EPA Responses to Interagency Comments on the Draft IRIS Assessment for Chloroprene

August 20, 2009

This document provides EPA responses to comments from the interagency review of the draft Toxicological Review of Chloroprene (dated February, 2009). Comments were received from OMB and all comments were carefully considered as indicated in the disposition document. This "Disposition" document is accompanied by a revised draft Toxicological Review and revised Charge for external peer review of the draft chloroprene assessment.

General Comments

- In chapter 6 of the tox review a very concise summary of the quantified RfC and cancer value is presented. However we note that page x of the forward states that the goal of this section is to present major conclusions and also characterize the overall confidence in the hazard and dose response by addressing the quality of the data and related uncertainties in addition the forward states that the "discussion is intended to convey the limitations of the risk assessment…" There does not appear to be any discussion of limitations and characterization of confidence of the quantified values as one would expect. The concise summary seems to be a reiteration of the values from section 5 but no discussion on confidence and limitations is provided. Adding such a discussion would be consistent with previous draft and final assessments.

  Response

  A discussion of the limitations and characterization of confidence in the RfC and cancer values as been added to Section 6.

- There seems to be a bit of a disconnect between the discussion in chapter 4 regarding the epidemiology studies and then EPA's reliance on these studies to support the cancer classification. 4.1.1.1 is clear that in most studies exposure assessment was poor and confounding of co-exposures is low. The concern about the lack of quantitative exposure assessment is reiterated in 4.1.1.3 and elsewhere. In addition NTP, in 2004, states that the evidence for carcinogenicity in humans is "limited". Yet EPA in section 4.7 and throughout the rest of the document, states that there is a "suggestive potential for causal association" for liver cancer. Considering the weaknesses of the studies, it is unclear how EPA is getting to this finding. EPA uses this statement to support the "likely to be carcinogenic to humans" finding. It seems from the animal data alone this statement could be made, yet EPA states that the human data also provide support. The justification for this based on the limitations of the studies is not clear.

Response

The 1st introductory paragraph in the Overview section (4.1.1.1) has been revised as it could be misinterpreted to be the EPA's synopsis of the weight of evidence, but was, in fact, a brief summary of previous reviews of only a few of the available epidemiological studies. The NTP report (2005) does state that evidence for carcinogenicity in humans alone is limited, but this also only considers two (Pell, 1978; Li et al, 1989) out of the nine epidemiological studies conducted to date. EPA has addressed the collective body of evidence including an assessment of the potential impact of previous study limitations on study findings and overall weight of evidence in Sections 4.1.1.2, 4.1.1.3, and 4.7.

Based on EPA's critical review of the eight cohorts reporting liver cancer data, a consistent increased risk of liver cancer incidence or mortality was observed in most of these studies (see Section 4.7.2.1.1. for further details). In addition, despite exposure assessment limitations, there was evidence of an exposure-response relationship when quantitative exposure data were examined across different cohorts. As stated in Section 4.7, demonstration of these key tenets of causality (Consistency, Strength of Association and Biological Gradient) offer suggestive evidence of causal association between chloroprene and liver cancer in humans.

Although limited (or a lack of) quantitative exposure data in some epidemiological studies precludes the use of these findings for quantitative dose-response analysis, those studies still can be considered in the overall weight of evidence determination. While the potential for residual confounding exists in all epidemiological studies, EPA agrees with the reviewers that the potential for confounding by many of the co-exposures and other unmeasured covariates is low as has been discussed in the toxicological review.

- EPA makes an argument that chloroprene produces epoxides, as does butadiene and isoprene, therefore chloroprene is mutagenic. When the reader gets to 4.7.3.1 this comes as a surprise as the support for this finding is not clearly presented in previous sections. More thorough discussion is needed regarding the creation and role of the epoxide metabolites that EPA believes are causing the mutagenicity. The current argument seems to be stating that since chloroprene produces epoxides, it must be mutagenic as both butadiene and isoprene act through a mutagenic mode of action. This is also inconsistent with page 6-2 which states that it is difficult to ascertain mutagenic potential (please note we are not suggesting that EPA delete the sentence on 6-2, but instead clarify why EPA is so sure chloroprene is mutagenic considering the genetic toxicology database). Many, many chemicals produce epoxide intermediates, but this has never led to the general assumption that therefore the chemical is clearly acting through a mutagenic mode of action—which seems to be the basis for the EPA determination here. More clarity is needed and we are pleased to see a charge question addressing this.

