely to have multiple catabolic pathways. This should be mentioned. However, species-specific sensitivities to metabolites could be an issue."

RESPONSE: *The metabolism of bromodichloromethane is fairly well described and ATSDR does not believe that the suggested generic statement contributes much information to the existing knowledge database.*

COMMENT (Section 3.1.4): The Reviewer states "Again a 30+ year old study is included without comment. How reliable are those data?"

RESPONSE: *There are no recent data that would allow for validation or repudiation of the older data.*

COMMENT (Section 3.1.5): The Reviewer states "This section is well written and this reviewer has not comments."

RESPONSE: *No revisions were suggested.*

COMMENT (Section 3.1.6): The Reviewer states "This is a critical aspect of this report particularly since so little human data exist. The authors should consider moving the critical aspects of this section to the introduction. The species/strain differences section seems superficial and vague as presently written considering so much emphasis will be placed on animal model data."

RESPONSE: *This section is placed after the discussion of the health effects and toxicokinetics so that the reader has some context prior to the discussion of animal to human extrapolation. In toxicological profiles, ATSDR uses the tenet that in the absence of data to the contrary, there are no species differences in the toxicity of a chemical. For chemicals in which there are toxicokinetic or mechanistic differences, the discussion of the applicability of animal data to humans is prominently discussed in the Relevance to Public Health (chapter 1), Health Effects, Toxicokinetics, and MRL derivation (Appendix A) sections of the toxicological profile.*

COMMENT (Section 3.2): The Reviewer states "Why is there no discussion of potential embryonic/fetal exposure in this section? Are there no studies reporting placental transfer? Even if not, the risk to a fetus with a naïve liver to clearly defined hepatotoxin seems to represent perhaps the most vulnerable system. This section needs to be amplified with a short section on the theoretical aspects of risks to pregnant women."

RESPONSE: *Embryo/fetal exposure was discussed in detail in Section 2.16. A reference to the observed developmental effects is included in Section 3.2.*

COMMENT (Section 3.3): The Reviewer states "This section takes license with the concept of biomarkers. Directly measuring the toxicant is hardly a biomarker. The fact that there are no true biomarkers should be indicated."

RESPONSE: *ATSDR disagrees that measurement of the parent compound in alveolar air, blood, and urine are not biomarkers of exposure for bromodichloromethane. The National Report on Human Exposure to Environmental Chemicals uses bromodichloromethane levels in blood for biomonitoring environmental exposures.*

COMMENT (Section 3.4): The Reviewer states "This is a critical section despite limited human data.

RESPONSE: *No suggested revisions.*

COMMENT (Chapter 4): The Reviewer states "This reviewer has no comment on chapter four."

RESPONSE: *No suggested revisions.*

COMMENT (Section 5.1): The Reviewer states "This section provides a comprehensive overview of known exposure sources, routes and vectors. In general these kinds of information are difficult to convey to the lay reader."

RESPONSE: *No suggested revisions.*

COMMENT (Section 5.2): The Reviewer states "Sufficient and detailed information is provided to explain sources and transport mechanisms that have the potential for human exposures. However, the one simplification that should be provided is the use of consistent units. Some of the table shift from ug/L, to ng/L to pg/mL. It is understood that the authors are simply taking the units as reported in each study and this is the appropriate procedure. However, for the sake of the lay reader, the authors should take the time and make the effort to add in parentheses the common unit value so the reader can make direct comparisons without trying to do simple mathematics in their head. In addition, some attempt should be made to link the actual exposure levels in real world situations for humans as in Table 5-12, to the LOEAL and NOEAL values that we determined by experimental studies in chapter three."

RESPONSE: *Table 5-11 was revised to use µg/L for water monitoring data; consistent units (ppb for air, water, and other food levels) were used in the other monitoring tables in Chapter 5.*

COMMENT (Tables 5.2 – 5.7): The Reviewer states "Table 5-17 reveals a consistent trend of declining blood bromodichloromethane levels in humans from 200-2001 to 2003-2004. This should be mentioned in the text and explained if possible. The extremely high concentrations that have been reported in blood and foodstuff seems to indicate the mean levels shown in table 17 may have little meaning. Therefore the range of values should also be included in all tables."

RESPONSE: *CDC (2015) does not report the range of blood bromodichloromethane levels; Table 5-17 in the profile includes the blood levels for the 50th, 75th, 90th, and 95th percentiles. Although blood bromodichloromethane levels appear to be decreasing over time, it is not known if this is a significant trend. Without information on national average bromodichloromethane levels in water and water use, it would be speculative of ATSDR to discuss possible explanations for this apparent decrease.*

COMMENT (Chapter 6): The Reviewer states "This section provides a comprehensive overview of known exposure sources, routes and vectors. In general these kinds of information are difficult to convey to the lay reader."

RESPONSE: *No suggested revisions.*

COMMENT (Section 6.1): The Reviewer states "Overview: Adequacy of the database: If human risks are the primary concern then it is clear that the current data is inadequate. This would be particularly true for human reproduction and specifically for pregnant women, early embryos and the fetus."

