**PEER REVIEW SUMMARY FINAL REPORT**

# External Peer Review Meeting on the *Toxicological Review of 1,2,3-Trichloropropane (CAS No. 96-18-4)*

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# TABLE OF CONTENTS

I. INTRODUCTION……...1

II. CHARGE TO REVIEWERS…….....3

III. GENERAL IMPRESSIONS……7

IV. RESPONSE TO CHARGE……13

A. GENERAL CHARGE QUESTIONS…….....13

B. ORAL REFERENCE DOSE (RfD)…….37

C. INHALATION REFERENCE DOSE (RfC)……...51

D. CARCINOGENICITY…….62

V. SPECIFIC OBSERVATIONS……...86

# I. INTRODUCTION

IRIS is an EPA data base containing Agency consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances.

IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose for non-cancer health effects resulting from oral exposure (the RfD), a reference concentration for non-cancer health effects resulting from inhalation exposure (the RfC), and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program developed *Toxicological Review of 1,2,3-Trichloropropane*, an assessment of which is currently available on the IRIS data base. 1,2,3-Trichloropropane was nominated for IRIS reassessment in 2002, by EPA's National Center for Environmental Assessment (NCEA) and the New Jersey Department of Environmental Protection, Division of Sciences, Research and Technology, because of the availability of significant new toxicity and carcinogenicity data. The draft document slated for the external peer review contains a chronic reference dose, a chronic reference concentration, and a quantitative cancer assessment.

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## II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of 1,2,3-trichloropropane that will appear on the Agency=s online database, the Integrated Risk Information System (IRIS). IRIS is a database of EPA=s scientific position on the human health effects that may result from exposure to various substances found in the environment. IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). There is currently no assessment on the IRIS database for the health effects associated with 1,2,3-trichloropropane exposure.

The draft health assessment includes a chronic Reference Dose (RfD) and Reference Concentration (RfC) and a carcinogenicity assessment. Below is a set of charge questions that address scientific issues in the assessment of 1,2,3-trichloropropane. Please provide detailed explanations for responses to the charge questions.

**(A) General Charge Questions**:

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of 1,2,3-trichloropropane.

3. Please discuss research that you think would be likely to reduce uncertainty in the toxicity values for future assessments of 1,2,3-trichloropropane.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

**Chemical-Specific Charge Questions:**

**(B) Oral reference dose (RfD) for 1,2,3-trichloropropane**

1. A chronic RfD for 1,2,3-trichloropropane has been derived from a 2-year oral gavage study (NTP, 1993) in rats and mice. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Increased liver weight was selected as the critical effect. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Is the rationale for this selection transparently and objectively described in the

document? Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect. Please comment on the use of increased absolute liver weight instead of relative liver weight to describe the liver weight change.

3. The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the point of departure (POD). All available models were fit to the data in both rats and mice for increased absolute and relative liver weight, increased absolute and relative kidney weight, fertility generating the 4$^{th}$ and 5$^{th}$ litter, and the number of live pups/litter in the 4$^{th}$ and 5$^{th}$ litters. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and adequately described? Is the benchmark response selected for use in deriving the POD scientifically justified and has it been transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfDs. For instance, are they scientifically justified and transparently and objectively described in the document?

5. Please comment on the transparency and scientific rationale and justification for the selection of the database uncertainty factor. Please comment on whether the application of the database uncertainty factor adequately represents the gap in oral reproductive and developmental toxicity data for 1,2,3-trichloropropane.

**(C) Inhalation reference concentration (RfC) for 1,2,3-trichloropropane**

1. A chronic RfC for 1,2,3-trichloropropane has been derived from the 13 week inhalation study (Johannsen et al., 1988) in rats. Please comment on whether the selection of this study as the principal study is scientifically justified. Is the rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Peribronchial lymphoid hyperplasia in the lungs of male rats was selected as the critical toxicological effect. Please comment on whether the selection of this critical effect has been scientifically justified. Is the rationale for this selection transparently and objectively described in the document? Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. The chronic RfC has been derived utilizing the NOAEL/LOAEL approach to define the point of departure. Please provide comments with regards to whether this is the best approach for determining the point of departure. Please identify and provide

rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfCs. For instance, are they scientifically justified and transparently and objectively described in the document?

5. EPA concluded that a database uncertainty factor of 10 was appropriate for the derivation of the RfC to account for the lack of a two-generation reproductive toxicity study and a developmental toxicity study. Please comment on whether the selection of the database uncertainty factor for the RfC is scientifically justified and has been transparently and objectively described in the document.

**(D) Carcinogenicity of 1,2,3-trichloropropane**

1. Under the EPA's 2005 *Guidelines for carcinogen risk assessment* (www.epa.gov/iris/backgr-d.htm), 1,2,3-trichloropropane is *likely to be carcinogenic to humans*. Please comment on the cancer weight of the evidence characterization. Do the available data support the conclusion that 1,2,3-trichloropropane is a likely human carcinogen? Has the scientific justification for the weight of evidence characterization been sufficiently, transparently, and objectively described? Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described?

2. Evidence indicating the mode of action of carcinogenicity of 1,2,3-trichloropropane was considered. The proposed mode of action includes bioactivation of 1,2,3-trichloropropane leading to the induction of mutations in cancer-related genes. A conclusion was reached that it is possible that this chemical is operating through a mutagenic mode of action, but the database contains limited evidence of in vivo mutagenic events that could lead to the observed cancer. Please comment on whether the weight of the scientific evidence supports this conclusion. Please comment on whether the rationale for this conclusion has been transparently and objectively described. Please comment on data available for 1,2,3-trichloropropane that may support an alternative mode of action.

3. A two-year oral gavage cancer bioassay (NTP, 1993) was selected as the principal study for the development of an oral slope factor (OSF). Please comment on the appropriateness of the selection of the principal study. Has the rationale for this choice been transparently and objectively described?

4. Data on tumors in multiple organs in F344 rats were used to estimate the oral cancer slope factor. Please comment on the scientific justification and transparency of this analysis. Please comment on the combination of etiologically similar tumor types, benign and malignant tumors of the same cell type, for quantitative purposes. Please specifically comment on EPA's inclusion of the data on forestomach tumors for

cancer quantitation in rats following the administration of 1,2,3-trichloropropane. Please comment on the estimation of a statistically appropriate upper bound on total risk (combined slope factor), which describes the risk of developing any combination of tumor types considered, and the quantitative process used to calculate the combined slope factor.

## III. GENERAL IMPRESSIONS

### *James V. Bruckner*

My general impressions are quite positive. See pertinent comments.

### *Richard J. Bull*

The toxicological review documents are generally constructed using a format that was developed more than 30 years ago, with some modification to incorporate explicit consideration of data related to pharmacokinetics and mode of action. In those 30 years, the type of information that ultimately bears on probable human risk has become more sophisticated. At their core, these documents should provide a systemic evaluation of the available data that is followed immediately by a clear interpretation of what the data indicate are likely adverse outcomes that may be associated with exposure to the chemical being considered. Then those data that inform the character of the dose-response curve as it is extrapolated to environmentally meaningful exposures need to be considered. Comparative metabolism, pharmacokinetics, and mode of action data are brought to bear on this point. These latter data need to be evaluated with the same rigor and clarity that is given to the descriptive toxicological data that forms the basis of the hazard assessment. When these data have been evaluated for their relevance and the missing pieces of information identified (e.g. have alternative modes of action been studied?), the information needs to be synthesized to provide a clear rationale for the decisions actually taken. The documents are fairly clear on the final decision, but the development of the logic supporting that decision is less than transparent. In prior IRIS documents I have reviewed (BDPEs), the structure was very awkward because it dealt with a large number of congeners that were discussed independently and probably should not have been. In the present case, however, it seems that the authors have not been properly instructed in how to incorporate information. Descriptive data are discussed repeatedly in detail and points related to mode of action seem to crop up randomly in the document. This presentation style tends to confound the substance of the mode of action arguments and, as a consequence, the path to the conclusions of the document is obscure.

It is suggested that it would be much better to summarize the conclusions of the data reviews in a coherent fashion (i.e. focusing on those results that are going to influence the final decision and why) in those sections where the data are presented and take only the major points/conclusions forward to subsequent sections. That would allow subsequent sections to concentrate on the important points needed to systematically develop the information that underpin the ultimate conclusions of the document. As the document is now constructed, the reader tends to read over the repetitive material and runs the risk of actually missing anything of importance that may be buried within it.

Some facts seem to appear spontaneously in the latter sections that actually need to be fully discussed earlier. One example of this is in the second paragraph of section 4.6.1. This paragraph belongs in metabolism section (3.3) and the discussion of carcinogenic effects, not the non-cancer effects section.

The sections on covalent binding and adduct identification also belong in the metabolism section (pp. 47-51) and then referenced in discussions of the mode of action. The fragmented discussion of metabolism in this document makes it necessary to jump back and forth between sections to develop any idea of how things fit together. The identification of DNA adducts and the dose-response characteristic of their formation is important in the development of the mutagenic mode of action argument. The document could have dealt with this issue in a more quantitative way in the mode of action section. Coupled with the genotoxicity data should be sufficient to form a conclusion that 1,2,3-TCP satisfies the criteria for a genotoxic carcinogen .

There are some conclusions in the evaluations of the oral vs. inhalation data that appear contradictory. For example liver weight data are more or less ignored with the inhalation data even though that endpoint forms the basis of the RfD derived from the oral data. It would seem reasonable to see the oral data as being confirmatory of the changes in liver weight seen with inhalation exposure and vice versa.

The other major issue is whether the cancer potency estimates are properly drawn from the rat rather than the mouse data. This is discussed in more detail in my response to the following charge questions, but the major issue is that this seems to ignore the general Agency policy of utilizing data from the most sensitive species unless there are specific data to suggest the responses of the more sensitive species are of minimal relevance to human exposures.

***Dale Hattis***

Overall, I think the proposed trichloropropane IRIS evaluation does a fair job of bringing together much of the accessible and relevant information and applies modeling tools in a reasonably appropriate evaluation. However I think the authors can and should make improvements which:

(1) strengthen their conclusions as to the mutagenic mode of action, drawing on the excellent available analogies with related compounds with similar or identical active metabolites,

(2) change the current recommendation not to apply the age-dependent adjustment factors to an affirmative recommendation to incorporate these factors in the light of a very strong likelihood of a mutagenic mode of action,

(3) incorporate indicated pharmacokinetic nonlinearities in the delivered dose of the DNA adduct forming metabolite(s) into the dosimetry used to model the cancer risk--greatly improving the fitting for both rat and mouse data and modestly increasing the assessed potency (approximately 2-3 fold), and

(4) show the modified potency estimates derived from the mouse as well as the rat data.

***Ralph L. Kodell***

I believe that the information on the toxicity of 1,2,3-TCP has been presented clearly and accurately. I think the processes used to derive the chronic RfD, chronic RfC and oral cancer

slope factor have been presented clearly, for the most part, although I have a few suggestions for adding clarity to the derivations. I disagree somewhat with the synthesis of the toxicity information and with the reasoning behind the inclusion of certain data and the exclusion of other data in deriving the RfD, RfC and oral slope factor. My responses to the charge questions detail my concerns.

In the Hazard Identification discussion, a substantial amount of text (with tables) is devoted to discussion of oral-exposure subchronic studies (pp. 10-22), which have no direct bearing on the chronic RfD. I spent a lot of time initially trying to understand and interpret the many tables of results only to realize that these data played only a supporting role. Perhaps there is a way to streamline the discussion of these results and give them less prominence. On the other hand, I think there are data that are directly relevant to the RfD, RfC and OSF that have not been given sufficient consideration in the document. I have mentioned some specifics in my responses to the charge questions.

***Harihara M. Mehendale***

The Draft document is generally well written, clear, as concise as it can be and understandable.

1. EPA's conclusion that the rodent point-of-contact tumors observed in the forestomach are not relevant to human populations is reasonable. However, the draft has not recognized the irrelevance of other tumor sites to humans. This may lead the agency to make overly conservative interpretations of the data. Many of the systemic tumor sites (zymbol, Harderian and preputial glands) lack human homologues rendering the animal tumor data irrelevant for dose-response considerations. Target organ specificity as well as lack of specificity for TCP metabolism to proximate carcinogen and sensitivity of tissues to the carcinogen cast doubt on the relevance of these data to humans.

2. How relevant are the carcinogenic data obtained from lifetime studies of TCP after administering TCP in corn oil vehicle? Similar studies of other chlorinated hydrocarbons such as chloroform and 1,2-dichloroethane (NCI 1976; NTP 1978; Kaunig et al. 1986) and comparison of the results after administering the test chemicals in corn oil and distilled water indicated production of tumors with corn oil vehicle whereas no tumors with distilled water as vehicles. Since TCP administered in distilled water produced no compound-related tumors, but did produce tumors when administered in corn oil by gavage, TCP would not be considered as a carcinogen. This may open up the possibility of setting cancer potency at the non-cancer level calculated by EPA.

3. The role of exposure vehicle (drinking water or corn oil) in producing DNA adducts and cell proliferation in BGC3F1 mice was addressed for TCP by La et al. (1996). In the forestomach DNA adduct formation was increased when mice were exposed to 6 mg TCP/kg/day) in corn oil (1,2,3-1 µmole/mole Guanine) compared to similar exposure via drinking water (86.8 µmole adducts/adduct mole Guanine). Corn oil gavage administration of TCP also increased liver and kidney tumors. While distilled water administration of the same dose did not increase cell proliferation in forestomach, glandular stomach, kidney, and liver, corn oil gavage administration of the same dose level produced significant increase in tumors in all four tissues.

4. Is the difference between vehicles used for administration of TCP really due to corn oil vs. drinking water or due to a bolus dose vs. low concentration administered? This needs to be addressed more clearly.

5. The cancer studies of TCP in rats and mice have an admittedly high (in some cases 100%) incidence if early mortality, much of which is due to point-of-contact tumors. While such findings may be helpful at screening substances for the presence of carcinogenic ability regardless of dose, they are generally regarded as of little or no use to characterize the carcinogenic potency in test species, much less to estimate dose-response relationships at far lower dose ranges that humans may experience.

A major limitation of these and similar studies is that the experimental doses exceed the "maximum tolerated dose" (MTD). For decades, authoritative bodies have recognized that to be of any value in characterizing cancer dose-response, lifetime studies need to be conducted at doses that do not overwhelm the physiological (including defense systems) capacity of the test subjects, precludes the ability to manifest carcinogenic activity later in life, and provides opportunity to describe dose-response relationships. The NTP cancer studies do not meet this criterion.

It is recognized that EPA took into account the only studies of TCP and cancer that had been reported. Perhaps, EPA had no alternative but to estimate cancer potency in the face of such flawed data, even if other governmental organizations charged with estimating unit cancer risks for chemical carcinogens might have postponed performing a risk assessment until more scientifically rigorous data became available.

I suggest describing fully the limitations of these studies in the cancer potency documentation so that users of this information may fully understand the degree of uncertainty incorporated into EPA's cancer potency estimate for TCP and take in consideration such uncertainty in formulating risk management approaches.

***Helmut Zarbl***

The present report represents the best effort attempt of the EPA to provide guidance of the human health risks associated with exposure to the compound 1,2,3-trichloropropane. The ability of the EPA to set RfD, RfC and OSL levels for human exposure represent a significant challenge given the limited amount of high quality suitable data available. In the case of oral (RfD) and inhaled (RfC) recommendations for acute toxicity, the approach of using the BMD to calculate point of departure doses and adding appropriate Uncertainty Factors was effective when the most sensitive endpoint was used. Thus the report is quite useful with regard to the noncancer endpoints.

While the classification of 1,2,3-trichloropropane as a probable human carcinogen is justified based on the weight of the evidence, the ability to recommend an OLS is hampered by the paucity of relevant data. The two-year study performed by the NTP was the only study that has an adequate sample size to attempt the derivation of an OSF. These data are less that ideal and have several problems. All tumors were induced by a high dose exposure given by gastric gavage

in corn oil. There is evidence to suggest that corn oil can synergize with carcinogens by acting as a co-carcinogen or a tumor promoter, therefore overestimating carcinogenicity. The highest of tumor frequency was at the point of contact in the forestomach, which in the presence of corn oil can lead to an overestimation of risk. Many distal tumors arose in organs (forestomach, hardarian gland, Zymbal's gland) that have no human homolog, and thereby overestimating human risk. The mouse was clearly more sensitive that the rat, but the rat was used to derive the OSF because the doses used in the mouse overshot the mark. Thus tumor incidence was close to saturation at all doses in the mouse. The decision to use the rat could lead to a significant underestimate of human risk.

There is a paucity of mechanistic and *in vivo* mutagenicity data. Thus, the assumption that the compound is a genotoxic carcinogen and the consequent default linear dose extrapolation can lead to an overestimation of human cancer risk .The decision to assume a genotoxic mode of action and use the rat data in the forestomach as the basis for deriving the OSL thus leads to a level of uncertainty that is not completely discussed in the Review. In fact the uncertainties are so large that it is unclear whether the EPA should attempt to estimate an OSL with the available data. Given the potential impact of the EPA Review, it might be best to indicate that no adequate recommendation can be made with the data available at this time.

***Lauren Zeise***

The document is reasonably well done. It would benefit by close technical editing to reduce repetition, straighten out logical inconsistencies.

The presumption that mutagenicity is only a plausible rather than a probable or likely mode of action in the face of the available evidence is not well founded. As discussed below, the evidence for a mutagenic action substantially outweighs the evidence against. Linear low dose extrapolation and the use of factors to address early in life susceptibility are both supported by the evidence.

The approach for establishing the RfD should be rethought. The RfD is based on a lower bound on the benchmark dose for the increased absolute liver weight in male rats in the NTP chronic bioassay. At the benchmark dose (3 mg/kg-d), non-cancer adverse effects were seen in the NTP study in both male and female rats (see responses to following charge questions). In addition, the majority of the animals developed treatment related tumors at the benchmark dose. Furthermore cancer risk estimated at the RfD of 0.004 mg/kg-d using the EPA potency is 2%. A further conundrum is that, had a low dose non-linear approach to dose response for the cancer endpoint been adopted, the RfD would be considerably lower. This is all to say that there is no margin of safety at exposures equivalent to the RfD, and indeed substantial risks may be incurred at that dose. This is inconsistent with the notion of an RfD as a reasonably safe level of exposure.