Response

EPA concluded that chloroprene induces a tumors through a mutagenic mode of action based upon the following support: chloroprene's conversion into an epoxide metabolite, formation of DNA adducts, evidence that chloroprene induces in vivo (base-pair transversions in proto-oncogenes observed in chloroprene-induced tumors in mice) and in vitro (positive results in S. typhimurium base-pair substitution mutation assays) genotoxicity, and similarities in tumor sites and sensitive species compared to closely related structural analogs (i.e., butadiene and isoprene). It is not solely based on a comparison to other similar chemicals, but rather a strong weight of evidence approach that includes chemical-specific data on which to base the proposed mode of action.

Previous sections (namely Sections 3.3 and 4.5) present the specific data on which these conclusions are based. In summary:

1. Conversion into reactive epoxide metabolites: Multiple in vitro studies have been conducted that show that chloroprene is converted into a reactive epoxide metabolite, (1-chloroethenyl)oxirane in liver and lung microsomes from multiple species, including B6C3F1 mice, Wistar and F344 rats, Syrian hamsters, and humans (Bartsch et al., 1979; Himmelstein et al., 2001b, 2004a; Cottrel et al., 2001) . Limited in vivo metabolic studies support the postulated metabolic pathway for chloroprene in Wistar rats (Summer and Greim, 1980).
2. Formation of DNA and other macromolecular adducts: Munter et al. (2002) observed that (1-chloroethenyl)oxirane formed adducts when incubated with both free DNA nucleosides and double stranded calf thymus DNA. The same adduct is observed when chloroprene is incubated with DNA and deoxyguanosine, and the reaction with deoxycytidine in double stranded DNA is significant as these adducts are difficult to repair and may be implicated in mutagenesis. (1-chloroethenyl)oxirane was also observed to form adducts with hemoglobin when incubated with mice erythrocytes.
3. Observation of in vivo and in vitro mutagenicity: Tissues from lung, forestomach, and Harderian gland tumors from mice exposed to chloroprene in the NTP chronic bioassay (1998) were shown to have a higher frequency of mutations in K- and H-ras proto-oncogenes than in spontaneous occurring tumors (Stills et al., 1999, 2001). Further, there was a high correlation between K-ras mutations and loss of heterozygosity in the same chromosome in chloroprene-induced lung neoplasms in mice (Ton et al., 2007). In general, bacterial base pair substitution mutation (*Salmonella typhimurium* strains TA100 and TA 1535) assays have been positive (Willems 1980; Bartsch et al., 1979) while the bacterial frame shift (*S. typhimurium* strains TA 97 and TA 98) assays have been negative (NTP, 1998; Willems 1980; Willems 1978). The observation of positive results in bacterial base pair substitution assays is in concordance with the finding that mutations in H- and K-ras oncogenes in select neoplasms of exposed mice manifest in base pair tranversions (Sills et al., 1999, 2001).

4. Similarities in tumor profiles with structurally related chemicals for which a preponderance of evidence suggests a mutagenic mode of action (i.e., butadiene and isoprene): A comparative analysis of sites of tumor incidence in rodents exposed to chloroprene, isoprene, or butadiene revealed qualitative and quantitative concordance of the chemical's tumorigenic effects and provides further evidence of for a similar mode of action for these chemicals. (Melnick and Sills, 2001).

To more clearly delineate EPA's proposed mode of action, additional text has been added to Section 4.5 in regards to in vitro formation of epoxides in lung and liver microsomes in multiple species and the observation of mutations in proto-oncogenes in chloroprene-induced tumors.

- The peer review panel should at a minimum include experts in metabolism as well as mutagenicity in addition to other expertise.

  Response

  The peer review panel is anticipated to include experts in inhalation/respiratory toxicology, carcinogenicity, genetic toxicology, and chloroprene toxicology. Expertise in these areas, especially chloroprene toxicology, will include any requisite expertise in metabolism as it relates to chloroprene's observed toxicity in animals and humans.

Comments on the Tox Review:

- Is it typical for atrophy to be considered a lesion? It is unclear why EPA refers to atrophy in the olfactory epithelium as a lesion. Similarly, is necrosis normally referred to as a 'lesion'. To improve transparency throughout, when referring to these endpoints we suggest replacing, throughout the document, "degenerative nasal lesions" with atrophy and necrosis of the olfactory epithelium.