RESPONSE: *No suggested revisions.*

COMMENT (Section 6.1): The Reviewer states "Figure 6.1 demonstrates the major caveat in that while the primary route of exposure is inhalation only five of the seventeen target organ category in inhalation has any data. Furthermore, nearly half of the inhalation studies focus mainly on hepatic and renal toxicity while reproductive toxicity, which is a prevalent finding in the oral rat studies, has none. In some minds, particularly lay readers, this bias in study may lend itself to a bias in the interpretation in determining targets of toxicity. This reviewer is not a lay reader and the impression here is that reproductive studies are under-represented based on the animal model data available.

Dermal studies to investigate dermal toxicity is only a footnote and according to the weight of evidence dermal exposure is second only to inhalation. That leads to a bias and this also needs to be explicitly discussed. It should also be pointed out that oral exposure data is generally already biased in that the exposures in all of those studies were not comparable to real-world oral exposures by humans. True, 85% of humans may be exposed orally…but seldom at the concentrations that results in adverse effects in animal experiments and never with oil gavage which increases the toxicity.
Somewhere in the report these caveats need to be specifically discussed."

RESPONSE: *ATSDR agrees that additional research is needed on reproductive/developmental toxicity, toxicity following inhalation and dermal exposure, and observed health effects in humans exposed to background levels of bromodichloromethane; these data gaps are identified in Section 6.2. Regarding the Reviewer's comment about gavage exposure, a statement was added to the Health Effects subsection that studies are needed comparing the toxicity of different subroutes of oral exposure to drinking water exposure, the prominent route of human oral exposure. A statement was also added in the Absorption, Distribution, Metabolism, and Excretion data needs section that studies evaluating potential metabolic saturation would be useful for interpreting the applicability of high-dose animal exposures to low-dose animal studies.*

COMMENT (Section 6.2): The Reviewer states "The need for additional data for MRLs for acute, intermediate and chronic exposures is acknowledged. Whether or not adequate MRLs for humans can be achieved using only rodent models is an additional question that is not addressed in this presentation."

RESPONSE: *No suggested revisions.*

COMMENT (Section 6.2, Health Effects): The Reviewer states "As outlined, specific additional studies will be required for human hepatic and renal targets. These could be extensions of previous rodent experiments. However, this approach would not be adequate for either reproductive or development

targets. For these target the only appropriate model would be the nonhuman primate animal model for a number of reasons. Evidence that the human placenta is a specific target of bromodichloromethane is completely ignored in this report and essentially no attention is given towards placental transfer and the embryo or fetal organs as a targets of toxicity. Overall, this is the greatest deficiency of this report. The need for additional data for neonates and children and identifying LOEALs and NOEALs for children is noted. However, these needs pale in comparison for the need to determine the potential effect of bromodichloromethane on early brain development in the higher primate which is quite different than that of the rodent models."

RESPONSE: *The need for nonhuman primate studies to evaluate reproductive/developmental toxicity was added to Section 6.2. The profile also identifies the need for studies in young animals and/or children to determine if toxicodynamic differences between children and adults might influence susceptibility.*

COMMENT (Chapter 7): The Reviewer states "This chapter explains standing rules, regulations and guidelines. Table 7.1 summarizes those same facts. The lay reader may have difficult in understand the superscripts in regard to potential cancer risks. This presentation did not focus on cancer but now at the end there is an indication that there is a cancer risk. This needs to be clarified at least with a more descriptive footnote."

RESPONSE: *It is noted that the lay reader is not the intended reader of Chapter 7. The footnotes for the cancer classifications are the U.S. Environmental Protection Agency (EPA) and International Agency for Research on Cancer (IARC) definition of B2 and 2B carcinogens. Discussion of the potential carcinogenicity and National Toxicology Program (NTP), EPA, and IARC cancer classification are included in Section 2.19 of the profile.*

COMMENT (Chapter 8): The Reviewer states "See the end of chapter two for additional references."

RESPONSE: *The Chen et al. (2004) and Pagé-Larivière et al. (2016) papers were added to the profile.*

GENERAL COMMENT

COMMENT: The Reviewer states "This is a well-written and sweeping overview of the existing scientific data relevant to the potential toxicity of bromodichloromethane. The language is appropriate for the lay reader and the table and figures are generally well organized and sufficiently explained. In only a few instance was it determined that more information, or more explanation would be of benefit. In no case were there instances of errors in compiling the data or presenting them."

RESPONSE: *No revisions suggested.*

COMMENT: The Reviewer states "The weight of evidence points to three primary targets of potential toxicity in the human. These are hepatic, renal and reproductive. This third area of potential environmental risk was either overlooked or ignored. This is the most serious deficiency in this report."

RESPONSE: *ATSDR disagrees with the Reviewer that the potential reproductive toxicity of bromodichloromethane was overlooked. Some effects that could be categorized as reproductive,*

including increased risk of spontaneous abortions, were discussed in the developmental toxicity sections of the profile.

COMMENT: The Reviewer states "The complete lack of nonhuman primate experimental data in contrast to the hundreds of reports using rodent models is stunning. This is particularly troubling since: 1) the evidence for reproductive targets is clear from many reports, 2) that rodents do not have the same reproductive targets as higher primates and 3) the known vulnerability of the embryo and fetus to hazards that act through hepatic mechanisms."

RESPONSE: *The need for nonhuman primate data, particularly for interpreting the increases in spontaneous abortions reported in some epidemiology studies, was added to Section 6.2 of the profile.*