No unit risk is established for the inhalation route. In the absence of a value for inhalation, risks by this route will not be calculated and are in practice may be treated as zero. An uncertain estimate based on the oral study and analogies to structurally related compounds is better than no

estimate. Approaches for estimating inhalation unit risk are suggested in responses to the following charge questions.

A more thorough analysis of mutagenic potential that carefully considers study design is needed. In this analysis, studies that have been published only as abstracts should not be used unless further documentation for these studies has been assessed and the studies are found to be reliable. The Toxicological Review gives weight to several study abstracts.

The issue has been raised that cancer risk could be overestimated because the unit risk estimate is primarily influenced by the bioassay findings for forestomach tumors after gavage treatment. Gavage treatment is seen as a problem for reasons laid out in the Toxicological Review. Dale Hattis suggested corrections based on adduct findings, which appears reasonable. Some have argued that the forestomach results should not be used for potency calculation. This would raise the issue of underestimation. For structurally related compounds tested by inhalation, gavage, dermal and dietary routes, tumors are seen local to the site of compound administration, as well as at distal sites (see table included in my response to the following charge questions). Another approach would be to make the dose adjustments suggested by Dale Hattis and to also adjust for possible contribution from corn oil gavage. Quantitative across route comparisons for structurally related compounds may provide support for this approach.

A sensitivity analysis should be conducted to check the effect of assuming that all tumors are incidental on the unit risk estimate. This is clearly an incorrect assumption since the substantial early death seen in most treatment dose groups was caused by tumor.

# IV. RESPONSE TO CHARGE

## (A) General Charge Questions

***1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?***

***James V. Bruckner***

This IRIS Toxicological Review is very well written. It reflects considerable effort by some dedicated authors. The accounts of different study protocols and findings are clear and concise, yet contain key information in most cases. There are exceptions where some topics deserve more attention. There is quite a lot of redundancy, but this is apparently the result of EPA's format requirements. The accounts of the most important findings from each investigation appear to be accurate, with some omissions of pertinent information from the toxicokinetic and carcinogenesis studies. These points are addressed under III. Specific Observations. There are also some topics for which more complete rationale should be provided.

***Richard J. Bull***

In general, the most of the conclusions of the document are supported. However, the document is confusing largely because the descriptive toxicological data are repeated throughout the document. Instead of providing a scientific basis for using the previously described data to make a decision, the authors just repeat the data, positive and negative. This is most unfortunate with respect to the genotoxicity data which needs to play a critical role in supporting the probable mode of action for cancer. Since there is no explicit consideration of the quality of data the extent to which this information supports the mutagenic mode of action is not clear. Nevertheless, my general opinion is that these data and the metabolism data demonstrating formation of specific DNA adducts are critical elements that support the conclusions of this document. The importance of these data is completely obfuscated in the discussion of uncertainty. These documents need to discuss uncertainties, but the logic of the pathway leading to the final decisions needs to be made clearer.

***Dale Hattis***

Generally, yes. But I have some suggestions for modified wording in some sections of the document (see responses to following charge questions).

***Ralph L. Kodell***

The review is clear and concise. I believe that EPA has accurately, clearly and objectively represented the scientific evidence for noncancer and cancer hazard. However, I question whether the evidence has been completely and logically synthesized. In my responses to the charge questions under B, C and D, I have raised specific questions regarding the exclusion of

certain data from consideration in deriving the chronic RfD, chronic RfC and oral cancer slope factor. I have also questioned the inclusion of certain other data in deriving the oral slope factor.

***Harihara M. Mehendale***

Yes. The Toxicological Review is logical, concise, and clear. EPA's response is objective and the agency has synthesized evidence for noncancer hazard of 1,2,3-trichloropropane(TCP). For the cancer hazard, I am not sure the interspecies variation, especially between the rats, mice, and, humans with regard to certain target tissues has been adequately represented and particularly, the way to handle it in the assessment of risk. The second major concern is the difference between the results of the principal study in rodents after bolus dosing in corn oil vs. dosing in drinking water. Not only the vehicles used for TCP administration have a direct impact on the outcome, but the toxicokinetic factors governing the impact on tissues of direct contact with TCP also influence the outcome. I am concerned that these factors have been fully dealt with.

***Helmut Zarbl***

The EPA has drafted a well written Toxicological Review of 1,2,3-trichloropropane that clearly describes what data sets were used, what assumptions were made, what risk assessment models were used, and how the authors reached their conclusions. The report is somewhat redundant in parts and perhaps could be condensed somewhat and reorganized in a way that single topics are covered in a systematic way.

The EPA has for the most part selected the most appropriate, complete and well designed studies as the source of data used in their analysis. In the case of the noncancer hazard, the data sets used were more complete and the analyses used were generally adequate. The review of the data was generally appropriate and hence the conclusions reached by the EPA are the appropriate and represent the best estimates based on available data.

In the case of the cancer hazard, the EPA faced a daunting challenge. The available data are limited and the studies available are not readily translated into standard risk assessment models. In the absence of *in vivo* genotoxicity data, the EPA decided to err on the side of caution and used a non-linear dose extrapolation in its evaluation. The carcinogenicity evaluations were based studies in two rodent species (rats and mice), with the compound given at high doses as a single bolus in corn oil, and largely on point of contact tumors in a tissue that has no homolog in humans. The conclusions reached by the EPA may therefore be challenged on several grounds.

***Lauren Zeise***

The Review is logical, but not always concise. It is fairly repetitious in spots. In other spots there are omissions. The evidence for hazard is generally well represented, but could be improved.

- The presumption that mutagenicity is only a plausible rather than a probable or likely mode of action in the face of the available evidence is not well founded. As discussed below, the evidence for a mutagenic action substantially outweighs the evidence against. There is the positive in vitro mutagenicity evidence, the formation of DNA adducts, the consistency of

adduct formation with understanding of mutagenic profile, and the compound's relationship with structurally similar compounds that produce structurally similar DNA reactive metabolites via similar pathways and produce tumors local to and distant from the site of compound administration by multiple routes. While it is possible that forestomach damage resulting from gavage administration and other non-mutagenic MOAs may play contribute to its carcinogenic activity, no viable explanations have been given as to how this can lead to such an overwhelming carcinogenic response – with low latency and high incidence and in multiple tissues both local to and distant from the site of compound administration.

- The discussion of non-neoplastic lesions for observed in the chronic NTP rat studies focuses on weight effects on the kidney and liver observed at 15 months. There were a number of non-neoplastic findings that the NTP concluded were compound related. Some of these have been noted in the Toxicological Review to be along a morphological continuum for the development of neoplasm. Nonetheless, in the discussion of the non-cancer findings (e.g., pages 26 and 31) at a minimum the non-neoplastic findings emphasized by the NTP as treatment related should be noted in the Toxicological Review. For the rat this would be increased severity of nephropathy (males only), and "increased incidences of basal cell and squamous hyperplasia of the forestomach, acinar hyperplasia of the pancreas, renal tubule hyperplasia, and preputial or clitoral gland hyperplasia." For the mouse this would be "increased incidences of squamous hyperplasia of the forestomach and eosinophilic foci in the liver." A concern, as discussed in the general comments above, is that the RfD is set at a level at which there may be substantial risk.

- For the subchronic inhalation study, a number of findings are presented without statistical significance evaluation because study authors did not do the evaluation. It is suggested that EPA conduct its own statistical evaluations for cases where the results are important and of most interest but the authors have declined to do or report them.

- The identification of the Miller et al. (1987) studies as "subchronic" can be questioned. In these studies animals were only treated for 9 days. These would appear to be more appropriately characterized as "short term" following EPA (2002, *A Review of Reference Doses and Reference Concentration Processes*). The study results are of interest. Would the discussion of it be better placed in the "other studies" section, beginning on page 46?

- The NTP medaka and guppy studies, though an experimental protocol, were designed as chronic carcinogenesis studies, and are on animals. Indeed the NTP has formally issued a peer reviewed report in its carcinogenesis report series and gives it the title "NTP Technical Report on the Carcinogenesis Studies of … 1,2,3-Trichloropropane … in Guppies … and Medaka..." The subsection "Waterborne Studies" under "4.4. Other Studies" in which these studies are discussed would seem better placed as a subsection in the animal chronic carcinogenicity section. After all these are chronic carcinogenicity studies with carcinogenicity findings conducted in two animal species. Also, while not having the same weight as the standard rodent bioassay, they nonetheless provide evidence of carcinogenicity and should be noted at least briefly and considered to add to the weight of evidence. The findings for these novel studies are quite interesting and tabulation of findings could be considered. It is of interest that the bile duct tumorigenesis was seen these studies, given that

hyperplasia was seen in at this site in NTP's subchronic rodent studies. Liver tumors were also seen in these medaka and guppy studies and also in the mouse studies, and these studies were not conducted using bolus dosing.

- The discussion of structure activity relationships where it first occurs should be expanded to include additional commonalities between DBCP and, as well as other structural analogs. As shown in the table below, 1,2,3-TCP and analogs cause tumors local to compound administration as well as at distal sites. As with 1,2,3-TCP via gavage, mortality was severe for the EDB and DBCP studies and may have precluded further observations of tumors at distant sites. These findings provide support for making presumptions regarding unit risk by the inhalation route in the absence of long term inhalation studies for 1,2,3-TCP, and also support the inclusion of forestomach following gavage in the weight of the evidence.

- Table notes:

Studies are by NTP or NCI unless otherwise noted

**Bold and underline** –Indicate tumors at sites distant from that of compound administration
Parentheses – Effect elevated in treated animals but not judged clearly compound related

- Studies that are only reported in abstract and which cannot be examined because of lack of documentation should not be relied upon. The results should not be included in the report unless substantiating documentation can be obtained. In particular, unreliable data presented in sections 4.5 and 4.7 should be removed.

| Target Site for Tumorigenesis | Gavage | | | | Diet | Inhalation | | | | Dermal | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1,2,3-TCP R 3,10,30 M 6,20,6 mg/kg-d | DBCP R 15 29 M 110 210 mg/kg-d | 1,2-DBA R ~40 M 62, 107 mg/kg-d | 1,2-DCA R 47,95 MM 97,195 FM 149,299 (78 wk study) | DBCP 0.3, 1, 3 mg/kg-d (Hazelton Labs, 1977) | DBCP 0.6, 3 (ppm) | 1,2-DBA 0, 10, 40 (ppm) (NTP) | 1,2-DBA 0, 20 (ppm) (Wong et al. 1982 –rats; Stinson et al. 1981 - mus ) | 1,2-DCA R 10,40, 60 M 10,30,90 (ppm) | 1,2-DBA (only male mouse studied) Van Duuren et al. 1979 | DBCP (only female mouse studied) Van Duuren et al. 1979 |
| Forestomach | MR FR MM FM | MR FR MM FM | MR FR MM FM | MR | MR FR MM FM | | | | | | FM |
| Oral cavity | MR FR (MM) FM | | | | | MR FR | | | | | |
| Kidney | MR | | | | MR FR | | | MR | | | |
| Liver | MM FM | | FR | | MR FR MM FM | | | MR FR | FM | | |
| Pancreas | MR | | | | | | | | | | |
| Mammary gland | FR | FR | | FR FM | | FR | FR FM | FR | MR FR FM | | |
| Uterus | FM | | | FM | | | | | | | |
| Clitoral gland | FR | | | | | | | | | | |
| Zymbal's gland | MR FR | | | | | | | | | | |
| Preputial gland | MR | | | | | | | | | | |
| Harderian gland | MM FM | | | | | | | | | | |
| Subcutaneous fibro | | | | MR | | | | | MR FR | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vascular, hemangio | | MR FR | MR | MR | | | MR | MR FR | MR | | |
| Lymph node | | | | | | | MR | | MR | | |
| Mesothelioma | | | | | | | MR | | MR | | |
| Adrenal cortex | | | | | | FR | | MR FR | | | |
| Nasal cavity | | | | | | MR FR FM | MR FR FM | MM FM | | | |
| Nasal turbinates | | | | | | MR | | | | | |
| Lung | | | MM FM | MM FM | | MM FM | MM FR FM | MR | FM | MM | FM |
| Skin | | | | | | | | | | MM | FM |

***2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of 1,2,3-trichloropropane.***

***James V. Bruckner***

I did not find any additional studies that need to be considered in the health assessment of 1,2,3-trichloropropane (TCP). I have included a number of references to studies of 1,2-dibromo-3-chloropropane (DBCP), 1,2-dichloropropane and other short-chain aliphatic hydrocarbons (halocarbons) that provide information to clarify and supplement the TCP document. These references are listed alphabetically at the end of Section III of my critique.

***Richard J. Bull***

Only one article was identified in a PubMed Search for the last 10 years that was not included in the bibliography. It is probably not a critical reference, but the citation is:

Chroust, K., M. Pavlova, Z. Prokop, J. Mendel, K. Bozkova, Z. Kubat, V. Zajickova, and J. Damborsky. 2007. Quantitative structure-activity relationships for toxicity and genotoxicity of halogenated aliphatic compounds: wing spot test *of Drosophila melanogastor*. Chemosphere 67(1):152-9.

I do provide some additional references that should be referred to at various points in the document under other charge questions relating primarily to issues of mode-of-action.

***Dale Hattis***

The document mentions the similar activated episulfonium metabolites produced by ethylene dibromide and dibromochloropropane but does not give it the weight it deserves. Carcinogenesis dose response observations for these similarly acting compounds could provide a important set of comparisons for the cancer potency conclusions for TCP.

***Relevant articles for DBCP include:***

**Glutathione-mediated binding of dibromoalkanes to DNA: specificity of rat glutathione-S-transferases and dibromoalkane structure.**
**Inskeep PB, Guengerich FP.**
**Carcinogenesis. 1984 Jun;5(6):805-8.**

1,2-Dibromo-[1,2-14C]ethane was bound irreversibly to DNA when glutathione S-transferase or rat liver cytosolic components were added to incubations of calf thymus DNA and glutathione at 37 degrees C. There was no DNA binding of 1,2-dibromoethane when glutathione was absent or in incubations of DNA with microsomal proteins with or without NADPH, thus supporting the proposal that the major route of DNA binding by 1,2-dibromoethane occurs via conjugation to glutathione. In vitro binding of 1,2-dibromoethane occurred most effectively when the YaYc (or 'B') isozyme of glutathione

S-transferase was included in incubations of DNA with 1,2-dibromoethane and glutathione. Other dihaloalkanes were incubated with DNA in the presence of glutathione S-transferase and [35S]glutathione. Of these, only 1,2-dibromo-3-chloropropane and tris-(2,3-dibromopropyl)-phosphate led to significant DNA binding of [35S]glutathione. 1,2-Dibromo-3-chloro-[1,3-14C]propane was bound to DNA when glutathione and glutathione S-transferase were present. However, even higher 1,2-dibromo-3-chloropropane binding to DNA occurred when cytosol or microsomes were included in incubations without glutathione. When glutathione was added to incubations containing cytosol and 1,2-dibromo-3-chloropropane, total DNA binding was decreased. Thus, the actual amount of DNA binding by dihaloethanes in vivo may be the result of a complicated balance among the opposing roles of glutathione conjugation in detoxicating and activating processes.

**Carcinogenesis Bioassay of 1,2-Dibromo-3-chloropropane (CAS No. 96-12-8) in F344 Rats and B6C3F1 Mice (Inhalation Study).**
**National Toxicology Program.**
**Natl Toxicol Program Tech Rep Ser. 1982 Mar;206:1-174.**

1,2-Dibromo-3-chloropropane (DBCP), a contaminant (0.05%) of the flame retardant tris(2,3-dibromopropyl)phosphate, has been used primarily as a soil fumigant to control nematodes. Unlike other halogenated nematocides, DBCP can be applied to soil without damaging growing perennials. Since it is slightly soluble in water at the concentrations used (30 ppm), DBCP can be either injected directly into the soil or added to irrigation water. By 1972, an estimated 12.3 million pounds were being used annually; in 1977, a total of 832,000 pounds were used in California, mostly on grapes and tomatoes. A carcinogenesis bioassay of technical grade 1,2-dibromo-3-chloropropane (DBCP), which contained trace amounts of epichlorohydrin and 1,2-dibromoethane, was conducted by exposing groups of 50 F344 rats and B6C3F1 mice of each sex by inhalation to concentrations of 0.6 or 3.0 ppm DBCP for 6 hours per day, 5 days per week, for 76 to 103 weeks. Untreated chamber controls consisted of 50 rats and 50 mice of each sex. Surviving high-dose rats were killed at week 84. Surviving high-dose female mice and low-and high-dose male mice were killed at week 76. Low-dose rats and female mice were killed at week 104. Accelerated mortality occurred in the high-dose groups of both species. Early deaths of high-dose rats and mice were associated with respiratory tract tumors. Interference with breathing and metastasis to the brain were major contributing factors in these deaths. Among male mice, accelerated mortality occurred in low-dose and control groups as well as in the high-dose group. Urogenital infection appeared to be associated with these deaths. Carcinomas, squamous-cell carcinomas, and adenocarcinomas of the nasal cavity and squamous-cell papillomas of the tongue each occurred in high-dose male rats at incidences significantly higher than those in the corresponding controls. Adenocarcinomas, adenomas, adenomatous polyps, and squamous-cell papillomas of the nasal cavity and adenomatous polyps of the nasal turbinates occurred in low-dose male rats with significantly increased incidences relative to controls. Carcinomas and adenocarcinomas of the nasal cavity, squamous-cell papillomas of the tongue, squamous-cell papillomas and carcinomas (combined) of the pharynx, and adenomas of the adrenal cortex each occurred in high-dose female rats at

incidences significantly higher than those in the corresponding controls. Also, adenomas and squamous-cell papillomas of the nasal cavity, adenomas of the adrenal cortex, and fibroadenomas of the mammary gland were increased significantly in low-dose female rats when compared with controls. Adenocarcinomas of the nasal cavity in high-dose female mice, papillary carcinomas in low-dose female mice, and carcinomas, squamous cell carcinomas of the nasal cavity, and alveolar/bronchiolar adenomas or carcinomas of the lung in high-dose male and female mice occurred at incidences significantly higher than those in the corresponding controls. Exposure to DBCP vapor was also associated with toxic tubular nephropathy in rats and mice of either sex and with proliferative changes in the nasal mucosa, lung, and forestomach in mice. Under the conditions of this bioassay, DBCP was carcinogenic for male and female F344/N rats, including increased incidences of nasal cavity tumors and tumors of the tongue in both sexes, and cortical adenomas in the adrenal glands of females. DBCP was carcinogenic in male and female B6C3F1 mice, including increased incidences of nasal cavity tumors and lung tumors. Levels of Evidence of Carcinogenicity: Male Rats: Positive Female Rats: Positive Male Mice: Positive Female Mice: Positive Synonyms: DBCP; dibromochloropropane; Nemagon; Fumazone