  Response

  A lesion is defined by Dorland's Medical Dictionary as "any pathological or traumatic discontinuity of tissue or loss of function of a part. Lesion is a broad term, including wounds, sores, ulcers, tumors, cataracts, and any other tissue damage". Both atrophy and necrosis of the olfactory epithelium are described as degenerative lesions in Pathology of the Fischer Rat: Reference and Atlas (Boorman, Eustis, Elwell, Montgomery, MacKenzie, eds, pp324-325. Academic Press, San Diego). Description of atrophy and necrosis as lesions in the toxicological review is scientifically valid and appropriate in this context.

For the purpose of deriving an RfC, both lesion types were combined into an inclusive critical endpoint called degenerative nasal lesions, and the toxicological review is clear and consistent in the characterization of this endpoint as a combination of the two lesion types. Therefore, "degenerative nasal lesions" was not replaced with "atrophy and necrosis of the olfactory epithelium" throughout the toxicological review.

- In the mode of action section, it would be informative to have discussion about the relative levels of epoxides produced, what tissues they are produced in and how this may crosswalk with the tumors seen in animals.

  Response

  A discussion of the in vitro and in vivo data regarding metabolism of chloroprene into (1-chloroethenyl)oxirane has been added to the mode of action section (Section 4.5.1) and is also included in Section 3.3. The majority of the data is derived from studies investigating metabolism in lung and liver microsomes from mice, rats, hamsters, and humans. Currently, no in vivo data are available for blood or tissue-specific epoxide concentrations. However, the metabolic profiles observed in the in vitro studies may partially explain why mice are observed to be the most sensitive species in regards to chloroprene's carcinogenicity.

- Page 4-54, seems to present data supporting the finding that the K-ras and H-ras mutations are not necessarily associated with malignant tumor formation and an expected dose-response is not seen. Some suggestions are made as to what other mutations may mean, but yet nothing is definitive. However in future sections, EPA seems to rely on this information for supporting a mutagenic mode of action. This seems inconsistent.

  Response

  The data presented in Section 4.5.1 do not support the finding that K- and H-ras mutations are not associated with malignant tumor formation. K-ras mutations were observed at much higher frequencies (80% vs. 30%) in chloroprene-induced lung tumors compared to spontaneously occurring tumors. The observed inverse dose-response relationship was specific to the predominate mutation (A*T transversion (CAA*CTA) at K-ras codon 61), which accounted for 60% of the total observed K-ras mutations in chloroprene-induced lung neoplasms. 80% (8/10) of low dose lung neoplasms and 70% (10/13) mid dose lung neoplasms had this mutation, whereas only 18% (4/22) high dose tumors were observed to have the mutation. No spontaneously occurring lung tumor was found to have this mutation. There are a number of factors that can explain the inverse-dose response relationship observed. In the lung, the lower frequencies in CTA transversions at high doses may be due non-ras mutation mechanisms of genotoxicity or carcinogenicity. Alternatively, differences in DNA-adduct formation, or adduct removal, may explain the inverse

relationship observed. Regardless, there is clear evidence that, at low to mid doses, mutations in K-ras proto-oncogenes are associated with the observed chloroprene-induced lung carcinogenesis. A discussion of these potential explanatory factors has been added to Section 4.5.1.

The predominate mutation observed in chloroprene-induced Harderian gland tumors was also an A*T transversion (CAA*CTA) at K-ras codon 61, observed in 93% (25/27) exposed animals. This particular mutation was only observed in 7% (2/27) spontaneously occurring Harderian gland neoplasms. K-ras codon 61 mutation frequencies were equally high in all dose groups (80-100%) indicating that the mutation in the K-ras oncogene codon 61 is consistently associated with chloroprene-induced Harderian gland malignancy.

EPA's reliance on this data supporting a mutagenic mode of action is scientifically justified, as there is clear and compelling evidence that chloroprene-induced carcinogenicity in the lung and Harderian gland is associated with mutations in K- and H-ras mutations.

- Page 4-65, line 28-31, this finding regarding the Marsh study seems inconsistent with language on page 4-14 where the authors concluded that their study provided no evidence of cancer risk associated with chloroprene exposures.