***Relevant articles for Ethylene Dibromide carcinogenesis include:***

**Formation of the DNA adduct S-[2-(N7-guanyl)ethyl]glutathione from ethylene dibromide: effects of modulation of glutathione and glutathione S-transferase levels and lack of a role for sulfation.**
**Kim DH, Guengerich FP.**
**Carcinogenesis. 1990 Mar;11(3):419-24.**
**Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.**

Hepatic S-[2-(N7-guanyl)ethyl]glutathione DNA adducts were determined in several strains of rats and mice after i.p. injection of a dose of 37 mg ethylene dibromide/kg body wt. More adducts were formed in rats than in mice, while no difference was noted among strains within each species. Removal of adducts in liver DNA was relatively slow in all animals tested. On the contrary, in vitro incubation of calf thymus DNA with ethylene dibromide and either rat cytosol or mouse cytosol gave rise to similar amounts of adduct, yet mouse cytosol showed much higher glutathione (GSH) S-transferase activity toward 1-chloro-2,4-dinitrobenzene. Human cytosol also activated ethylene dibromide, with the extent of conjugation being approximately half that of rat cytosol. Pretreatment of rats with phenobarbital or beta-naphthoflavone induced GSH S-transferases but did not increase the in vivo formation of DNA adducts, suggesting that concomitant induction of cytochrome P450 might abolish the effect of induction of GSH S-transferase by increasing the oxidation of ethylene dibromide. Butylated hydroxytoluene induced GSH S-transferase and also markedly increased DNA adduct levels. Disulfiram, a known cytochrome P450 inhibitor, significantly increased the formation of DNA adducts whereas it did not affect GSH S-transferase activity. Depletion of GSH by pretreatment of rats with diethylmaleate or buthionine sulfoximine resulted in decreased in vivo DNA adduct levels and the degree of reduction was well correlated with the extent of GSH

depletion. In vitro incubation of tritiated S-(2-hydroxyethyl)GSH with calf thymus DNA in the presence of 3'-phosphoadenosine-5'-phosphosulfate and rat liver cytosol did not result in significant binding to DNA, suggesting that sulfation of the alcohol does not readily occur to add a leaving group and regenerate an episulfonium ion. These results suggest that induction of the Phase II enzyme GSH S-transferase can be detrimental in the case of ethylene dibromide and that decreases in GSH levels reduce DNA alkylation in rats.

**Direct-acting alkylating and acylating agents. DNA adduct formation, structure-activity, and carcinogenesis.**
**Van Duuren BL.**
**Ann N Y Acad Sci. 1988;534:620-34.**

**Induction of DNA repair in rat spermatocytes and hepatocytes by 1,2-dibromoethane: the role of glutathione conjugation.**
**Working PK, Smith-Oliver T, White RD, Butterworth BE.**
**Carcinogenesis. 1986 Mar;7(3):467-72.**

1,2-Dibromoethane (EDB) is a widely used industrial chemical, and a well-known mutagen and carcinogen. EDB is biotransformed either by cytochrome P450-dependent oxidation, leading to the formation of bromoacetaldehyde, or by enzyme-catalyzed conjugation with glutathione, giving rise to reactive half-sulfur mustard compounds and their derivatives. In vitro mutagenicity and DNA binding studies suggest that the latter pathway is the primary source of genotoxic metabolites from EDB. In this study we have examined EDB-induced unscheduled DNA synthesis (UDS) in F-344 rat pachytene spermatocytes and hepatocytes. EDB (10-100 microM) induced UDS in both hepatocytes and spermatocytes in vitro. In contrast, only hepatocytes exhibited UDS when isolated from rats given EDB (100 mg/kg) 2 h earlier, and only then if the compound was given i.p. rather than orally. Preincubation of hepatocytes or spermatocytes with inhibitors of cytochrome P450-mediated oxidation had no effect on EDB induction of UDS in vitro. In contrast, depletion of cellular glutathione strongly inhibited EDB-induced UDS in both cell types in vitro. Treatment of rats with 175 mg metyrapone/kg (an inhibitor of hepatic mixed-function oxidases) 1 h prior to administration of EDB in vivo had no effect on EDB-induced UDS in hepatocytes, but led to a positive UDS response to EDB in spermatocytes in vivo. This suggests that the mixed-function oxidase pathway of metabolism is the primary route of clearance of EDB and that inhibition of cytochrome P450-mediated oxidation led to a more extensive tissue distribution of the parent compound. These data also suggest that the pathway which produces genotoxic metabolites from EDB in hepatocytes and spermatocytes, in vitro and in vivo, involves the conjugation of EDB to glutathione and its subsequent metabolism.

**Glutathione-mediated binding of dibromoalkanes to DNA: specificity of rat glutathione-S-transferases and dibromoalkane structure.**
**Inskeep PB, Guengerich FP.**
**Carcinogenesis. 1984 Jun;5(6):805-8.**

1,2-Dibromo-[1,2-14C]ethane was bound irreversibly to DNA when glutathione S-transferase or rat liver cytosolic components were added to incubations of calf thymus DNA and glutathione at 37 degrees C. There was no DNA binding of 1,2-dibromoethane when glutathione was absent or in incubations of DNA with microsomal proteins with or without NADPH, thus supporting the proposal that the major route of DNA binding by 1,2-dibromoethane occurs via conjugation to glutathione. In vitro binding of 1,2-dibromoethane occurred most effectively when the YaYc (or 'B') isozyme of glutathione S-transferase was included in incubations of DNA with 1,2-dibromoethane and glutathione. Other dihaloalkanes were incubated with DNA in the presence of glutathione S-transferase and [35S]glutathione. Of these, only 1,2-dibromo-3-chloropropane and tris-(2,3-dibromopropyl)-phosphate led to significant DNA binding of [35S]glutathione. 1,2-Dibromo-3-chloro-[1,3-14C]propane was bound to DNA when glutathione and glutathione S-transferase were present. However, even higher 1,2-dibromo-3-chloropropane binding to DNA occurred when cytosol or microsomes were included in incubations without glutathione. When glutathione was added to incubations containing cytosol and 1,2-dibromo-3-chloropropane, total DNA binding was decreased. Thus, the actual amount of DNA binding by dihaloethanes in vivo may be the result of a complicated balance among the opposing roles of glutathione conjugation in detoxicating and activating processes.

**Comparative in vivo genotoxicity and acute hepatotoxicity of three 1,2-dihaloethanes.**
**Storer RD, Conolly RB.**
**Carcinogenesis. 1983 Nov;4(11):1491-4.**

Hepatic DNA damage was demonstrated by alkaline DNA unwinding/hydroxylapatite batch chromatography in male B6C3F1 mice treated with non-necrogenic doses of 1,2-dichloroethane, 1-bromo-2-chloroethane, and 1,2-dibromoethane. Intraperitoneal administration of 0.5 mmol/kg of 1-bromo-2-chloroethane and 1,2-dibromoethane produced similar levels of DNA damage. A 4-fold higher dose of 1,2-dichloroethane (2.0 mmol/kg) was required to produce a comparable effect.

**Carcinogenesis Bioassay of 1,2-Dibromoethane (CAS No. 106-93-4) in F344 Rats and B6C3F1 Mice (Inhalation Study).**
**National Toxicology Program.**
**Natl Toxicol Program Tech Rep Ser. 1982 Mar;210:1-163.**

A carcinogenesis bioassay of 1,2-dibromoethane, a widely used nematocide and leaded gasoline additive, was conducted by exposing groups of 50 F344 rats and B6C3F1 mice of each sex by inhalation to concentrations of 10 or 40 ppm of the 1,2-dibromoethane for 78-103 weeks. Untreated controls consisted of 50 rats and 50 mice of each sex exposed in chambers to ambient air. Throughout the study, mean body weights of high-dose rats and high-dose mice of either sex were lower than those of the corresponding untreated controls. Survival of the high-dose rats of either sex and of the low- and high-dose female mice was significantly shorter than that in the corresponding controls. The principal cause of early death in control and dosed male mice was ascending, suppurative urinary

tract infection that resulted in necrotic, ulcerative lesions around the urethral opening, chronic or suppurative cystitis (often with urinary tract obstruction), and ascending suppurative pyelonephritis. Carcinomas and adenocarcinomas of the nasal cavity were observed with significantly increased incidences (P<0.001) in high-dose rats of either sex relative to controls. The incidences of adenocarcinomas and adenomas of the nasal cavity were also significantly increased (P<0.001) in low-dose rats of either sex. Adenomatous polyps of the nasal cavity showed significantly increased incidence (P<0.001) in low-dose male rats. The combined incidence of alveolar/bronchiolar adenomas and carcinomas was statistically significant (P=0.024) for high-dose female rats. Hemangiosarcomas of the circulatory system (mainly spleen) and mesotheliomas of the tunica vaginalis occurred in high-dose male rats with significantly increased incidences (P<0.001) relative to controls. The incidence of fibroadenomas of the mammary gland was significantly elevated (P<0.001) in dosed female rats relative to controls. The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma were significantly increased(P<0.001) in high-dose male mice relative to controls. These tumors were also increased in high-dose female mice (P=0.007 for adenomas and P<0.001 for carcinomas). Hemangiosarcomas occurred in low- and high dose female mice at incidences significantly greater (P<0.001) than the incidence in the controls (0/50). High-dose female mice also had significantly increased incidences of subcutaneous fibrosarcomas (P<0.001) and of nasal cavity carcinomas (P=0.013). Low-dose female mice also showed a significantly increased incidence (P<0.001) of mammary gland adenocarcinomas. Exposure to 1,2-dibromoethane was also associated with hepatic necrosis and toxic nephropathy in rats of either sex, testicular degeneration in male rats, retinal degeneration in female rats, and epithelial hyperplasia of the respiratory system in mice. Under the conditions of this bioassay, 1,2-dibromoethane was carcinogenic for F344 rats, causing increased incidences of carcinomas, adenocarcinomas, adenomas of the nasal cavity, and hemangiosarcomas of the circulatory system in males and females; mesotheliomas of the tunica vaginalis and adenomatous polyps of the nasal cavity in males; and fibroadenomas of the mammary gland and alveolar/bronchiolar adenomas and carcinomas (combined) in females. 1,2-Dibromoethane was carcinogenic for B6C3F1 mice, causing alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas in males and females; and hemangiosarcomas of the circulatory system, fibrosarcomas in the subcutaneous tissue, carcinomas of the nasal cavity, and adenocarcinomas of the mammary gland in females. Levels of Evidence of Carcinogenicity: Male Rats: Positive Female Rats: Positive Male Mice: Positive Female Mice: Positive Synonyms: ethylene dibromide; EDB; ethylene bromide.

**Report on carcinogenesis bioassay of 1,2-dibromoethane (EDB).**
**[No authors listed]**
**Am Ind Hyg Assoc J. 1979 Feb;40(2):A31-5.**

In a carcinogenesis bioassay of the brominated hydrocarbon 1,2-dibromoethane (also called ethylene dibromide or EDB), a gasoline and antiknock additive and soil and grain fumigant, oral administration by stomach tube caused cancers in rats and mice. In both sexes of both species, EDB induced squamous cell carcinomas of the forestomach. Blood

vessel cancers in male rats, liver cancers in female rats, and lung cancers in male and female mice also were attributed to EDB dosage.

**Carcinogenesis in rats of combined ethylene dibromide and disulfiram.**
**Plotnick HB.**
**JAMA. 1978 Apr 21;239(16):1609.**

Also of some possible relevance for further work is our own paper reporting the modeling of local glutathione depression and recovery for another carcinogen that causes tumors in the forestomach:

**Ginsberg, G. L., Pepelko, W. E., Goble, R. L., and Hattis, D. B. "Comparison of Contact Site Cancer Potency Across Dose Routes: Case Study with Epichlorohydrin," Risk Analysis Vol. 16, pp. 667-681, 1996.**

***Ralph L. Kodell***

I cannot identify any additional studies that should be considered. However, in my specific comments, I have raised questions as to why certain studies that were discussed were not considered further for deriving the RfD, RfC and oral slope factor.

***Harihara M. Mehendale***

I am not aware of any additional studies that should be considered for cancer and non-cancer assessments for 1,2,3-trichloropropane.

***Helmut Zarbl***

As indicated above, the major deficiency in the draft report was a dependence of incomplete data sets for carcinogenicity assessment. While there is a paucity of data, the EPA did not consider all available data. Most obvious is the fact that Toxicological Review does not include an analysis of findings from a recent NTP study of 1,2,3-trichloropropane carcinogenicity studies done in fish two species of fish guppies and Medaka (National Toxicology Program Tech. Rep. Ser. 528: 1-190, 2006), which indicate liver carcinogenicity in chronic exposure studies. The latter might have been used to counter some of the arguments that can be made against using the rodent data in isolation, by providing additional evidence for liver carcinogenicity in another vertebrate species.

A major criticism of the NTP rodent carcinogenicity studies is that the compound was administered as a bolus in corn oil, which resulted in point of contact tumors. Corn oil has been shown to synergize with the test chemical to promote carcinogenesis. This argument could be addressed by carefully reviewing the histopathology of the forestomach of rodents treated with the vehicle control for any abnormalities. Absence of any lesions could assuage, while the presence of lesions could validate the argument that use of this vehicle may have influenced the data used for risk assessment.

### *Lauren Zeise*

Dermal administration of the 1,2,3-TCP metabolite 1,3-dichloroacetone resulted in skin tumors. The study is discussed in the mode of action analysis section 4.7 but also deserves mention in the animal cancer evidence section, and is a factor to consider in the weight of the evidence evaluation.

The IARC (1995) finding of the compound as group 2A should be noted in the weight of evidence discussion. In making this call it upgraded the evidence based in part on mode of action information "The metabolism of 1,2,3-trichloropropane is qualitatively similar in human and rodent microsomes. … 1,2,3-Trichloropropane is mutagenic to bacteria and to cultured mammalian cells and binds to DNA of animals treated in vivo." Similarly the National Toxicology Program's Report on Carcinogens identifies the compound as "reasonably anticipated to be a human carcinogen" based on "sufficient evidence of malignant tumor formation at multiple sites in multiple species of experimental animals." This could also be noted. [references: International Agency for Research on Cancer. 1995. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 63. Dry cleaning, Some Chlorinated Solvents and Other Industrial Chemicals, World Health Organization, IARC, Lyon; National Toxicology Program (NTP, 2007) Report on Carcinogens, Eleventh Edition; U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC.]

The WHO / IPCS opinion regarding the genotoxicity studies reported in abstract should be considered and the EPA should consider making a similar caveat if the studies remain in the document.

***3. Please discuss research that you think would be likely to reduce uncertainty in the toxicity values for future assessments of 1,2,3-trichloropropane.***

***James V. Bruckner***

Additional research, to clarify the identity and role of the metabolic pathway(s) that produce cytotoxic and/or carcinogenic metabolites of TCP, is sorely needed. Investigation of putative proximate toxicants is also necessary. The findings of Weber and Sipes (1990) raise more questions than they answer in this regard, though the results of their work published in 1992 provide some clarification. Standard metabolism and toxicokinetic experiments should be performed in mice and rats to characterize the time-courses of blood and target organ disposition of TCB. Such data could then be utilized in the future to develop a PBPK model, with which to extrapolate animal data to humans. Metabolism and binding experiments in freshly isolated cells and microsomes from rodent and human tissues should also be conducted.

Several additional investigations are needed to provide data to serve as bases for non-cancer and cancer hazard assessments of TCP. An oral developmental toxicity study is lacking, as are a multigenerational reproduction inhalation toxicity study and an inhalation cancer bioassay. It would also be a worthwhile to conduct a 2-year cancer bioassay in which mice and rats receive TCP in their drinking water. This is now the primary source of exposure of the general population. Such an exposure regimen mimics "real life" exposure much more closely than oral corn oil bolus dosing. La et al. (1996) demonstrated that TCP adduct formation and cellular proliferation are lower when mice ingest TCP in their water.

***Richard J. Bull***

There is sufficient descriptive toxicological data to characterize the most probable harmful effects of 1,2,3-TCP. These data provide a basis for estimating probable cancer risk to humans based upon default assumptions. There are virtually no data to provide quantitative insight into relative human sensitivity to this compound. Comparative characterization of its metabolism to reactive metabolites in human tissues relative to rodents and developing the descriptive information would allow more quantitative comparisons of the toxicokinetics in humans relative to the test species could provide one dataset that would improve estimates of the risk to humans who may be exposed to this compound.

The document overplays the question of whether mutations can actually be identified that are responsible for the cancer. That is not as straight-forward as suggested. Specific problems with utilizing ras-mutation spectra in this context are referred to in the following question as it was a major point in discussing uncertainties as to mode of action. Here I caution against thinking that looking at mutation spectra is necessarily as diagnostic with of a genotoxic carcinogen as implied in the document. The same mutations are found in spontaneous tumors and many more besides. One can identify potential mutation sites based on errors in replication of DNA that contains the adduct, in

vitro. That information can be used to look for changes in DNA sequences of key genes that are consistent with the error(s) seen with in vitro replication of DNA. However, other mutations will be produced by other metabolites of the compound or will arise spontaneously in the course of tumor development that makes tracking down the mutation ultimately responsible for initiating the tumor problematic. Such work is important, but at present consistency or lack of consistency of mutations produced in a single oncogene is clearly not diagnostic. The suggestion that it ras mutations, in particular, can be diagnostic places the Agency in an impossible position for proving mutagenic modes of action. This discussion requires significant modifications in several places within the document.

In the absence of actual association of a carcinogenic response to specific mutations that are produced by the chemical, one has to rely on general evidence of the compounds genotoxic properties. As indicated earlier, a major weakness of this document is that it failed to critically evaluate the available data and this undermined the ultimate conclusions of the document. For example, much of the 'negative' data cited abstracts or other poorly documented studies. With these removed from consideration, the genotoxicity data appear reasonably convincing. However, judgments need to be made relative to the validity of various tests and what they specifically suggest about probable modes of action. This means the quality of each data set needs to be evaluated, comparative concentrations/doses that were used in the tests, consideration of the probable mechanisms giving rise to a particular response and reasons sought to explain why some tests were positive and others were negative. This type of analysis will allow one to more clearly justify the documents conclusions about mode of action.