  Response

  The objective of the toxicological review is not to agree or disagree with study authors, but to objectively assess the epidemiological evidence in individual studies and collectively as a whole. The sentences on p. 4-65 are consistent with EPA's assessment of the Marsh et al study described on p. 4-14. In sum, a thorough review of the data indicates a suggestion of a dose-response trend (an important tenet of causality) in liver cancer incidence/mortality across various exposure metrics in that study and two others.

- Page 4-65, line 33, the discussion of the limitations seems to frame the limitations as all leading to an underestimate of risk. Does this statement also capture all the limitations regarding the lack of control for co-exposures and the poor exposure assessment in the studies? More transparent discussion of these limitations would be helpful.

  Response

  The text has been restructured to clarify that the underestimation was referring just to the healthy worker effect. EPA has discussed limitations for each epidemiological study in great detail in Section 4.1.1.2. *Individual Occupational Studies*. EPA has previously included a transparent discussion of the potential magnitude and direction of different types of bias in various studies, when this information was available. For example, in addition to the healthy worker effect discussion, please see Section

4.1.1.3 for further details regarding the potential for confounding due to smoking and vinyl chloride exposures.

- Page 4-66, line 6, at a minimum, please clarify that EPA is saying that there is a suggestive potential for a causal association. Same comment for page 4-68, line 12.

  Response

  The text was revised to state that epidemiological data are suggestive of a causal association for liver cancer following chloroprene exposure based solely on human data. This also supports the animal data and the weight of evidence decision that chloroprene is a likely human carcinogen.

- Page 4-68, line 7, please clarify which data EPA are relying upon to state that there is consistent evidence of an association with liver cancer. Did all studies show a meaningful association? Its not clear Marsh would agree with this finding. Also on line 10, EPA states that these effects are less likely to be impacted by bias. Exactly what is EPA referring to here? Please clarify.

  Response

  Please consult Table 4-11 and Sections 4.7.2.2 (paragraph Entitled *Consistency*) for further information on the assessment of consistency across epidemiological studies for liver cancer. As noted in the text "Four different studies have shown an association between chloroprene exposure and liver cancer incidence and mortality (Bulbulyan et al. 1998, 1999; Li et al., 1989; Leet and Selevan, 1982), while a fifth study showed suggestive evidence when examined in relation to detailed exposure data (Marsh et al, 2007b)". In addition, the three studies examining cumulative exposure consistently reported very large relative risk estimates for both the intermediate and high exposures groups relative to low or unexposed groups.

  The text has been clarified to explain that large relative risks (noted across several studies) are less likely to be impacted by bias.

- 4.7.2.2.2, when discussing strength of association, shouldn't EPA mention other limitations of the studies (exposure classification concerns, confounding, co-exposures) not just the healthy worker effect?

  Response

  Discussion of the limitations of the individual studies, and the potential effects of those limitations on the strength of association, as well as other determinants of causal association, have been added to Section 4.7.2.1.1 (formerly Section 4.7.2.2.).

- Page 4-71
    - line 3- does clear information exist regarding the presence of these epoxide metabolites at target sites? Is there any evidence to support this proposal? Presentation of such information would be helpful.
    - Line 8- suggest deleting line 8 as this statement is obvious and does not speak at all to the support for chloroprene acting as a mutagenic compound.
    - Line 11, the argument that because chloroprene produces epoxide intermediates, as does butadiene and isoprene, and both are carcinogenic seems like weak support for a mutagenic MOA. Does it produce the same epoxide intermediates at the same tissues as butadiene and isoprene? What can be said about similarity of the mutations produced in vitro and in vivo? A more specific discussion is needed otherwise it seems as though EPA is setting a precedent that any chemical that produces an epoxide, must be therefore be acting through a mutagenic mode of action.
    - Line 13, similarly, similarity is tumor sites and sensitive species (which is common for many chemicals that are carcinogenic) does not seem to provide strong support for an argument that there must be a mutagenic mode of action.

<u>Response</u>

There are currently no in vivo blood or tissue specific epoxide concentrations available; however, chloroprene has been shown to be metabolized to its epoxide metabolite in liver and lung microsomes in vitro in a number of species (mice, rat, hamster, and human).

The support for the proposed mutagenic mode of action for chloroprene is not solely dependent on a comparison to butadiene or isoprene: chemical specific data regarding in vitro epoxide formation, formation of macromolecule adducts, and in vivo and in vitro genotoxicity are presented throughout the document. The specific data are clearly, consistently, and exhaustively delineated in both Sections 4.5 and 4.7.3.2.

Data pertaining to tumor site concordance between chloroprene, isoprene, and butadiene is informative in support of a mutagenic mode of action. Comparison of chloroprene to these two chemicals in regard to tumor site concordance/mode-of-action is scientifically valid as all three are closely related structural analogs and exhibit similar metabolic profiles (i.e., metabolism into DNA-reactive epoxide). The similarities in sites of tumor induction between the chloroprene, butadiene, and isoprene provide further evidence (in relation to the chemical-specific data for chloroprene: DNA-adduct formation, in vivo and in vitro genotoxicity) for a similar mode of action for these chemicals (i.e., mutagenicity).

Comparison of chloroprene's tumor profile with those of butadiene and isoprene as evidence of mutagenicity is only one piece of the total weight of evidence for chloroprene's proposed mode of action, and should be considered in aggregate with

the chemical-specific data also presented (i.e., chloroprene's epoxide formation, DNA-adduct formation, and in vivo and in vitro mutagenicity).

- Page 4-74
    - line 3-6, again more specificity is needed here. Tumor profile and species sensitivity seem like very weak arguments for a finding that there is a shared mutagenic mode of action. We also note that in 2004, NTP only went as far as to say that "oxidation of epoxide intermediates has been postulated.." and NTP does not make a finding of a mutagenic mode of action.
    - line 10- in stating that the MOA applies to all tumor types, does this include thyroid tumors?
    - Line 16-17, again EPA should be specific about which epoxides in which tissues support this analogy
    - Line 23-31- Shouldn't EPA also discuss the specificity of mutations as it compares to those from chloroprene- is there comparability with isoprene and butadiene?
    - Line 32, in this section EPA should be clear that dose-response concordance was not seen and is not consistent with the dose-response seen for tumors.

<u>Response</u>

Again, the weight of evidence for chloroprene's proposed mode of action is not solely based on the tumor profile concordance with other epoxide forming structural analogs, but rather on a spectrum of observations including chloroprene's metabolism into an epoxide, DNA-adduct formation, in vivo and in vitro mutagenicity, and similarities in tumor induction compared to other structurally related compounds. This data is fully presented and discussed in Sections 4.5 and 4.7.3.2.

In regards to NTP stating that "oxidation of epoxide intermediates has been postulated" in their 11th Report on Carcinogens (2005), multiple studies (Himmelstein et al., 2001b; Cottrell et al., 2001; Munter et al., 2003)) have reported on chloroprene's metabolism to the reactive epoxide (1-chloroethenyl)oxirane in lung and liver microsomes from multiple species, and are appropriately cited in the toxicological review.

Also with regards to mode of action, NTP makes no determination of any mode of action. The issue of EPA's proposed mode of action for chloroprene has been appropriately and extensively supported in the toxicological review.

The mode of action is proposed to apply to all tumor types.

EPA appropriately illustrates the specificity of K- and H-ras mutations in chloroprene-, isoprene-, and butadiene-induced lung, Harderian gland, and forestomach tumors, relative to spontaneously occurring tumors.

The EPA is clear that an inverse dose-response relationship between chloroprene dose and K-ras codon 61 mutation frequency is observed. However, it is also noted that at the low dose and mid dose, 80% (8/10) and 71% (10/13), respectively, of lung neoplasms have this particular mutation. Also, nearly all analyzed Harderian gland tumors (80-100%) exhibit this mutation regardless of dose. These data illustrate that chloroprene-induced neoplasms are consistently associated with K- and H-ras mutations, although other mechanisms of lung carcinogenicity may be active at higher doses.

- Page 4-75
    - Line 20, again more specificity regarding common epoxide metabolites and the tumor locations and mutation responses would be useful.
    - Line 29- the section on early life doesn't seem to belong here under mode of action elements. If EPA keeps it, the conclusion should be clear that there are no data that exist to support an increased early life risk.

Response

See responses above.

The section of early life susceptibility is included as it directly pertains to the determination that chloroprene is proposed to have a mutagenic mode of action and therefore increased early-life susceptibility should be assumed. EPA is clear in conveying that no chemical-specific data exist to develop separate risk estimates for childhood exposures.