***Dale Hattis***

PBPK modeling of comparative dosimetry in animals and people; at least comparative measurement of liver metabolism/production and degradation of episulfonium intermediate. Perhaps use of carcinogenesis data for ethylene dibromide and dibromochloropropane (which produce the same or similar glutathione-derived DNA adducts) to make supplementary alternative assessments of the likely cancer potency of TCP for both mice and rats (see the references provided in response to question 2 above).

***Ralph L. Kodell***

I believe that mode of action studies could reduce the uncertainty in the toxicity values in future assessments of 1,2,3-TCP. Specifically, in vivo gene-mutation studies would be useful, as no studies have been conducted that show evidence of gene mutation.

***Harihara M. Mehendale***

I am not aware of any new research that is likely to reduce the uncertainty factors.

### *Helmut Zarbl*

The dependence of incomplete data sets for carcinogenicity assessment is the major source of uncertainty. In the absence of these data the assumptions made and the models used are subject to criticism. Therefore, risk assessment could benefit from several additional studies in the future. Rodent studies, particularly in the mouse need to be repeated to capture the correct dose range for assessing carcinogenicity. Studies should also be performed by administering the compound via a different exposure route and in the absence of confounders such as corn oil in the vehicle. Further studies are required to further evaluate species specific differences in metabolism, mechanisms of action, and genotoxicity. Moreover, studies performed in a non-rodent mammalian species model whose metabolism of the compound and recapitulates that of humans would be useful.

### *Lauren Zeise*

The current approach does not provide the basis for estimating inhalation cancer risk for 1,2,3-TCP. A small research project, limited in scope, could review the quantitative differences in oral and inhalation cancer activities of structurally similar compounds and on the basis of these findings derive a unit risk for inhalation potency. Alternatively an inhalation unit risk could be derived from the oral value based on pharmacokinetic and site of action considerations.

A variety of studies could be performed, including an inhalation cancer bioassay to further nail down the estimate, in vivo studies noted as lacking in the Toxicological Review as well as biomonitoring of exposed workers for both exposure and effect markers to address the mode of action issue.

Somewhat better statistical approaches could be developed for combining risks of multiple tumors. This would benefit the current assessment as well as future EPA assessments, but would not have a large impact on the uncertainty.

The degree that the forestomach should be included in unit risk calculations has been raised. Issues regarding continuing contact with the stomach, the possible interaction with corn oil, and irritation due to dosing – all important considerations – have been raised. One possibility proposed by some reviewers is to exclude findings in the forestomach from consideration because of possible confounding. The same arguments can be made for EDB and DBCP, and to inform this issue, route comparisons of potency on a mg/kg basis can be made for these compounds. Turning to the TD50 tables (e.g., http://potency.berkeley.edu/chempages/1%2C2-DIBROMO-3-CHLOROPROPANE.html), results for gavage and inhalation on an mg/kg basis for DBCP show similar potencies by the two routes. The analysis of Reed et al. (Health Risk Assessment of 1,2-Dibromo-3-Chloropropane (DBCP) in California Drinking Water, 1987, UC Davis), which takes into account non-linearity in the dose response relationship (in contrast to TD50 analyses) also shows results in diet, inhalation and gavage are fairly similar. A relatively small effort to systematically compare potencies by different routes would provide a better understanding of the potential for confounding by gavage

administration for these compounds. Before moving toward excluding any tumor site for the purpose of potency estimation a careful quantitative consideration of the issue should be undertaken.

***4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?***

***James V. Bruckner***

It is stated in line 13 of the 2nd paragraph of page 94 that there are numerous ADME references. The ADME and mode of action databases are sorely lacking in a number of respects. There have apparently been no oral or inhalation studies in which absorption or the time-courses of blood and tissue disposition have been delineated. Therefore, there is little information on internal or target organ doses of TCP for relevant exposures.

The uncertainty of whether to use forestomach tumor in rats needs better/more completion discussion. The utilization of mouse tumor data should also be considered and discussed.

***Richard J. Bull***

The sections on uncertainty are important additions to IRIS documents. In my view, discussion of uncertainties in these documents should not be limited to justification of the uncertainty factors assigned to points of departure (POD).

Panel members were asked to specifically comment on the usefulness of Tables 5-1 and 5-2 in the discussion of uncertainties. In my view these tables are very important to the document, but primarily for summarizing the available dose-response information for a variety of endpoints. Thus, Table 5-1 essentially supports the selection of a particular endpoint as the most sensitive. For that reason, I think it would be better placed at the end of section 4.2.1.2. On the other hand, Figure 5-2 is really more of a translation of the discussions of uncertainty into adjustments of BMDs or NOAELs by uncertainty factors. Therefore, it is more properly a summary of the conclusions reached in section 5 and belongs at the end rather than the beginning of the section. I am not certain why is necessary to repeat this as Figure 6.1. The Agency may have reasons for this placement, but I do not think it needs to be displayed twice.

The discussion of uncertainties identifies most of the key questions. An uncertainty not adequately discussed is how the lack of adequate dose-response data from the most sensitive species affected the cancer risk assessment. There are other aspects of the uncertainties that could have been more logically developed from the data that are available. The result is the application of some uncertainty factors that are not entirely justified.

The following comments, however, point to why it is important to expand the discussion of uncertainties beyond the simple derivation of uncertainty factors. Uncertainty needs to

be based on a better discussion and utilization of the information that is available on the chemical in question and with related chemicals, if chemical-specific data are limited. In the present case, there are data that provide estimates of relative rates of formation of a particular adduct with DNA at varying doses and by varying routes of administration that were not exploited to the full. As was pointed out by Dale Hattis, these data can be used to develop better insight into the dosimetric aspects of the cancer risk assessment. The uncertainties arise from the fact that there are other metabolites that are capable of adducting DNA, and their involvement in the actual carcinogenic response cannot be excluded. As is expanded upon below, there are also uncertainties as to whether mutagenicity is the primary influence on carcinogenic responses in certain target organs. This need not detract for the overall conclusion that 1,2,3-TCP is a mutagenic carcinogen. However, it may be a reason for selecting among the different target organs for purposes of low-dose extrapolation.

Extending this argument, one can point to differing capabilities among species to activate related compounds by the same general pathways (see e.g. Watanabe, K, Liberman, R.G., Skipper, P.L., Tannenbaum, S.R. and Guengerich, F.P. 2007. Analysis of DNA adduct formed in vivo in rats and mice from 1,2-dibromoethane, 1,2-dichloroethane, and dibromomethane and dichloromethane using HPLC/accelerator mass spectrometry and relevance to risk assessments. Chem. Res. Toxicol. 20(11):1594-1600) that can further inform the dosimetry issues. Note the level of adduction is lower in hepatocytes of mice than that of rats. Since mice are more sensitive than rats to liver cancer induced by 1,2,3-TCP it indicates that there are some important intrinsic pharmacodynamic differences between mice and rats relative to the hepatocarcinogenicity of this compound. This finding is hardly surprising since the hybrid mice used in the NTP bioassays are much more sensitive to liver tumor induction than the F344 rat and, for that matter, than other strains of mice.

In the end, one might find uncertainties that are quantifiable provided above and may negate in part the need to apply uncertainty factors. However, the examples illustrate that although the metabolic variables identified above are undoubtedly important in the relative sensitivity of mice and rats, it is clear that other variables are also playing a role. The sensitivity of each species in particular target organs reflects genetic and physiological differences that control the relative species/strain-sensitivity to a particular effect. Going through this exercise then provides a scientific justification for uncertainty factors even though some of the differences in species sensitivity have been quantified. The Agency tends to categorize uncertainties into certain bins – e.g. a factor of 3 for PK variability and factor of 3 for PD variation. The discussion of uncertainties should be sensitive to findings that are inconsistent with these assumptions and modify these factors accordingly, based on all the well supported data that bear on the issue. If such arguments cannot be developed, it justifies the application of the policy-driven uncertainty factors.

Treatment of mice with the low dose of 6 mg/kg resulted in maximal or near maximal yields of forestomach tumors. The dose response of rats at this tumor site did display some dose-dependency, but generally produced a lower incidence than observed in mice

even at 10 or 30 mg/kg. Therefore, mice are more uniformly sensitive at this site (but not in the oral cavity). The conversion of dose to unit surface area from mouse data will further increase the estimates of risk at low dose. The Agency needs to justify not using the most sensitive species on other grounds than pointing to inadequate data at lower doses that have less than maximal tumor responses. For example, the mouse forestomach tumors may not be appropriate for estimating risk in humans should be explored for reasons discussed further below (perhaps a difficult point to sustain as the same critical site seems to be involved in both species). An alternative would be to develop some estimate of how much the lack of low-dose data potentially biases the risk assessment for 1,2,3-TCP relative to other compounds that have been evaluated using the Agency policy of selecting the most sensitive species for estimating cancer risk. Can the conclusion be bolstered by examining relative responses of mice and rats to chemicals that are clearly related to 1,2,3-TCP (e.g. DBCP or bromodichloropropane).

The document identifies uncertainties in the database related to developmental toxicity. This is justified in part, but the document describes a fairly extensive two-generation study conducted by the oral route of administration that evaluated multiple outcomes of multiple breedings of the $F_1$ generation. These data provide pretty clear evidence that reproductive performance declines with repetitive breedings. While not necessarily the result of a genetic damage in germ cells, these data clearly informs the question relative to reproductive/developmental toxicity. An UF 10 was applied in developing the RfC. A UF of 3 would be much more credible. This is discussed further in my responses to the following charge questions.

The question of the relevance of the forestomach tumors was not fully discussed. From an editorial point of view, the paragraph should be written more carefully to make clear what is being said. For example, "The oral cavity, pharynx, and glandular stomach <u>of the human</u> are histologically….". More importantly, the potential role of hyperplasia of the forestomach epithelium in the development of cancer needs to be discussed more thoroughly. Based on the 120-day NTP study, this was ruled out for rats in this tissue. However, increased cell proliferation in the forestomach is a common <u>early finding</u> with related compounds (Ghanayem, BI, Maronpot, RR and Matthews, H.B. 1986. Association of chemically-induced forestomach cell proliferation and carcinogenesis. Cancer Lett. 32(3):271-278) and is certainly an alternative mode of action that should be considered. Is there a possibility that intubation damage is more likely in mice and may that have contributed to the apparent greater sensitivity of mice to cancer induction in this organ?

On page 113, the last sentence indicates that the lack of information linking the mode of action to the observed carcinogenicity mitigates the application of ADAFs for estimating risks associated with early-life stages. This needs explanation. The reverse would be more logical (i.e. conclusive mode of action data should mitigate the needs for applying such a factor if animals in early life stages were no more sensitive than adult animals to that mode of action). In this particular case, the data supporting the application of ADAF's based upon a mutagenic mode of action seems justified based on the Agency's cancer risk assessment guidelines. The reluctance to apply such a factor appears to be

based upon an inconsistency in the ras mutation spectra in tumors induced in the forestomach. The inappropriateness of this conclusion is discussed in the following charge questions.

Ras mutations are very common in tumors in various organs. Ras is not necessarily the target gene of 1,2,3-TCP. It has been found that the mutation spectra of ras in tumors often shifts with age irrespective of treatment (see e.g. Bull, RJ., Orner, G.A., Cheng, R.S., Stillwell, L., Stauber, A.J., Sasser, L.B., Lingohr, M.K. and Thrall, B.D. 2002. Contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene. Toxicol. Appl. Pharmacol. 182:55-65). Moreover, the activation of downstream effectors of ras signaling are frequently modified irrespective of whether there are ras-mutations or not (Kalkuhl, A., Troppmain, J., Buchmann, A., Stinchcombe, S., Bueneman, C.L., Rapp, U.R., Kaestner, I.K., and Schwarz, M. 1998. p21(Ras) downstream effectors are increased in activity or expression in mouse liver tumors but do not differentiate between RAS-mutated and RAS-wild-type lesions. Hepatology 27:1081-1088). Finally, ras-mutation spectra in mouse liver tumors have been found to be modified to a greater extent by some established non-genotoxic carcinogens than genotoxic ones (Fox, T.R., Schumann, A.M., Watanabe, P.G.,Yano, B.L., Maher, V.M. and McCormick, J.J. 1990. Mutational analysis of the H-ras oncogene in spontaneous C57BL/6 x C3H/He mouse liver tumors and tumors induced with genotoxic and non-genotoxic hepatocarcinogens. Cancer Res. 50:4014-4019; Anna, C.H., Maronpot, R.R., Pereira, M.A., Foley, J.F., Malarkey, D.E. and Anderson, M.W. 1994. ras proto-oncogene activation in dichloroacetic acid, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F1 mice. Carcinogenesis 15:22555-22611). Therefore, the absence of mutations in ras that are consistent with the one DNA adduct known to be formed from 1,2,3-TCP provides no substantive insight into the question of whether the compound is a mutagenic carcinogen or not. It just means that ras-mutations could not be linked to the treatment based on the investigations of miscoding that would be expected from <u>one</u> of the genotoxic adducts formed from the metabolism of 1,2,3-TCP in one gene that frequently becomes activated (mutated, increased copy number or activation of a ras-dependent pathway) in the course of tumor development over time.

***Dale Hattis***

This seems qualitatively reasonable, although the lack of any attempt at quantification reduces its usefulness.

***Ralph L. Kodell***

I believe that the key sources of uncertainty have been identified and characterized, and that the choices and assumptions made in the discussion of uncertainty have been transparently and objectively described. However, in response to question 4 under B and C, I have indicated disagreement with some of the uncertainty factors applied in deriving the RfD and RfC.

I agree that age-dependent adjustment factors (ADAFs) that EPA guidance recommends for carcinogens that act through a mutagenic mode of action and are assumed to convey early-life susceptibility should not be applied (p. 77). The data are not conclusive regarding the postulated mutagenic mode of action of 1,2,3-TCP.

***Harihara M. Mehendale***

The identification and characterization of uncertainty in chapters 5 and 6 of the assessment document are clear, transparent, and adequately discussed in the document.

***Helmut Zarbl***

In general, the draft report has clearly outlined sources of uncertainty and indicated how these could affect the conclusion reached by the EPA. However, in the case of cancer risk, the report fall short in providing all arguments that could be made based on the data used and the assumptions made in the analysis.

Several of the decisions with respect which studies to use and what assumptions to make could lead to serious overestimates of risk. For example, the report underestimates the potential for the vehicle used influence carcinogenicity. The decision to use the forestomach tumor incidence in the rat as the basis for assessing risk is also problematic. The argument could be made that this is a point of contact tumor that does not accurately reflect risk by more relevant routes of exposure.

On the other hand, the decision was made to base cancer risk calculations on forestomach data in the rat, even though the mouse was clearly much more sensitive. The reason for this decision was the fact that the tumor frequency in the mouse was close to saturation at the lowest dose used in the NTP study. Since the lack of a dose response precludes use of the models selected by EPA for their analysis, the decision was made to use the rat data. This decision could lead to a serious underestimate of risk. The EPA should have considered alternative modeling approaches that are less dependent on a wide dose-response range. Alternatively they could have considered introducing an addition uncertainty factor to account for the fact that the species used (rat) is clearly not the most specific.

The net effect is that the conclusion reached with respect to uncertainty at best do not accurately reflect the large uncertainty in the cancer risk, and at worst are misleading.

***Lauren Zeise***

- There is a good discussion of the uncertainties in sections 5 and 6. Clearly there is much that is not known about the MOA and pharmacokinetics in animals, let alone humans. Perhaps the document could elaborate further on the pharmacokinetic uncertainties.

- With respect to human variability, the current approach assumes that each and every one of us faces the same identical cancer risk when exposed to the same dose of compound, without considering possible differences in genetic, disease status, lifestyle that all are likely to contribute to interindividual differences.

- There of course are large uncertainties regarding what sites in humans may be affected.

- It is unclear why there are several sites seen with 1,2,3-TCP that are not seen with structural analogs.

## (B) Oral Reference Dose (RfD) for 1,2,3-Trichloropropane

***1. A chronic RfD for 1,2,3-trichloropropane has been derived from a 2-year oral gavage study (NTP, 1993) in rats and mice. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.***

***James V. Bruckner***

I agree with the selection of the NTP (1993) bioassay as the principal study, as well as this document's authors' scientific reasons for choosing it. The study and many of its results have been clearly and objectively described. As noted under my specific comments, the authors should add the findings for the 3 and 10 mg/kg/day groups at the final sacrifice. Also, it is important to describe any clinical chemistry and morphological changes seen in the liver at the 3 dosage-levels at the interim and final sacrifices, since the liver is utilized as the primary target organ.

***Richard J. Bull***

It appears that the NTP gavage study provides the best data available for deriving the oral RfD. It is the only chronic study. The results are generally consistent with (i.e. supported by) subchronic studies. Considerable emphasis is placed upon absolute and relative liver and kidney weights, but correlative clinical chemistry data are not provided. It is noted on page 28 that there were changes in serum enzyme levels, but the reader is not provided any indication of the magnitude of these changes and the dose-response. It is indicated that these data were sporadic in the rat. As many of these parameters are related to necrotic damage to the liver (occasionally the heart and kidney as well) they should be presented. However, I was unable to determine if the lack of changes of serum enzyme levels in the rat was due to the fact that necrotic damage was not observed. It is important to distinguish increases in liver and kidney weight in the absence of pathology. If the changes in organ weights are not associated with pathology, they well may reflect reversible effects (i.e. reflecting induction of enzymes involved in xenobiotic metabolism) that are of considerably less concern.

There are some effects identified that were not adequately reported in the review of the data, but later sections of the document implied they were observed. For example, on page 80 it is stated that an increase in levels of creatine kinase were apparent in the chronic NTP study and this was related to the inflammation-associated myocardial necrosis in rats. It was not possible to assess the dose-response of this parameter based upon the information in the document, nor to determine whether it was actually associated with myocardial necrosis in the NTP study. This raises concerns related to the selection of the critical effect.

***Dale Hattis***

Choice of the 2 year oral gavage mouse study is entirely reasonable.