- Page 4-76, line 1, its unclear what the "therefore" is referring to and what is being used to come to this conclusion- is this still part of the early life subheader? Please clarify.

Response

The paragraph is included to communicate the weight of evidence supports a mutagenic mode of action (epoxide formation, DNA reactivity, in vivo mutagenicity) and no chemical-specific early life susceptibility data exist, ADAFs should be applied in accordance with the Supplemental Guidance for Assessing Susceptibility from Early –Life Exposures to Carcinogens (US EPA, 2005).

- Page 4-77, line 1, we suggest deleting this paragraph as it is not clear it belongs here and it is completely redundant of previous language.

Response

Paragraph directly refers to the proposed mutagenic mode of action and the assumption of early life susceptibility and application of ADAFs, and is appropriate in its current location (i.e. Possible Childhood Susceptibility).

- Table 5-1 both table notes * and ** appear to be the same. Also, here and in the text, it is unclear why EPA has decided to combine the atrophy and necrosis endpoints? What is the rationale for this? More discussion is needed and a charge question regarding this approach should be considered.

  Response

  Text has been corrected to indicate that * = $p < 0.01$

  Text has been added to Section 5.2.1 explaining why atrophy and necrosis were combined into one endpoint. This issue has been incorporated into the second charge question under the RfC section.

- Page 5-5, EPA should clarify that they are treating these effects as portal of entry effects. If this is not the case (unclear whether extrathoracic is the same thing) please clarify why not. In addition a charge question should be added regarding the adjustment factor approach.

  Response

  Clarification has been added to text. A charge question regarding the adjustment factor is not necessary, it is standard EPA policy to use dosimetric adjustment factors (DAFs) in the absence of a PBPK model.

- Table 5-2.
  - Should this also present AIC values as they were used as a decision parameter?
  - Footnote h states that high dose groups were not used for necrosis and atrophy. One would think that before model is chosen for use, a decision would be made as to the quality of data that should be used and which data points are valid. It seems that if all the data are relevant and there are no concerns with quality or outliers, it is unclear why EPA would throw out the high-dose group simply to improve model fit. If throwing out the data then change how the model fit, doesn't this mean that the data were having an impact on the shape and slope of the curve? Thus it is unclear how EPA could make the statement that the data are not informative to the shape of the dose response curve. Wouldn't these data be very relevant to shape and slope of the curve? We would suggest that EPA not throw out relevant data to make the model fit. If the model does not fit, using all

data, then EPA should not use the model, and if need be a NOAEL approach should be used.

Response

AICs are provided in the tables in Appendix B for comparison purposes between different models for the same endpoint. It is unnecessary to provide them in Table 5-2 because the table's intent is not to compare between models. Providing the chi-squared and p-value for the chosen models in 5-2 is informative, as they give an indication of individual model fit for individual endpoints.

In regard to dropping the high dose group, according to EPA Technical Guidance:

> A simpler and sometimes advisable approach to use when none of the available models provide an adequate fit is to omit the data at the highest dose and refit the models to the remaining data". The rationale for eliminating data at the highest dose as opposed to lower doses is that the data at the highest dose should be the least informative of responses in the lower dose region of interest (i.e., near the BMR). For example, different modes of action may predominate in the different regions of response. The process of eliminating the data at the highest dose can be repeated until an adequate fit is obtained.

All models were run against the full datasets for the endpoints indicated and no appropriate model fit was obtained (i.e. p-value > 0.10). Therefore, according to the above technical guidance, the high dose group was dropped and the models were rerun, this time with some models achieving adequate fit. The high dose group is informative to the shape and slope of the dose response curve, but as is stated above, it is the least informative of responses in the area of interest (i.e. the low dose region of the curve). The wording of footnote h was not intended to indicate that the high dose group is not informative to the over all shape and slope of the dose response curve. In order to improve clarity, footnote h was edited to read: High dose group was dropped in order to obtain adequate model fit.

- Page 5-7, please clarify that the UF's are default values. Also for the database deficiency factor, it is not clear why EPA is choosing 3x over 10x when EPA cites the lack of a two-gen study as a major limitation. Please clarify.

Response

Where a default uncertainty factor was applied, it has been noted in the text.

EPA has determined that the database for chloroprene is sufficiently strong to not warrant the application of a 10-fold database UF. The lack of a multigenerational developmental toxicity study is a limitation in the database, therefore a 3-fold UF was applied to account for this deficiency.

- Section 5.4.4
    - Transparency would be greatly improved if EPA were to present similar findings for what unit risk would result if EPA had instead chosen the rat data rather than the most sensitive mouse.
    - It is unclear why lung tumors are treated as systemic effects, rather than portal of entry effects. EPA should clearly explain this and also present the lung data both ways—so that reviewers can transparently understand the results of this determination. It is unclear which value is used for the overall unit risk determination. In addition, EPA should add a specific charge question asking about the appropriateness of this approach.
    - Table 5-6 and 5-7, in the lung row it is unclear why the human equivalent column has 2 values.
    - Page 5-19, line 13, more clarity is needed regarding why EPA states 10^{-2} is the lowest risk necessary. This section is confusing as it seems that EPA is on one hand not following the cancer guidelines and on the other hand it is the recommended approach. Why would it appear to differ if it is recommended?
    - Page 5-20 line 7, this paragraph should discuss the values with lung tumors treated both as a systemic risk and not as a systemic risk. Should also mention what the finding would have been if rats were used. Also it is not clear why the sensitivity analysis is not included. Why not provide this as an appendix?

<u>Response</u>

It is not always straightforward to judge which species/sex combination demonstrates the most sensitive response without modeling all of the relevant data. However, in this case, EPA determined there was no need to model the rat data for that determination due to the higher tumor rates in mice, shorter latency in mice, and greater variety of affected sites including rarer tumor types. Since it is unknown which of the tested species is more predictive of human cancer risk from exposure to chloroprene, use of the most sensitive response follows from the cancer guidelines.

Further text has been added to Section 5.4.3 explaining the rationale for treating lung tumors as both portal-of-entry and systemic lesions for the purpose to deriving human equivalent concentrations. The derivation results for both approaches (i.e., calculation of HEC BMD/BMDL values and unit risks) for lung tumors are presented in Tables 5-6 and 5-7 and footnote f explains that values in italics indicate BMDs/BMDLs when lung tumors are treated as systemic lesions. The text in Section 5.4.4 states which dosimetric adjustment was used when presenting the overall risk values. A charge question regarding the treatment of lung tumors alternatively as systemic or portal-of-entry has been added.

The cancer guidelines do not cover several topics in enough detail. The sensitivity analysis mentioned in the same paragraph of the ToxReview as cited here, and reported with the results of combining risks across sites, indicated that for these data there was no incompatibility of the risk combining procedure with the low-dose extrapolation method recommended by the cancer guidelines.

The paragraph immediately after the 2 paragraphs detailing the derivation results for female and male mice tumor data discusses what the results for the overall risk would have been had lung tumors been treated as portal-of-entry effects. See response above concerning the rat data. Regarding the sensitivity analysis, it would extend an already long document without providing significant information beyond the result already reported.

- Page 5-21
    - Line 5 please clarify in the text that 'as appropriate' means that it should be based on actual exposure data
    - Line 21, clarify that the assumption is constant exposure from birth to 70 years

<u>Response</u>

Use of "as appropriate" is confusing. Text has been deleted.

Text has been added clarifying the assumption is constant exposure from birth to 70 years

- Table 5-8 (and similar changes in the text on page 5-23)
    - Why wouldn't variability either increase or decrease?
    - Under species/gender- please clarify that EPA used the most sensitive species and gender
    - Please also talk about the uncertainty regarding whether lung tumors are portal or entry or systemic effects. It may also be important to note that epi studies do not show lung cancer effects, thus this may be a portal of entry effect.

<u>Response</u>

Human population variability could increase or decrease the low-dose risk estimate, Table 5-8 has been edited to indicate as such.

Clarifying text has been added indicating that EPA used the most sensitive species and gender (female mice).

A discussion of the uncertainty in treating lung tumors alternatively as portal-of-entry or systemic effects has been added. Although an increased risk of lung cancer

incidence and mortality was observed in several epidemiologic studies, there was inadequate evidence to make a determination of the carcinogenic potential of chloroprene with regards to increased lung cancer in humans. Regardless, it is unclear how this would indicate that the lung tumor effects in mice are definitively portal-of-entry effects.