***Ralph L. Kodell***

The NTP study was selected as the principal study because it was a well- designed chronic study, conducted in both sexes of two species (rats and mice), with sufficient numbers of dose groups and numbers of animals, and appropriate toxicological endpoints. The study has been transparently and objectively described. I believe the NTP study has been scientifically justified as the principal study.

***Harihara M. Mehendale***

Chronic RfD for TCP was based on a 2-year oral gavage study (NTP, 1993). This has been scientifically well justified. Selection of the 2-year gavage study as the principal study has also been scientifically justified.

***Helmut Zarbl***

The selection of the NTP study is appropriate and justified on the grounds that it is the best designed study performed under optimum conditions and standards. The study evaluation is both clear and described objectively. The main problem, which is clearly discussed in the report, is the fact that carcinogenicity reduced survival of animals in the non-cancer evaluation, possibly confounding the results. This of course raises the concern of deriving an RfD at doses that are carcinogenic.

***Lauren Zeise***

The selection of the NTP study has been justified to a certain extent, but the discussion could be improved. The discussion on page 63, paragraph 3 indicates that the chronic NTP studies employed lower doses than the subchronic studies. It further suggests that the decreased survival may be the most likely reason that similar effects on the heart, kidney and liver observed in the subchronic studies may not have been observed in the chronic study. But this discussion does not hold up to scrutiny. For example, relative heart weight was significantly effected in the subchronic NTP study at all dose levels in the male mouse – 8, 16, 32, and on up – and in all but the lowest level in the female mouse, whereas at the 15 month interim evaluation in the chronic study there were sufficient animals available for analysis and this effect was not seen at either 20 or 60 mg/kg. Similarly relative weight for rat kidney was affected at 16, 32 and on up in subchronic but not at the 20 in the chronic in the female mouse study. Relative liver weight was affected at roughly 60 mg/kg but not lower in both NTP subchronic and chronic studies in male and female mice. Examining the rats relative liver weights are decreased at 10 mg/kg in the chronic for both sexes, and not below and at 16 but not 8 in the female subchronic and 32 but not 16 in the male subchronic (although absolute liver weight effects are seen at 8 mg/kg in the chronic). Ultimately care must be taken in

discussing the effects and the basis for study selection to make sure the generalizations are fully supported.

***2. Increased liver weight was selected as the critical effect. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Is the rationale for this selection transparently and objectively described in the document? Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect. Please comment on the use of increased absolute liver weight instead of relative liver weight to describe the liver weight change.***

***James V. Bruckner***

The selection of increased liver weight as the critical effect has not been scientifically justified. It is not stated on page 79 or 80 that rat data are being utilized. This may be the most sensitive non-cancer effect, but it is usually not considered to be toxicologically-significant. Many halocarbons and other VOCs (including non-toxic ones) produce an increase in liver weight, due to reversible hypertrophy of hepatocytes. Reversible microsomal enzyme induction (as with TCP) and proliferation of smooth endoplasmic reticulum often accompany hypertrophy. It is stated in line 4 of page 80 that increased liver weight may be part of a continuum of adverse hepatic effects. This is a highly speculative statement. This statement is preceded (at the bottom of page 79 and top of page 80) by accounts of histopathological and clinical chemistry changes indicative of hepatocellular toxicity. The NTP clinical chemistry battery did not show evidence of liver injury in the rats (See pgr. 1 on page 26). Decreased serum ALT implies decreased hepatocellular damage/death. There is no mention in the document of non-cancerous morphological changes in the liver at the terminal sacrifice. Ingestion of 125 mg TCP/kg/day was required to elevate liver cytoplasmic enzymes in serum and cause histopathological changes in the liver of male and female rats examined at the 15-month interim sacrifice. It is stated in lines 6 & 7 in the first paragraph of page 13 that liver necrosis was seen in 1 of 10 male rats at 32 and at 63 mg/kg/day. Are these statistically significant changes?

It is appropriate to use absolute rather than relative liver weight when there has been a significant reduction in body weight gain. This was recognized by the NTP (1993) (See last 2 lines on page 11).

***Richard J. Bull***

The use of increased liver weight in the rat as the critical effect is difficult to assess. The pathology data for this organ was not tabulated or graphed. The clinical chemistry data was also not explicitly presented so that it could be reviewed. Although these data are not explicitly used in determining a POD, they are essential to the evaluation of changes in liver weight as the critical effect. In fact my initial comments were based upon a misreading of this text, making it very apparent why these data need a much higher visibility within the document. One should be able to make a side by-side comparison of the dose response for liver weight increases with the development of pathology or indirect indicators of such pathology, such as the serum enzyme levels. If pathology is

not observed as doses increases, a conclusion that changes in liver weight are purely adaptive is justified.

The selection of increases in liver weight of the rat in the derivation of the oral RfD raises the question of why the liver hypertrophy observed in rats exposed to 1,2,3-TCP by inhalation were discounted. In this case, the document indicated that no pathology was observed in the liver, a finding that apparently contradicts finds observed when 1,2,3-TCP was administered by corn oil gavage.
In my view, the increased kidney weight data should also be presented in parallel with dose-response data on the development of pathology as well as quantitative indicators of modified in renal function. This is doubly important because this information is important in discussing potential modes of action for increased yields of renal tumors in male rats.

Pathology is also observed in the liver of the mice, which is apparently supported by serum enzyme changes, that appears to correlate with increases in liver weight in this species. These data are not provided in tabular or graphical representations of this information. The mice did develop hepatic tumors so these changes should be discussed as potential contributors to the mode of action involved in the development of liver tumors.

***Dale Hattis***

The data and explanation in the document is not complete enough to come to a clear conclusion on this. Neither Table 5-1 or Appendix B appear to provide comprehensive information on the modeling of all measured endpoints for all models run for the different datasets in both species.. Of the data that are quoted in Table 5-1, BMD/BMDL calculations for a 10% change in mean liver weight is apparently selected in preference to a 1 standard deviation change without explanation, even though the 1 standard deviation change reportedly yields a slightly lower BMD and BMDL. The mystery deepens somewhat when the supporting model run data for these options are examined in Appendix B. For the Hill model runs represented as the source of both parameters, the runs appear to have been based on identical data for the means and standard deviations of the absolute liver weights. No data are presented there on the quantized parameters (fraction of animals with either a 10% or a 1 standard deviation increase in liver weight). Chapter 4 does give a more complete review of data for other endpoints, however, and from those data it does appear that the liver weight change observations do represent the most sensitive endpoints. More comprehensive discussion of the choice of the selected endpoint vs available alternatives would be helpful.

***Ralph L. Kodell***

Increased liver weight (in rats) was identified as the critical effect because liver toxicity appeared to be the most sensitive effect. The document states that designating the liver as the target organ is consistent with the observed binding of 1,2,3-TCP metabolites to hepatic proteins and nucleic acids. Increased kidney weights were also considered for

benchmark dose modeling and comparison, but they resulted in larger BMDLs than the liver data. Treatment-related effects were detected among hematological parameters in rats, but these were not considered biologically relevant on their own, because they were thought to be the result of the chemically-induced tumors. Two reproductive endpoints from the 1990 NTP reproduction/fertility study in mice were also modeled, but they resulted in larger BMDs and BMDLs.

In the NTP chronic study, liver weights were measured in only 8-10 animals per dose group evaluated at the 15-month interim sacrifice. The highest dose groups in the rat and mouse studies were terminated early for both sexes because of high mortality, and no tissue weights were taken in the highest dose group of either species except at 15 months. The dose-response data on increased liver weight that were discussed and used to derive the oral RfD were 15-month data. The selection of increased liver weight as the critical effect has been scientifically justified, and the rationale is transparent and objective. However, I question why an adjustment of the RfD to full-lifetime exposure (24 months = 104 weeks in rats) to 1,2,3-TCP wasn't made. I did not see that this issue was even addressed. If it is assumed that the unobserved 24-month effect would be equivalent to the 15-month effect, then this needs to be stated. The document states that absolute liver weight was selected instead of relative liver weight because it is a more direct measure of liver weight change and because relative liver weight can be affected by decreased body weight with an increase in dose (p. 82). It's true that relative liver weight can be affected by decreased body weight with an increase in dose, but I think that's a rationale *for* using it instead of *against* using it. I think it's appropriate to use absolute liver weight, but a better rationale is needed. The BMDLs for absolute are smaller than those for relative (Table 5-1). Perhaps that's all that needs to be said as justification.

***Harihara M. Mehendale***

Increased liver weight as the critical effect has been scientifically justified.

***Helmut Zarbl***

The selection of liver weight as the endpoint was selected because it was the most sensitive measure of toxicity. Selection of liver weight was further justified on the basis of a well behaved dose response in both sexes of mice and rats, in both sexes and the fact that increased liver weight, an established criterion for toxicity. Increased hepatic weight was also associated with an increased necrosis and decreased enzyme synthesis in the subchronic study, and with increased serum enzymes and liver lesions in the chronic study. Moreover, exposure of leads to increased formation of DNA and protein adducts of 1,2,3-trichloropropane, and liver is a target organ for carcinogenesis. Together these studies were used to argue that the liver is a valid target for modeling risk. However, a careful evaluation of liver pathology is needed to rule out an adaptive response and justify this selection, especially since there is little evidence of liver damage from clinical chemistry/serum enzymes. Liver pathology was not discussed in the report. In fact the argument that increased organ weights can be adaptive is used as an argument against selection of increased liver weights in the inhalation study.

### *Lauren Zeise*

The discussion of non-cancer effects in section 4.6.1 and section 5.1.1 on choice of principle study and critical effect taken together do a fairly good job synthesizing the evidence for non-cancer effects in different organ systems, and rightly emphasizes the evidence supporting an overall finding of hepatocellular damage. However, several of the major non-cancer effects noted by NTP for its chronic studies are not discussed. For the rat NTP notes increased severity of nephropathy (males only), and "increased incidences of basal cell and squamous hyperplasia of the forestomach, acinar hyperplasia of the pancreas, renal tubule hyperplasia, and preputial or clitoral gland hyperplasia." For the mouse NTP notes "increased incidences of squamous hyperplasia of the forestomach and eosinophilic foci in the liver." These endpoints should be discussed and considered. Curiously, had a low dose non-linear response been assumed, the RfD would have been based on cancer effects and would have been considerably lower. This is another illustration that exposures at the draft RfD should be avoided.

As noted in the Toxicological Review, forestomach hyperplasia is along the morphological continuum leading to cancer. This is also the case for several other sites with tumor induced by 1,2,3-trichloropropane, but this has not been noted in the Toxicological Review. **As is usual practice, hyperplasia is not taken into account in the cancer potency calculation. But it is also not typically considered in the non-cancer findings for the purpose of RfD calculation.** If hyperplasia is considered to be a non-cancer endpoint, then it is clearly an endpoint that should be considered in determining critical effects for non-cancer assessment. Large statistically and biologically significant increases in hyperplasia were seen in the lowest dose groups in the male and female rat and mice studies. For this reason the effects identified for chronic non-cancer endpoints in these studies can be questioned and these effects should be given consideration for possible inclusion in Table 5-1.

Liver is an obvious target for the toxic effects of the compound. As noted in the review, the clinical chemistry markers in the subchronic study support liver damage as an endpoint, as well as absolute and relative-to-bodyweight changes in liver weight. Rather than arguing for any particular endpoint as being the most appropriate, the BMD and BMDLs for the different indicators of liver toxicity could be considered, including BMDs for pseudocholinesterase in the subchronic study. Taking these measures together and rounding, a point of departure between 1.5 and 2 may be appropriate. But again, consideration should also be given to other endpoints not tabulated in Table 5-1 but considered by NTP to be important.

***3. The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the point of departure (POD). All available models were fit to the data in both rats and mice for increased absolute and relative liver weight, increased absolute and relative kidney weight, fertility generating the 4th and 5th litter, and the number of live pups/litter in the 4th and 5th litters. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and adequately described? Is the benchmark response selected for use in deriving the POD scientifically justified and has it been transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.***

***James V. Bruckner***

Why were absolute and relative liver and kidney weights of mice utilized in BMD modeling when they were not altered by TCP?

BMD modeling is scientifically justifiable and offers some advantages over the use of NOAELS and LOAELS. It is however, a conservative approach for selecting a RfD. In view of this and my aforementioned questions about the toxicological significance of modest changes in liver weight, I believe that a 10% weight change is too low to use as a point of departure in this modeling exercise. An additional level of conservatism was added by choice of the lower 95% confidence interval. The BMD modeling itself has been adequately described and appears to have been conducted appropriately.

***Richard J. Bull***

In general, BMD modeling is preferred to utilizing NOAELs and LOAELs as points of departure. The data sets appear to be adequate for that purpose.

There is a concern that the POD for changes in mean live pups per litter was selected to be a 1% change. Unlike the rationale that was applied to using a 10% response in liver weight, an effect this small is clearly well below one that could actually be measured experimentally. When this 10-fold factor is coupled with an uncertainty factor of 3 added to the liver weight endpoint for lack of "adequate" developmental toxicity data, the importance of this endpoint has been significantly exaggerated. If this latter factor is applied to any data set, it should be added to the POD derived from the reproductive/developmental toxicity study, not the POD for liver weight increases.

The document obscures the sources of data in the discussion of using the increases in liver weight as the endpoint used to develop the non-cancer POD. The liver weight data from the rat was used, but one has to comb through the text to determine if there are pathology data to support the use of this endpoint as a POD. As indicated above, ability to compare the dose-response information of a sensitive endpoint that is postulated as a precursor of pathology should demonstrate that pathology is progressively linked to that

endpoint when the exposure period was increased or higher doses are administered. While serum enzyme changes did not support the relationship, there was some indication of decreases in pseudocholinesterase activity in serum of rats in the text. That information also needs to be displayed in tabular or graphic form so that it can be compared to other findings of hepatocellular damage.

***Dale Hattis***

In general BMD modeling is the best approach currently recognized by the Agency for determining points of departure for RfD assessments. However, as mentioned in my answer to point 2 immediately above, the modeling inputs and results have not been comprehensively described. For the selected endpoint, we simply don't know what if any models were run other than the Hill model (however this model seems to more than adequately fit the data, so this quibble is not very important in that case). However it is unclear how exactly the model was applied to determine the benchmark doses for the precise quantal endpoints described, as the model itself seems to have only been applied to mean data.. And the choice between the 10% liver weight endpoint and the 1 standard deviation shift endpoint is completely unclear, as the latter appears to yield a slightly lower BMDL.

***Ralph L. Kodell***

I believe BMD modeling is the best approach when adequate dose-response data are available, as in the present case. The document provides output in Appendix B-1, apparently from all models that fit adequately ($p>0.1$). Presumably, none of the models fitted to the data on mouse organ weights achieved adequate fits, as the results are not provided. I wonder why these data were not included in a LOAEL/NOAEL assessment, for comparison purposes in deriving the POD, assuming the reason they were not used to derive BMDs is that the models didn't fit. I believe an explicit explanation ought to be given. I think this is especially important in light of the results of the chronic oral gavage study. The RfD is derived based on liver-weight changes in rats, but the chronic study did not show a dose-related effect on liver tumors in rats. On the other hand, the chronic study did show a dose-related effect on liver tumors in mice.

With continuous endpoints like organ weights, it is not as straightforward to select a BMR as with quantal data. The EPA has included two approaches for the liver-weight data: 10% change in the mean and 1-SD change in the mean. The resulting BMDs and BMDLs are comparable by the two approaches (Table 5-1). For live pups/litter a stringent 1% change in the mean was selected for the BMR because of the frank toxicity of the endpoint. For the fertility endpoint the BMR was apparently set at 10% (Appendix B-1, pp. 161,163), but I did not see this discussed in the document. For all modeled endpoints except fertility (BMR not discussed), I believe the benchmark response for deriving the POD has been transparently and objectively described. The Hill model was used for the organ-weight data. Because there were only four doses to fit four parameters, apparently the exponent in the Hill model defaulted to 1. I think it would be helpful to mention that, so that it is clear that there is no over-fitting. For the variance

function, it would be helpful to mention that the parameter alpha is the homogeneous variance when the parameter rho is set to zero as in this case. This would make it easier to follow the sequential likelihood-ratio testing strategy for selecting the final model. Fertility data were modeled with a probit-logdose model. Data on pups/litter were modeled using a quadratic regression model. The models and modeling were not discussed in the document. I think it would be useful to add some textual description rather than just rely on the output shown in Appendix B-1.

***Harihara M. Mehendale***

Chronic RfD derived from utilizing the BMD modeling to define the point of departure (POD) has been adequately and scientifically justified.

***Helmut Zarbl***

The RfD derived by the EPA using BMD to define the point of departure is both adequate and well justified for the available. The analysis examined all endpoints and selected absolute liver weights in male rats because this was the most sensitive point of departure. The value obtained is only slightly lower than the previously defined value. Of concern is the fact that the RfD was derived dose that were carcinogenic. The use of a 1% change in live pups is for the POD was of concern because it is probably below a level that can be accurately measured unless very large numbers of animals are used in the analysis. It might also be useful to include the results obtained using a NOEAL/LOEAL approach.

***Lauren Zeise***

The benchmark dose modeling is fairly well described and is a reasonable approach to establishing a point of departure, although the description of the cancer modeling was given in more detail and easier to understand in terms of just what was done. To provide some context and a better understanding of the findings, it may be helpful to include in the tabulations the LOAEL and NOAELs as well, or at least show the plots on a figure near the Table 5-1 so the results can be easily compared. Regarding whether the modeling has been adequately done, it would is instructive to compare NOAELs and LOAELs with the BMDs to look for any pathologies in the data, as often occur with non-cancer data sets. Where this occurs it may be preferable to consider use of a NOAEL or MOAEL for that data set.

Earlier in the document NOAELs and LOAELs are identified. But it is unclear why they are being identified - in section 4.2 of the Toxicological Review for subchronic non-cancer endpoints. The values are not carried through to calculation of subchronic RfDs, but provide a useful context for the benchmark dose derivations. The selection of these subchronic indicators of toxicity in section 4.2 could be strengthened. For each NOAELs and LOAELs, identified, the justification could be strengthened.

***4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfDs. For instance, are they scientifically justified and transparently and objectively described in the document?***

***James V. Bruckner***

A 10-fold interspecies uncertainty factor is not justified. As previously described (See Specific Comments), livers of mice and rats have substantially higher CYP450 and glutathione (GSH) S-transferase activities than humans. Substantially larger quantities of reactive metabolites will be produced in the liver of rodents. As described in my specific comments, in vitro experiments (with human and rat microsomes and cells) have conclusively shown that rats metabolically activate significantly more DBCP to covalently binding protein and DNA adducts and experience greater cytotoxicity. Epoxide hydrolase, the enzyme that detoxifies episulfonium ions, is expressed to a greater extent by human hepatocytes.

A 3-fold intraspecies uncertainty factor is still justified in the absence of human and animal data on the toxicodynamics of TCP.

***Richard J. Bull***

The selection of the interspecies and intraspecies UF appears to conform to Agency policy. Questions related to UF applied for database deficiencies were addressed under questions 3 and 5.

***Dale Hattis***

The choice of uncertainty factors seems relatively standard and defensible.

***Ralph L. Kodell***

The default $UF_A$ value of 10 was selected because of the lack of information on toxicokinetic and toxicodynamic differences between rats and humans with respect to 1,2,3-TCP. However, available data in *mice* indicate that higher DNA adduct formation and cellular proliferation were observed in tissues of animals exposed to 1,2,3-TCP via oral gavage than in comparably dosed animals exposed via drinking water. It seems reasonable to assume that the cellular response leading to the increased liver weights (e.g., hypertrophy, if not cell proliferation) would differ similarly between routes of exposure, and that the same toxicokinetic difference between routes of exposure would apply to rats and humans. Because the primary human route of exposure is via drinking water, I believe this argues for a smaller value of $UF_A$ to cover mainly toxicodynamic uncertainty. I suggest that the value of 10 be reduced to 3, or that a route-to-route adjustment (e.g., based on La et al., 1996) be made before the animal-to-human extrapolation to partially offset the toxicokinetic uncertainty component of the factor of 10.

The default $UF_H$ value of 10 was chosen because of insufficient information to choose otherwise. This is justified.

No $UF_S$ factor was applied because the critical effect was measured in a chronic study. However, the effect was not a full-lifetime effect; it was measured at the 15-month interim sacrifice. The highest dose groups in both the rat study and the mouse study were terminated prior to 24 months, and organ weights were not taken at termination. I believe that an adjustment from 15 to 24 months should be made, or an uncertainty factor should be applied. If not, then there needs to be an explanation as to why this need not be done.

A factor $UF_L$ was not applied because BMD modeling was done and the BMR value of 10% change in absolute liver weight was selected under the assumption that it represents a minimal biologically significant effect. In my own thinking, a BMR of 10% should be interpreted more as a LOAEL response than a NOAEL response. Thus, I think a $UF_L$ ought to be applied, but not necessarily the default of 10. I recommend a $UF_L$ value of 3.

***Harihara M. Mehendale***

Selection of the uncertainty factors applied to the POD has been transparently justified.

***Helmut Zarbl***

A total uncertainty of 300 was applied to the POD to derive the RfD. These included as a UF of 10 for cross species extrapolation, a UF of 10 for intraspecies variation, and a UF of three for database deficiencies. These were appropriate and well justified, although the reasons for selection of a UF of 3 for database uncertainties were less obvious and should be elaborated in the review.

***Lauren Zeise***

The selection of uncertainty factors is scientifically justified, transparent, and well described. However, the end result does not appear to be sufficiently conservative for reasons I raised above.

***5. Please comment on the transparency and scientific rationale and justification for the selection of the database uncertainty factor. Please comment on whether the application of the database uncertainty factor adequately represents the gap in oral reproductive and developmental toxicity data for 1,2,3-trichloropropane.***

***James V. Bruckner***

This 3-fold uncertainty factor appears to be justified, in light of the genotoxicity of TCP and the lack of developmental data. This scientific rationale has been clearly presented. It would be worthwhile to mention that closely-related compounds (e.g., DBCP, EDB, 1,2-dichloropropane) all are spermatotoxic in most species, including man. This may account for the progressive decrease in number of offspring of mice (NTP, 1990). Decreases in testes' weights were seen in the subchronic inhalation studies by Johannsen et al. (1988) and Miller et al. (1987a). No histopathological changes were reported in the latter study. Were histopathological changes seen at the 15-month or terminal sacrifices by NTP (1993)?

***Richard J. Bull***

I am generally skeptical of the database uncertainty factor as it is currently applied by the Agency. I do not object to the application of such a factor at the risk management step of the decision making process. At the risk assessment step, it introduces a very different kind of question than the other uncertainty factors that is related to the incomplete information available on an endpoint that is considered to be important in environmental health. The other factors deal with uncertainties related to extrapolation of an established effect across species or do low dose or as an adjustment related to short durations of exposure. Essentially, there are straightforward scientific bases for making these adjustments.

Setting the question of when the database adjustment factor should be applied aside, I find the application of the data base U.F. to the liver effect to be illogical in this instance. Reproductive/developmental data were presented in the document that was derived from a pretty thorough study whose major fault is that a complete evaluation of potential reproductive and developmental effects was not carried through in the second generation. However, the evaluation of reproductive performance in the F1 generation was much more rigorous than is normally done, even in 2-generation designs. In my experience 2-gen studies are not particularly sensitive anyway and I suspect the measurement of reproductive performances out to the fifth mating is likely to be much more sensitive than a typical 2-generation study. The point is that a significant and consistent effect was observed. Therefore, I think if an UF is necessary at all, it should be applied to this dataset and not applied to the liver weight POD. Applying it here is logical because 1) there was a clear effect and 2) I can accept the possibility that this effect could be exacerbated in the next generation. The liver weight changes are not related to the effect at all, so application to the POD for that endpoint is simply illogical.

***Dale Hattis***

Yes, I think the database uncertainty factor chosen is reasonable.

***Ralph L. Kodell***

A $UF_D$ value of 3 has been applied for database deficiencies. I do not believe this is justified. The reasoning given in the document is that the database lacks information on developmental toxicity associated with 1,2,3-TCP, and that the two-generation study indicates that the developing fetus may be a target of toxicity. However, the comparison of BMDs and BMDLs in Table 5-1 indicates that the corresponding values are all higher for the reproductive endpoints than for the critical liver effect. So, the POD corresponding to the critical effect should be protective of the developing fetus. I recommend that no $UF_D$ value be applied.

***Harihara M. Mehendale***

Selection and application of the database uncertainty factors has been adequately justified.

***Helmut Zarbl***

However, the EPA did not consider adding a UF of 10 for increased susceptibility during development. Instead the EPA used a database uncertainty factor of 3 to account for incomplete data. The reason for this value was not justified. Would not evidence to suggest increased development toxicity, albeit incomplete and not derived from a standard development study, warrant an UF of 10? Review should present a detailed justification of why a UF of 3 was used in developing the RfD.

***Lauren Zeise***

The database uncertainty factor is described given the limitations in addressing oral developmental toxicity with the available studies, however, again, it is problematic to have such a large prediction of cancer risk at the RfD.

## (C) Inhalation Reference Concentration (RfC) for 1,2,3-Trichloropropane

***1. A chronic RfC for 1,2,3-trichloropropane has been derived from the 13 week inhalation study (Johannsen et al., 1988) in rats. Please comment on whether the selection of this study as the principal study is scientifically justified. Is the rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.***

***James V. Bruckner***

The 13-week study by Johannsen et al. (1988) has not been peer-reviewed, but seems to be the best choice in light of the paucity of long-term inhalation data. The design of the investigation and the account of the study results seem reasonable, although I have not reviewed the report. The authors of the current document need to justify their reasoning on page 89 for selecting this particular study.

***Richard J. Bull***

The 13-week study of Johannsen in rats does appear to be the appropriate selection.

***Dale Hattis***

This seems to be the best study for the purpose.

***Ralph L. Kodell***

There is no chronic inhalation study of 1,2,3-TCP on which to base a chronic RfC. The two combined 13-week inhalation studies of Johannsen et al. (1988) in rats provide the only subchronic data. These appear to be appropriate studies on which to base the chronic RfC. However, it's not clear why the first of Johannsen's single-generation reproductive study described on pages 44 and 45 was not discussed further. From the text, it seems that neither of Johannsen's reproductive studies demonstrated obviously treatment-related reproductive effects. However, in the first study, only 10 out of 20 females mated at 15 ppm compared to 17 out of 20 in the 0 ppm group. The text states that no statistical significance was evident, but this effect is statistically significant in a two-group comparison (17/20 vs 10/20 gives $Z=2.36$, $p<0.01$). I believe this needs better explanation, and that the toxicological endpoints in the Johannsen single-generation reproductive studies need to be given more consideration in selecting the principal study and critical effect for deriving the POD.

***Harihara M. Mehendale***

RfC has been derived from the 13 week inhalation study. This has been based on sound scientific rationale. This has been objectively described in the document.

### *Helmut Zarbl*

The Johannsen was study was selected as the source of data for deriving the RfC. In the absence of chronic inhalation data, the 13-week study provided the best alternative and a biologically relevant endpoint. However this study was not peer reviewed and is not a chronic study, limiting the value of any conclusions drawn. Moreover, the study seems to suggest reproductive effects which were not explored further in the EPA report.

### *Lauren Zeise*

The limited data available for RfC calculation are a particular concern. There are no long term studies to support the derivation. The justification for the selection of the Johannsen et al. study is reasonable, transparent, and objective. It would be of interest to see tabulations similar to those done for the oral exposure studies comparing LOAELs and NOAELs for the various endpoints in the other inhalation studies.

***2. Peribronchial lymphoid hyperplasia in the lungs of male rats was selected as the critical toxicological effect. Please comment on whether the selection of this critical effect has been scientifically justified. Is the rationale for this selection transparently and objectively described in the document? Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.***

***James V. Bruckner***

The rationale for selection of peribronchial lymphoid hyperplasia over increased absolute liver weight is not very strong. What is the toxicological significance of the former change? It would seem that the latter effect would be chosen for both the RfC and RfD, for sake of consistency. It is not clear to this reviewer whether the lymphoid hyperplasia is a predecessor of, or is associated with subsequent development of pulmonary tumors. It has not been established whether chronic TCP inhalation causes lung tumors, but some structurally-related compounds (that appear to be metabolized similarly) do (e.g., DBCP, EDB).

***Richard J. Bull***

It does seem reasonable to focus on the peribronchial lymphoid hyperplasia as the endpoint of interest. However, as noted above, it is internally inconsistent to dismiss the evidence of hepatocellular hypertrophy out of hand, which appears to occur at higher incidence at the same doses when this is the endpoint that was selected for the development of the oral RfD.

The major question raised by the inhalation study relative to liver effects is that no pathology was observed in the liver. That goes to the discussion raised in the RfD section of the document about the importance of associating pathology as a progressive and inevitable outcome or implication of modifications of liver weight. Consequently, the conclusion can be observed to be justified. On the other hand the lack of pathology when liver weight changes were seen in the inhalation studies raises issues related to why pathology was observed in a similar time frame in the NTP studies where corn oil gavage was used to administer 1,2,3-TCP. The question that is raised is whether corn oil gavage is affecting this outcome as it has been clearly demonstrated to synergistically affect liver pathology when used to administer other halogenated hydrocarbons (see extensive data on chloroform: Bull et al. 1986. Enhancement of the hepatotoxicity of chloroform in B6C3F1 mice by corn oil: Implications for chloroform carcinogenesis. Environ. Health Perspect. 69:49-58; Larson et al. 1995. Induced regenerative cell proliferation in livers and kidneys of male F3-44 rats given chloroform in corn oil by gavage or ad libitum in drinking water. Toxicology 95:75-86). The differences in vehicle and mode of administration have been shown to have implications for the development of liver tumors in mice (Pereira, M.A. and M.A. Grothaus. 1997. Chloroform in drinking water prevents hepatic cell proliferation induced by chloroform administered by gavage in corn oil to mice. *Fundam. Appl. Toxicol*. 37:82-87). Therefore, the lack of pathology induced in the liver of rats treated with 1,2,3-TCP by inhalation raises issues related to the suitability

of using liver weight changes produced by the oral treatment using corn oil gavage. While I do not think these data entirely negate the logic for using liver weight as a POD for the oral RfD, these questions need to be discussed as uncertainties in related to the derivation of the RfD. That is especially true of liver weight changes are treated differently in the derivation of the RfC and RfD.

***Dale Hattis***

The selection of the critical effect in this case seems reasonable.

***Ralph L. Kodell***

The rationale for choosing peribronchial lymphoid hyperplasia as the critical toxicological effect is that it occurred in both male and female rats and might be correlated with the observed increased lung weight. Peribronchial lymphoid hyperplasia in the lungs of male rats is an appropriate effect to consider in choosing the critical toxicological effect. However, I believe there are other toxicological endpoints that ought to be given more consideration than they have been given in selecting the critical toxicological effect. The first 13-week of Johannsen et al. showed statistically significantly increased absolute and relative liver weights in male and female rats at the higher concentrations of that study (Table 4-16). The relative liver weights showed a concentration-response in both sexes. It is stated (p. 37) that the second study which was done at lower concentrations identified a NOAEL for organ weight changes at its highest concentration, 1.5 ppm. These data are not provided. The rationale for not considering the increase in liver weights is that lesions and serum levels indicative of liver damage were not evident. However, given that an increase in liver weights was chosen as the critical effect in deriving the chronic RfD, it seems appropriate to consider the concentration-response data on the same effect from the inhalation studies in deriving the chronic RfC. The rationale for not including hepatocellular hypertrophy in male rats as a potential critical effect is that it was considered potentially adaptive in the absence of additional overt toxicity in the liver. This has no effect on the ultimate outcome, as the NOAEL and LOAEL are the same as for peribronchial lymphoid hyperplasia. As mentioned above, I believe the toxicological endpoints from the Johannsen reproductive study ought to be considered as well.

***Harihara M. Mehendale***

The selection of peribronchial lymphoid hyperplasia in the lungs of male rats as the critical effect is well justified.

***Helmut Zarbl***

Since 1,2,3-trichloropropane is a known irritant to the human airway, selection of the peribronchial lymphoid hyperplasia was a reasonable endpoint, since enlargements in other organs were deemed adaptive due to lack of histopathology. The latter argument would however be in conflict with the use of increased liver weight, in the absence of

pathology or clinical chemistry support of liver damage, in the derivation of the RfD. This discrepancy needs to be addressed in the report. Peribronchial lymphoid hyperplasia was also selected because the effect was seen in both males and females rats and may be linked to hyperplasia. However there is no discussion of the histopathology in these lymphoid tissues. Were they indicative of a response to tissue damage, increased recruitment of specific lymphoid cells or cell types? Was there evidence of increased lymphokines? A more detailed evaluation of the phenotype would increase confidence in its selection as the endpoint for POD estimations.

***Lauren Zeise***

The justification given is reasonable, but the selection of the endpoint because of possible correlation with lung weight changes is not sufficiently compelling absent a more in depth discussion of the importance of this endpoint. Again, it would be of interest to see a more in depth discussion comparing inhalation to oral findings, on a mg/kg basis, given the considerably greater oral data base.

***3. The chronic RfC has been derived utilizing the NOAEL/LOAEL approach to define the point of departure. Please provide comments with regards to whether this is the best approach for determining the point of departure. Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.***

***James V. Bruckner***

In light of the lack of a dose-response relationship for the female rats and no dose-related increase between 15 and 50 ppm, the NOAEL/LOAEL approach seems to be reasonable. It appears to this reviewer that this dose-response deficiency may argue against selecting peribronchial lymphoid hyperplasia as a critical effect.

***Richard J. Bull***

It is not entirely clear why benchmark dose modeling could not be done with the peribronchial lymphoid hyperplasia data. The response in male rats was dose-related up to 15 ppm. Would it not be possible to model these data by dropping the 50 ppm dose and obtain an acceptable fit? There was no analysis or discussion of what caused the "bad" fits. There are difficulties with the data in female rats, but their responses were in the same dose range, suggesting that their sensitivities are not substantially different than that of the males. It seems doubly odd to complain that the NOAEL may actually range from 0 to as high as 24% response. This is not a problem that is peculiar to TCP, so the Agency should consider whether this is the appropriate place to raise this issue. This "error" was calculated by the projection of one model or the other (not specified in the document insofar as I was able to determine). The only way to handle this issue is to explore additional/different approaches to benchmark dose modeling. It is not appropriate to criticize the NOAEL approach based on a BMD estimate that is not explained in some detail.

***Dale Hattis***

I am not at all clear as to why a NOAEL/LOAEL analysis was applied in this case, rather than the BMD type modeling used for the oral RfD analysis. I think a BMD analysis should generally be preferred.

***Ralph L. Kodell***

It is stated that benchmark dose modeling was not utilized because the peribronchial lymphoid hyperplasia incidences were not amenable to modeling due to the inconsistent dose response at the three highest doses in both males and females, with model outputs that did not adequately fit the data. I dislike using the NOAEL/LOAEL approach when dose-response data are available. However, if the models do not fit, then NOAEL/LOAEL is the default. On the other hand, it might be possible to achieve adequate fits by simply removing the highest dose group. I believe this ought to be tried

for peribronchial lymphoid hyperplasia. As stated above, I believe that other endpoints ought to be considered in selecting the POD, some of which appear amenable to benchmark dose modeling. As was done for the RfD, I believe that BMDs and BMDLs or LOAELs and NOAELs ought to be derived for these other endpoints and compared to the NOAEL (or BMD) result for peribronchial lymphoid hyperplasia. A comparative table for the chronic RfC like Table 5-1 for the chronic RfD would be helpful.

***Harihara M. Mehendale***

The chronic RfC was derived utilizing the NOAEL/LOAEL approach to define the point of departure (POD). This is the best choice given limited options of quality data.

***Helmut Zarbl***

This part of the analysis was somewhat weak. Due to inconsistencies in the dose response curves made to the BMD approach impractical, and hence the decision was made to use the NOAEL/ LOAEL approach. Unfortunately, the NOAEL had a low confidence value due to the small sample size (15 animals), introducing a large uncertainty factor for extrapolation from the LOAEL. Although the approach taken is probably a reasonable alternative, the attempt to benchmark modeling, perhaps leave out high dose data points (e.g. 50 ppm) should also have been considered for comparison.

***Lauren Zeise***

The use of NOAEL/LOAEL approach to derive the RfC has been adequately justified on the basis of irregularities in the dose response relationship for the critical effect. This can lead to pathologies in the BMD estimation. Still it would be good as a sensitivity analysis to see how the values derived for the inhalation exposures compare with those for oral, on a mg/kg basis.