- Page 5-23, lines 1-3 is unclear, please clarify. Why would presuming concordance support the relevance of one rodent species for the other?

  Response

  Sentence was edited to clarify that while the observation of concordance between rodent species and humans was observed for liver tumors, lack of other site-specific concordance does not diminish concern for human carcinogenicity beyond liver tumors.

- Page 6-4, this should discuss the uncertainty regarding treating lung tumors as a systemic effect. Also line 31-33 is confusing- please clarify as intent is unclear.

  Response

  A discussion of the specific uncertainties surrounding treating lung tumors as systemic lesions would be redundant of previous sections. Therefore, the text has been edited to reference Section 5.4.3 and 5.4.7 for a more detailed and complete discussion of the uncertainties surrounding this issue. Text on lines 31-33 has been deleted.

- Appendix B- we do not see any values in bold as mentioned on page B-1. Please also present a summary of the rat BMD modeling for comparison to see what difference the choice of species makes.

  Response

  The best fitting model for individual endpoints has been highlighted by bold text as mentioned on page B-1.

  As indicated in Section 5.2.1., all portal-of-entry and systemic nonneoplastic lesions that were statistically significantly increased at the lowest exposure concentration (12.8 ppm) were considered candidates for the critical effect and analyzed using BMD methods. There was often no species concordance in type of lesions observed during histopathological analyses. For example, histiocytic cell infiltration was observed in the lung of female mice, but not in rats. Olfactory effects were seen in both species, but only at the low dose in rats. Olfactory effects in mice were only statistically significant at the mid- or high dose and were not analyzed using BMD. All endpoints modeled with BMD software are included in Appendix B.

Comments on the draft Charge:
(in addition to the suggestions provided in the above section)

- EPA should add a very clear charge question regarding whether, considering the limitations of the epidemiology studies, reviewers support the EPA finding of "suggestive potential for a causal association" between liver cancer and chloroprene exposure.

  Response

  The first charge question under the Carcinogenicity section should be sufficient to capture any concern the reviewers have regarding the characterization of the human epidemiology data. No further charge questions regarding the weight of evidence of the human epidemiology data are considered necessary.

- C2 - please clarify (here and elsewhere), as per comments above that the degenerative lesions are atrophy and necrosis. In the 2nd sentence here, please include a clause at the beginning of the sentence that says "Considering the severity of the endpoints." This will ensure that reviewers explicitly address the severity of the findings and how this may impact the use of the endpoint. Please also add a question about EPA's approach of combining the 2 endpoints.

  Response

  The description of degenerative nasal lesions expressly defines them as being characterized as atrophy and necrosis of the olfactory epithelium in the first sentence of charge question B2.

  The severity of the endpoint should not be a determinant in whether the choice of the endpoint is considered appropriate for the critical effect. A minimally severe endpoint is still appropriate as a critical effect as the effect could be a precursor event. The clause "Considering the severity of the endpoints" was not be added to the beginning of the second sentence.

  A second charge question pertaining to the combination of the two individual lesions into one endpoint is not considered necessary.

- C3 - please add a question regarding whether the modeling approach accurately captures any concerns the reviewers may have regarding the severity of the endpoints chosen.

  Response

  The questions pertaining to BMD modeling already included in the charge should be sufficient capture any concern the reviewers have regarding the modeling approach

used in the assessment. No further charge questions regarding modeling approaches are considered necessary.

- Section D:
    - As per comments above, please add a separate question about the epi data and their reliance as suggestive potential for a causal relationship.
    - As per comments above, after asking about the mutagenic mode of action finding, please also ask about whether the weight of evidence supports ADAF application.
    - EPA should ask about whether mice were the correct species and whether female was the appropriate choice.
    - Please also specifically ask about the treatment of lung tumors as systemic rather than solely portal of entry.

<u>Response</u>

See response above.

No charge question is necessary for application of the ADAFs. After a determination of a mutagenic mode of action, ADAFs are applied in accordance with EPA guidance (Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens, US EPA, 2005).

The question pertaining to the use of data on tumors in multiple organs in $B6C3F_1$ mice for derivation of the inhalation unit risk should be sufficient to capture any concern the reviewers have regarding choice of the most sensitive species/sex.

A charge question regarding the treatment of lung tumors alternatively as systemic and portal-of-entry has been added.