***4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfCs. For instance, are they scientifically justified and transparently and objectively described in the document?***

***James V. Bruckner***

Selection of the interspecies and intraspecies uncertainty factors is clearly described and scientifically justified.

***Richard J. Bull***

The UF applied to the inhalation data generally correspond to Agency policy.

***Dale Hattis***

The uncertainty factors selected for the RfC assessment seem reasonable.

***Ralph L. Kodell***

A $UF_A$ value of 3 was applied to cover uncertainty about toxicodynamic differences between rats and humans, toxicokinetic differences having been addressed by the applied dosimetry method used to convert to a human equivalent concentration. This is justified.

The default $UF_H$ value of 10 was applied because of insufficient information to predict variation in susceptibility among the population. This is justified.

The default $UF_S$ value of 10 was applied for extrapolating from subchronic to chronic exposure duration, because peribronchial lymphoid hyperplasia may be more severe at lower doses with prolonged exposure, and because additional effects may occur with chronic exposure. Given that two separately conducted subchronic studies were combined to derive the NOAEL, using a default value of 10 seems prudent.

A $UF_L$ was not applied because the POD was a NOAEL.

***Harihara M. Mehendale***

Selection and application of the uncertainty factors applied to POD for derivation of RfCs is scientifically adequately justified.

***Helmut Zarbl***

A total uncertainty factor of 3000 was applied to the POD, including 10 for database deficiencies, 10 for intraspecies, 10 for the LOAEL to NOAEL extrapolation and 3 for extrapolation from rats to humans. The use of a UF of 3 for cross species extrapolation is not well justified, referring to convention. The LOEAL to NOEAL extrapolation was needed because the EPA decided not to do benchmark dose modeling. Since this is

preferred over the LOEAL/NOEAL approach it is difficult to determine the relevance of this UF in the absence of evidence that benchmark modeling could not yield comparable estimates.

***Lauren Zeise***

The selection of uncertainty factor was appropriate given the limited data available with which to estimate a chronic RfC for this hazardous compound.

***5. EPA concluded that a database uncertainty factor of 10 was appropriate for the derivation of the RfC to account for the lack of a two-generation reproductive toxicity study and a developmental toxicity study. Please comment on whether the selection of the database uncertainty factor for the RfC is scientifically justified and has been transparently and objectively described in the document.***

***James V. Bruckner***

A 10-fold safety factor is excessive, in light of the 300-fold factor that has already been provided for. The 300-fold factor includes a 10X factor for extrapolating from a subchronic study to chronic exposure. The 2-generation oral reproductive study should give adequate assurance of TCP's potential to inhibit reproduction. A factor of 3 might be retained for lack of an adequate developmental study.

***Richard J. Bull***

The UF of 10 for lack of a two-generation study is excessive. First it is applied to the overall RfC and ignores the fact that a fairly substantial and probably more sensitive modified 2-generation study was conducted by the oral route of exposure. As indicated above, that study indicated changes in fertility and decreased live pups/litter in the fourth and fifth mating of the $F_1$ generation. The major complaint appears to be that these same endpoints were not followed up in the $F_2$ generation. As a 1% decrease in the number of live pups/litter was considered the point of departure and this endpoint still did not qualify as the critical endpoint in the oral studies, the combined effect is a factor of 100. If the Agency is arguing that the expectation is that reproductive/developmental effects are more probable with inhalation exposure, that rationale needs to be explicitly laid out. It seems more logical to assume a systemic effect is equivalent between routes of exposure as long as the systemic doses are normalized (there has been no attempt to do the latter in this document).

***Dale Hattis***

The choice here seems to follow relevant guidance.

***Ralph L. Kodell***

I do not think a $UF_D$ value of 10 is justified. The concern that the database lacks a multigenerational reproductive study and a developmental toxicity study may be somewhat alleviated by the results of the oral gavage studies with 1,2,3-TCP. A comparison of BMDs for chronic toxicological endpoints and two-generation reproductive endpoints indicated to me that the POD selected based on chronic endpoints would probably be protective with respect to reproductive and developmental effects. I think that if other toxicological endpoints currently available are used to derive potential PODs for comparison with the peribronchial lymphoid hyperplasia POD, some of the concern about database deficiencies may be alleviated. I recommend that the $UF_D$ valued

be reduced from 10 to 3 unless additional concerns are raised if and when the currently available data on additional endpoints are modeled.

***Harihara M. Mehendale***

The database uncertainty factor of 10 is adequately justified and is appropriate. Lack of 2-generation reproductive toxicity study and a developmental toxicity study is scientifically justified.

***Helmut Zarbl***

This UF is appropriate and well justified in the report.

***Lauren Zeise***

The selection of the factor was justified scientifically explained transparently and objectively in the report. One can question whether it is large enough given the lack of a well done chronic study for this compound.

## (D) Carcinogenicity of 1,2,3-trichloropropane

***1. Under the EPA's 2005 Guidelines for carcinogen risk assessment (www.epa.gov/iris/backgr-d.htm), 1,2,3-trichloropropane is likely to be carcinogenic to humans. Please comment on the cancer weight of the evidence characterization. Do the available data support the conclusion that 1,2,3-trichloropropane is a likely human carcinogen? Has the scientific justification for the weight of evidence characterization been sufficiently, transparently, and objectively described? Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described?***

***James V. Bruckner***

I believe the bioassay data clearly support the conclusion that TCP, in sufficient doses, is likely to be a human carcinogen. The weight of evidence has been succinctly, but clearly and objectively presented, as has the justification for conducting a quantitative cancer risk assessment.

***Richard J. Bull***

Yes, 1,2,3-TCP should be considered a probable human carcinogen. The weight-of-evidence argument was logical to the extent it was developed. The fish tumor data should be considered as part of the weight-of-evidence as a probable human carcinogen. It is essentially confirmation of carcinogenic activity in a third species and should be used in this determination rather than being relegated to a vague group of "other studies."

Clearly, there would be difficulty in utilizing the fish data for quantitative risk assessment since there are major issues related to obvious pharmacokinetic differences of chemical exposure via immersion as opposed to ingestion. However, an inability to normalize exposure does not detract from the usefulness of the fish data to contribute to weight-of-evidence determination of probable carcinogenicity to humans.

***Dale Hattis***

Yes. However, I would strengthen it somewhat as to the mode of action (see responses to following charge questions). It seems to me that the evidence for a mutagenic mode of action is clear and convincing, partly because of the analogy with DBCP and EDB. By comparison, there does not seem to be an alternative mode of action hypothesis that has anything close to convincing supporting evidence.

***Ralph L. Kodell***

I believe that the data support the conclusion that 1,2,3-TCP is a likely human carcinogen. In the NTP (1993) chronic oral gavage study, tumor incidences were elevated with increasing exposure levels at several sites in both sexes of rats and mice, including both point-of-contact sites and distant sites. However, the primary exposure to

humans is through drinking water, whereas the exposure to rodents was via oral gavage. DNA adduct studies in mice exposed to equivalent doses of 1,2,3-TCP by corn oil gavage and by drinking water indicated that adduct levels were higher in the gavage-exposed animals (Table 4-25; from La et al., 1996). In addition, cellular proliferation appeared to increase in a dose-related fashion for gavage-treated animals, but little if any was observed in drinking-water exposed animals. Nevertheless, the fact that tumors were induced at distant sites suggests that 1,2,3-TCP is likely to be carcinogenic via drinking-water exposure (and inhalation exposure) as well as oral gavage, even though the dosimetry may differ between routes of exposure. It is pointed out in the document that the relevance of forestomach tumors in rodents may be of concern because humans do not have a forestomach. I share that concern. However, other parts of the human alimentary system where tumors were observed are similar to rodents, and the fact that tumors were induced in multiple distant organs in both sexes of the two species by what may be a mutagenic mode of action is compelling. I think the scientific justification for the weight of evidence characterization has been sufficiently, transparently, and objectively described. I did not see an explicit justification for deriving a quantitative cancer assessment, just a discussion of why a linear-low-dose extrapolation was used (default option, p. 102). In light of the differences observed by La et al. (1996) in adduct formation and cellular proliferation between oral gavage and drinking water exposures, it seems that some sort of adjustment for route of exposure ought to be made, say, assume that 1,2,3-TCP is only half as potent by the drinking water route.

***Harihara M. Mehendale***

The available data support the conclusion that TCP is likely to be a human carcinogen. The scientific evidence supporting this conclusion has been adequately presented and justified in the document.

***Helmut Zarbl***

The EPA's classification of 1,2,3-trichloropropane as a probable human carcinogen base on the weight of the evidence is fully justified. The compound induces carcinomas at the point of contact and at distal organ sites in both male and female rats and mice. Despite the fact that some of the organs targeted have no human homology, there is sufficient evidence for shared major organs such as liver. There is limited information to suggest that the compound is genotoxic, albeit much of it indirect. However, there is a notable absence of *in vivo* genotoxicity data. Also missing is evidence of carcinogenicity in rodents by exposures other than gavage in corn oil. Although not evaluated in this report, a recent NTP study has also found evidence of hepatocarcinogenicty in fish exposed environmentally (in aquaria). Mechanism-of-action data are suggestive of genotoxicity, but solid in vivo data are lacking. There is also structure activity data from closely related compounds that are genotoxic and carcinogenic.

***Lauren Zeise***

The finding likely to be carcinogenic to humans is clearly supported by the evidence outlined in the report. In discussing the potential for the induction of cancer via the inhalation route the statement is made that no information is on the compounds carcinogenicity is available for the inhalation route. A slight clarification is suggested. There is no direct information, but the information discussed on page 66, as well as structural analogies to DBCP and other analogs, as discussed above, provide strong indirect evidence that the compound is likely to be a human carcinogen by the inhalation route.

Section 4.7.1 provides a summary of the overall evidence before the full discussion of the evidence is provided. Left out of the discussion in this section is the supporting evidence from genotoxicity and other mode of action related studies. It may make for a better flow in the discussion to place the summary at the end of the evaluation of the carcinogenicity in section 4.7. The current layout results in a considerable amount of repetition. For example the tumors induced by the compound are listed in two different places on the same page (p. 66), one list in section 4.7.1 and a second in section 4.7.2. The list also appears in several other locations in the document.

Even though repetitious, taken as a whole the scientific justification for the weight of evidence characterization of likely is transparent, sufficient and adequate. As noted above, the structural analogue 1,2-dichloroethane also provides support in addition to DBCP (noted in point 3 at the bottom of p. 66). In addition the discussion of lack of confidence in the mode of action support for carcinogenicity because the Saito-Suzuki et al. 1982 dominant lethal test and the two micronucleus tests, one reported by Doublas et al. in 1985 only in abstract, and the other by Crebelli which was negative for all ten chemicals testing positive in other genotoxicity tests is a concern.

With regard to the Crebelli et al. 1999 study, the authors note “No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes over the control values were observed at any sampling time with any of the 10 halogenated hydrocarbons assayed. The comparison of the results obtained in this study with the findings provided by in vitro micronucleus assays on the same chemicals, reported by other authors, indicate that mouse bone marrow is weakly sensitive to the genotoxic effects induced by halogenated hydrocarbons in other test systems. This suggests that the role of such an assay in carcinogen screening may be questionable for this chemical class. An examination of mouse bone marrow micronucleus test results with the halogenated aliphatic hydrocarbons classified as carcinogens by IARC supports this conclusion.”

With regard to the negative micronucleus study by Douglas et al. the IPCS Concise International Chemical Assessment Document for 1,2,3-TCP (http://www.inchem.org/documents/cicads/cicads/cicad56.htm#8.5) notes the following “Negative test results for a micronucleus test in mouse bone marrow (Douglas et al., 1985) and unscheduled DNA synthesis in rat hepatocytes in vivo (Mirsalis et al., 1983)

mentioned in two abstracts cannot be validated because of lack of documentation (e.g., dose, test conditions)."

The study design and results of the Saito-Suzuki et al. dominant lethal test be carefully considered in light of the weight given to the study. As noted above all studies reported only as abstracts should not be given weight unless separate documentation can be obtained.

***2. Evidence indicating the mode of action of carcinogenicity of 1,2,3-trichloropropane was considered. The proposed mode of action includes bioactivation of 1,2,3-trichloropropane leading to the induction of mutations in cancer-related genes. A conclusion was reached that it is possible that this chemical is operating through a mutagenic mode of action, but the database contains limited evidence of in vivo mutagenic events that could lead to the observed cancer. Please comment on whether the weight of the scientific evidence supports this conclusion. Please comment on whether the rationale for this conclusion has been transparently and objectively described. Please comment on data available for 1,2,3-trichloropropane that may support an alternative mode of action.***

***James V. Bruckner***

I concur that the weight of evidence supports mutagenesis, as the primary mode of action of TCP. Mutagenesis and cytotoxicity are clearly due to reactive metabolites, not the parent (unmetabolized) compound. This concept of the mode of action is not well developed. As mentioned in my specific comments, chronic irritation and cell death very likely play an important role in carcinogenesis at the initial respiratory and alimentary portals of entry. Recurring necrosis and regenerative hyperplasia are also likely to be contributory in some other internal organs.

***Richard J. Bull***

The basic argument is valid, but poorly made. A continuum of evidence should be presented beginning with evidence that metabolites of 1,2,3-TCP interact with DNA, evidence that the chemical is genotoxic that largely depends on in vitro data. With some organizational changes in the document, such an argument can be adequately and succinctly developed in a half page of writing rather than regurgitating all the material that is on pages 74-77.
However, this can only be done if the genotoxicity data are more systematically evaluated for consistency and more specifically evaluated in how they support a genotoxic mode of action as these data are presented in the previous sections. Questions such as do the data support a mutagenic mode of action? Do they support a clastogenic mode of action? Or both? Are the inconsistencies among studies attributable to differences in design or methodology? The document implies that negative data in the absence of S9 metabolic activation somehow is contradictory to evidence of activity in the presence of S-9, for example. This section is impossible to evaluate as it is now constructed. One piece of evidence that should be more explicitly used in the mode of action argument is the indications that the metabolite of 1,2,3-TCP, 1,3-dichloropropanone, is an tumor initiator in mouse skin. While it cannot be said for certain that this is the metabolite responsible for the tumors, it does provide clear in vivo evidence that a metabolite of 1,2,3-TCP can act by a mutagenic mechanisms to produce cancer.

As indicated above, there are sufficient data to indicate that 1,2,3-TCP should be considered a genotoxic carcinogen based upon criteria established under the current risk assessment guidelines. However, alternative modes of action have not been sufficiently considered. These should be considered by tumor site. This is most important with respect to forestomach tumors as discussed more fully in responses to the following charge questions. The document should

also recognize that there are often contributions/modifications of carcinogenic responses by non-genotoxic mechanisms even by ‘mutagenic‘ carcinogens. These are generally considered high-dose effects, but that is not always the case. In the present case, I think that high-dose non-genotoxic effects do contribute to liver and kidney tumors observed. However, in the case of forestomach tumors there well may be a low dose contribution of a non-genotoxic mechanisms that needs to be fully explored. There are many data to suggest that administration of several related halogenated compounds by corn oil gavage does increase cell replication rates in the forestomach at early time points (e.g. Ghanayem, BI, Maronpot, RR and Matthews, H.B. 1986. Association of chemically-induced forestomach cell proliferation and carcinogenesis. Cancer Lett. 32(3):271-278.)

***Dale Hattis***

I would strengthen the statement of the conclusion to “very likely” in part because of the known mutagenic properties of the episulfonium activated metabolite, the dose response data on the DNA adducts in relation to carcinogenesis, and the analogy with other mutagenic carcinogens ethylene dibromide, dibromochloropropane, that produce similar or the same type of episulfonium activated intermediates via reactions with glutathione. Moreover I would modify the dose response analysis to reflect likely saturation of the activating metabolism via either depletion of glutathione or saturation of the glutathione transferase enzymes.

I obtained the underlying paper for the La et al. (1995) DNA adduct observations that were partially summarized in Table 4-24 on page 50. It can be seen in Tables 1 and 2 on the following pages that in 7/7 cases for male rats and 8/9 cases for male mice, a 10 fold increase in administered dose gives rise to less than a 10 fold increase in observed adduct levels. This indicates some degree of saturation of metabolism, either via saturation of the relevant glutathione transferase(s) or partial depletion of glutathione. It is possible to use the general Michaelis-Menten enzyme equation to analyze the data from each organs studied to quantify the likely saturation of metabolism on a systemic basis. For each organ we define:

$$\text{Adduct Level}_{\text{organ}} = \frac{V_{\text{organ}} * \text{Dose}}{K_m + \text{Dose}}$$

where Km is the dose at which the rate of activating metabolism is half of its maximum level.

Now such saturation can be analyzed either on an organ-specific basis (yielding the local Km results recorded in the last columns of Tables 1 and 2) or on a systemic basis—the latter assuming that the metabolism (and the saturation) is primarily in the liver where most metabolism is generally assumed to occur in physiologically-based pharmacokinetic (PBPK) modeling. For both rats and mice, the local organ-specific analysis suggests that there is a much greater degree of saturation (lower Kms) in the forestomach than elsewhere in the rodents. Combining all the data for each species, least-squares fits yields systemic estimates of about 38 and 99 mg/kg for the overall Kms for saturable activating metabolism in rats and mice, respectively. Either the local or the systemic results could be used to generate alternative estimates of delivered dose for use in modified dose response modeling for carcinogenesis (see below).

Table 1
DNA Adduct Levels in Relation to Dose for Different Rat Organs, And Estimated Local Km's for Saturable Metabolism

| Organ | Dose (mg/kg) | Adducts (µmole/mole guanine) | Std deviation | Std error | 30/3 Adduct Ratio | Suggested Km (local) (units of external mg/kg) |
|---|---|---|---|---|---|---|
| Forestomach | 3 | 3.7 | [a] | 0.92[a] | | |
| | 30 | 14.6 | | 3.62 | 3.9 | 14.6 |
| | | | | | | |
| Glandular stomach | 3 | 3.8 | | 0.94 | | |
| | 30 | 20.4 | | 5.06 | 5.4 | 28.3 |
| | | | | | | |
| Kidney | 3 | 6.6 | 1.4 | 0.7 | | |
| | 30 | 38.9 | 5 | 2.5 | 5.9 | 35.8 |
| | | | | | | |
| Liver | 3 | 5.4 | 0.7 | 0.35 | | |
| | 30 | 47.6 | 21 | 10.5 | 8.8 | 198 |
| | | | | | | |
| Pancreas | 3 | 5.3 | 1 | 0.5 | | |
| | 30 | 37.8 | 12.8 | 6.4 | 7.1 | 64.1 |
| | | | | | | |
| Spleen | 3 | 0.8 | 0.06 | 0.03 | | |
| | 30 | 7.1 | 1.8 | 0.9 | 8.9 | 210 |
| | | | | | | |
| Tongue | 3 | 4 | | 0.99 | | |
| | 30 | 20.4 | | 5.06 | 5.1 | 25 |

[a]Cases where no standard deviation is given represent the results of measurements on pooled samples from different animals. Their standard error is estimated from the square root of the average sum of squares of the coefficients of variation (standard deviation divided by the mean) for organs where there were separate measurements on 4 animals.

Table 2
DNA Adduct Levels in Relation to Dose for Different Mouse Organs, And Estimated Local Km's for Saturable Metabolism

| Organ | Dose (mg/kg) | Adducts (µmole/mole guanine) | Std deviation | Std error | 60/6 Adduct Ratio | Suggested Km (local) (units of external mg/kg) |
|---|---|---|---|---|---|---|
| Forestomach | 6 | 19.8 | | 7.38 | | |
| | 60 | 41 | | 15.28 | 2.1 | 8.1 |
| Glandular stomach | 6 | 28.1 | | 10.48 | | |
| | 60 | 208.1 | | 77.57 | 7.4 | 148.1 |
| Kidney | 6 | 4.4 | 2.9 | 1.45 | | |
| | 60 | 32.5 | 11.3 | 5.65 | 7.4 | 146.6 |
| Liver | 6 | 12.1 | 4.6 | 2.3 | | |
| | 60 | 59.3 | 21.7 | 10.85 | 4.9 | 45.9 |
| Brain | 6 | 0.43 | 0.11 | 0.055 | | |
| | 60 | 3 | 0.2 | 0.1 | 7.0 | 118.6 |
| Spleen | 6 | 0.61 | | 0.23 | | |
| | 60 | 7.8 | | 2.91 | 12.8 | Not meaningful |
| Heart | 6 | 0.38 | | 0.14 | | |
| | 60 | 2.4 | | 0.89 | 6.3 | 86.6 |
| Lung | 6 | 0.77 | 0.16 | 0.08 | | |
| | 60 | 5.3 | 0.2 | 0.1 | 6.9 | 113.3 |
| Testes | 6 | 0.32 | 0.14 | 0.07 | | |
| | 60 | 1.2 | 0.6 | 0.3 | 3.8 | 26.4 |

[a]Cases where no standard deviation is given represent the results of measurements on pooled sample from different animals. Their standard error is estimated from the square root of the average sum of squares of the coefficients of variation (standard deviation divided by the mean) for organs where there were separate measurements on 4 animals

***Ralph L. Kodell***

The available mode-of-action data on 1,2,3-TCP have been extensively discussed. I believe the weight of evidence supports the conclusion that "it is possible that this chemical is operating through a mutagenic mode of action, but the database contains limited evidence of in vivo mutagenic events that could lead to the observed cancer." The rationale for this conclusion has been transparently and objectively described, and concerns about its validity have been discussed. Unfortunately, studies have not been conducted that show evidence of gene mutation. Available data that either cloud the evidence for a mutagenic mode of action or potentially support an alternative mode of action include the following. The single, major DNA adduct found in many tissues where tumors were observed, including the forestomach, was shown to be S-[1-(hydroxymethyl)-2-($N^7$-guanyl)ethyl]glutathione. However, the mutations found in the forestomach are not consistent with the miscoding properties of this major adduct, and in vivo assays have provided both positive and negative evidence of genotoxicity (Table 4-26). As is stated in the document, the formation of DNA adducts of 1,2,3-TCP in tumors other than where tumors formed is an area of uncertainty associated with the suggested mode of action (p. 73). Data are not available to make a determination about whether other modes of action, such as cytotoxicity followed by regenerative cell proliferation, are plausible.

***Harihara M. Mehendale***

Even though there is no *in vivo* evidence for mutations after exposure to TCP, there is sufficient converging scientific evidence for this mode of action. Even though there is limited evidence for this mode of action in vivo, there is sufficiently compelling scientific weight of evidence to assume this mode of action for carcinogenic effect of TCP. Therefore, the conclusions of document are justified.

***Helmut Zarbl***

As already indicated above, the data in support of a genotoxic mechanism of action are limited. Most significant is the lack of *in vivo* mutagenicity data, other than the Ames test and the fly wing spot test (SMART). The fact of the matter is that direct *in vivo* mutational assays are seldom performed, and are usually deduced from more indirect assays. Clearly the compound is metabolized to reactive intermediates that form adducts with protein and DNA, induces DNA strand breaks, and induces a variety of mutational endpoints, but mechanism of action is inferred from what is known about its metabolisms and the reactive intermediates formed during metabolism. Together, these lines of evidence would seem to make a strong case for a genoticity. However, establishing etiology on the basis of mutation spectra in oncogenes of tumors induced by the compound is not justified, and accumulating evidence caution against the use of such correlations to infer etiology or mechanism. Mutations in tumors may be the product of mutagenesis, but they are just as likely to be the result of selection. These types of data should not be used in risk assessment unless mutation rates in the gene of interest are measured directly in the target cells after the exposure. However, the absence of adducts

consistent with a mutagenic mechanism of action in the target organ is of concern and may suggest that at least in the forestomach, a different mode of action may be operative.

***Lauren Zeise***

The discussion of the mode of action is not sufficiently strong. While the current hypothesis has yet to be proven, it nonetheless is strongly supported by the available evidence. The argument synthesizing the position about the hypothesized mode of action starts by enumerating the data supporting the mode of action. While it misses a few points raised elsewhere in the document, like the strong structural similarity to DBCP a chemical with strong evidence supporting a mutagenic mode of action, it nonetheless lays out the supporting information reasonably well. It then focuses on negative assays and the implication is that these argue against the hypothesis. Six "negatives" are listed.

- The first two focus on assays without metabolic activation systems. Rather than being evidence against the mode of action, these studies actually support it since the hypothesis involves activation of the compound. These first two bullets therefore should be reframed and moved up to the support side of the argument.

- The third bullet refers to the micronucleus assay conducted by Tafazoli and Kirsch-Volders on 1,2,3-trichloropropane along with a four other mutagenic halogenated aliphatic compounds. In this study 1,2,3-trichloropropane was not positive, but none of the other compounds "was able to induce a clear and reproducible linear dose-dependent increase in micronucleus frequencies" although they did show weak response. The authors concluded that "The results of the present work suggested that the comet assay might be a more suitable and sensitive screening method than the micronucleus test for this particular class of compound."

- The fourth bullet refers a study reported in abstract that, as noted above, was discounted by the WHO review as lacking documentation.

- The fifth is a single 1979 study of chromosomal aberrations that may be a true negative. Still the more recent and replicated NTP study found CAs in CHO cells.

- Finally the last bullet refers to an in vivo micronucleus test that the WHO discounted because of inadequate documentation and a single negative dominant lethal study.

Thus the "con" list contains only a couple, of true but non-replicated "negatives." The text then aptly points out that areas of uncertainty exist because standard batteries have not been performed and in vivo testing has been limited and spotty, and that there are not studies prospectively demonstrating gene mutation in vivo. While this is all true, and we do not have confirmation that the compound is acting via a genotoxic mechanism, there still is much stronger evidence than not that a mutagenic mode of action is "likely" and more than just "possible." There is substantial in vitro mutation data and in vivo DNA adduct findings including in target sites – laid out in the document in multiple places and - as well close structural similarities to compounds with strong evidence of mutagenic

MOAs. There is the lack of good evidence for other MOAs. Thus on page 77, given the available evidence, the statement that the compound "may be acting through a mutagenic mode of action" understates the overall weight of the evidence. It would be more concise to report that the compound "is likely to act by a mutagenic MOA.

***3. A two-year oral gavage cancer bioassay (NTP, 1993) was selected as the principal study for the development of an oral slope factor (OSF). Please comment on the appropriateness of the selection of the principal study. Has the rationale for this choice been transparently and objectively described?***

***James V. Bruckner***

This was the only chronic study. Fortunately, it was well designed and conducted.

***Richard J. Bull***

The NTP study was the only option.

***Dale Hattis***

This choice of cancer bioassay is fine. There does not appear to be anything like a comparably appropriate option.

***Ralph L. Kodell***

The NTP oral gavage study chronic study was selected because it was a well-designed study, conducted in both sexes of rats and mice with an adequate number of dose groups and animals per dose group, and examination of appropriate toxicological endpoints. In fact, it is the only chronic study on 1,2,3-TCP that was discussed in the document. Tumor incidences were elevated with increasing doses at several sites across both sexes of the two species, both at point-of-contact and distant organ sites. The study has been transparently and objectively described. I believe the NTP study has been scientifically justified as the principal study.

***Harihara M. Mehendale***

The use of the 2-year gavage study as the principal study is scientifically defensible for cancer risk assessment of TCP. It is a well conducted study. The only controversial or debatable issue is the relevance of corn oil used as the vehicle that has been associated with cancer studies of other chlorinated hydrocarbons such as chloroform. Aside from the vehicle used in such studies, bolus nature of dosing regimen adds another wrinkle because of direct contact of the relatively large dose of test chemical with the target tissues and enzyme systems involved with the metabolism of the test chemical. These issues should be discussed to the fullest extent, especially since the document also contains the results of the studies on the effects of TCP given in drinking water.

***Helmut Zarbl***

A two-year study performed by the NTP often represents the most complete and carefully controlled study of carcinogenicity. In the case of 1,2,3-trichloropropane, this is the only

study that has an adequate sample size to attempt the derivation of an OSF. Unfortunately, in this case the data are less that ideal and have several problems:

1. All tumors were induced by a high dose exposure given by gastric gavage in corn oil. There is evidence to suggest that corn oil can synergize with carcinogens by acting as a co-carcinogen or a tumor promoter, therefore overestimating carcinogenicity.

2. The highest frequency of tumors were at the point of contact in the forestomach, which in the presence of corn oil, can lead to an overestimation of risk

3. Many distal tumors arose in organs (forestomach, hardarian gland, Zymbal;s gland) that have no human homolog, and possibly overestimating human risk.

4. The mouse was clearly more sensitive that the rat, but the rat was used to derive the OSF because the doses used in the mouse overshot the mark. Thus tumor incidence was close to saturation at all doses in the mouse. The decision to use the rat could lead to a significant underestimate of human risk.

The decision to assume a genotoxic mode of action and use the rat data in the forestomach as the basis for deriving the OSL leads to a level of uncertainty that is not completely discussed in the report. This section should be expanded to include a more complete picture of how using the forestomach data could affect risk estimates.

***Lauren Zeise***

The NTP gavage studies do provide adequate basis for cancer dose response modeling and are appropriately selected for this purpose. The rationale is transparently and objectively described. Further support for the approach could be garnered by a systematic look at structurally related compounds and route similarities and differences in carcinogenic potency. As shown in the table above, EDB and DBCP, which similarly form episulfonium ions and produce strong effects local to the site of compound administration as well as at distant sites. The studies of these compounds provide additional support for the use of the gavage study for unit risk estimation.

***4. Data on tumors in multiple organs in F344 rats were used to estimate the oral cancer slope factor. Please comment on the scientific justification and transparency of this analysis. Please comment on the combination of etiologically similar tumor types, benign and malignant tumors of the same cell type, for quantitative purposes. Please specifically comment on EPA's inclusion of the data on forestomach tumors for cancer quantitation in rats following the administration of 1,2,3-trichloropropane. Please comment on the estimation of a statistically appropriate upper bound on total risk (combined slope factor), which describes the risk of developing any combination of tumor types considered, and the quantitative process used to calculate the combined slope factor.***

***James V. Bruckner***

It is reasonable to combine benign and malignant tumors of the same cell type of the same organ. I do not concur with inclusion of data on forestomach tumors for cancer quantification. Oral bolus dosing with a corn oil vehicle is not relevant to actual human exposures to the chemical in food or drinking water. Bolus dosing places an artificially high concentration in direct contact with the mucosa. The corn oil delay systemic absorption, keeping the TCP in contact with the mucosa for an extended period. This results in chronic irritation and inflammation, with their attendant inflammatory mediators and growth promoters. It should also be recognized that a human's stomach differs from the rodent's stomach in that the forestomach is a holding compartment that keeps TCP in contact with the tissue for a relatively long period of time (Proctor et al., 2007).

***Richard J. Bull***

Conducting low dose estimates of unit risk for cancer using the rat data is fine. However, the evidence of carcinogenicity in mice should not be so casually dismissed by the simple statement of uncertainty in the last paragraph on page 123. At the very least some risk values projected from the mouse data (e.g. use a one-hit model from the lowest dose) should be done in an effort to estimate how much of an uncertainty this introduces into the estimate of cancer risk. As pointed out earlier, the mouse is identifiably more sensitive from the data that exists and this will be increased further if the usual dose per unit surface area correction is made. Doses corrected for surface area are lower in the mouse than the rat even if precise modeling of dose-response is not possible. This is an obvious point that requires more exploration and discussion in the section that deals with uncertainties in section 6.2.3.

Concerns related to increased yields of forestomach tumors in studies of halogenated hydrocarbons administered by corn oil gavage are discussed in No. 2 above.

***Dale Hattis***

I think in all the cases of these specific queries the analysts have made reasonable choices. However in the light of the clear saturation of the DNA adduct formation observations (Tables 1 and 2 above) I think the analysts should have used the results of simple Michaelis-Menten modeling to transform the administered doses to multiples of "low-dose equivalents" when

projecting low dose risks. This is particularly true for the forestomach tumors, where saturation appears to be most prominent.

Essentially the idea of "low dose equivalents" is to remove the effects of high dose metabolic saturation from the dose response model fitting and the derivation of the estimated low dose slope factor (i.e., the carcinogenesis potency estimate). The effect of metabolic saturation is to diminish the effective adduct-generating metabolism at higher doses by the factor 1/(Dose + Km) from the Michaelis-Menten equation given earlier. The "low dose equivalent" is an estimate of the mg/kg dose that would have been sufficient to generate the adducts expected at a specific dose level if there were no saturation effect (that is, if the number of available enzyme molecules were undiminished by receptor occupancy). The low dose equivalent must necessarily be lower that the actual administered dose. Essentially we use the internal dosimetry indicated by the La et al. (1995) data to do an end run around the need to develop an elaborate PBPK model to assess internal doses in relation to external doses.

Tables 3 and 4 show the effects of varying Km assumptions on the effective "low-dose equivalents" for delivered DNA-adduct production indicated by the local and systemic Kms estimated for males of the two species. As might be expected, as estimates of Km rise, the saturation effect diminishes until for very high Km's the "Low Dose Equivalents" approach the external doses. The first row of bolded numbers in these tables represents the expectations for the local Km's estimated from the forestomach adduct data in each species. The second row of bolded numbers uses the estimates of systemic Kms derived from a weighted least squares analysis of the data from all available organs. The numbers in the final three columns of these rows can be directly input to dose response models in place of the administered doses to achieve dose response projections for low doses that are not distorted by the degree of saturation of activating metabolism indicated by the La et al. (1995) DNA adduct data.

Table 3
Low Dose Equivalents (mg/kg) for Various External Dose Groups Used in the Male Rat Bioassay Based on Different Assumptions for Km

| Km (mg/kg administered. dose) | Low Dose mg/kg Equivalents for 3 mg/kg admin. dose | Low Dose mg/kg Equivalents for 10 mg/kg admin. dose | Low Dose mg/kg Equivalents for 30 mg/kg admin. dose |
|---|---|---|---|
| 1 | 0.75 | 0.91 | 0.97 |
| 3 | 1.50 | 2.31 | 2.73 |
| 10 | 2.31 | 5.00 | 7.50 |
| **14.6** | **2.49** | **5.93** | **9.82** |
| 30 | 2.73 | 7.50 | 15.00 |
| **37.7** | **2.78** | **7.90** | **16.71** |
| 100 | 2.91 | 9.09 | 23.08 |
| 300 | 2.97 | 9.68 | 27.27 |
| 10,000 | 3.00 | 9.99 | 29.91 |
| 1,000,000 | 3.00 | 10.00 | 30.00 |

Table 4
Low Dose Equivalents (mg/kg) for Various External Dose Groups Used in the Male Mouse Bioassay Based on Different Assumptions for Km

| Km (mg/kg administered. dose) | Low Dose mg/kg Equivalents for 6 mg/kg admin. dose | Low Dose mg/kg Equivalents for 20 mg/kg admin. dose | Low Dose mg/kg Equivalents for 60 mg/kg admin. dose |
|---|---|---|---|
| 2 | 1.50 | 1.82 | 1.94 |
| 6 | 3.00 | 4.62 | 5.45 |
| **8.1** | **3.45** | **5.77** | **7.14** |
| 20 | 4.62 | 10.00 | 15.00 |
| 60 | 5.45 | 15.00 | 30.00 |
| **99** | **5.66** | **16.64** | **37.38** |
| 100 | 5.66 | 16.67 | 37.50 |
| 300 | 5.88 | 18.75 | 50.00 |
| 10,000 | 6.00 | 19.96 | 59.64 |
| 1,000,000 | 6.00 | 20.00 | 60.00 |

To illustrate the direction and approximate magnitude of change in estimated low dose risks that these revised dose estimates would imply, Tables 5 and 6 compare the MLE and 95% upper confidence limits of the slope factors (q1's and q1*'s) for "alimentary system total squamous neoplasms," using a simple one-stage carcinogenesis model, implemented in a simple Excel program published by Haas (1994).[*] This model was just applied to the whole-life tumor incidence data provided in summary form in the draft IRIS document. For this illustrative purpose I did not attempt to reproduce the full Weibul time-to-tumor model that EPA used in the final analysis. However I expect that the magnitude and directions for change in the results would be similar with the more elaborate time-to-tumor model. I will provide EPA with the Excel spreadsheet where all these numbers were derived as a supplement to this report.

As is usual, the model proceeded by first attempting to fit all data points to the model. The resulting P values were used to guide judgments of the acceptability of the one-stage model to the data (because of the convex/saturating shape of the dose response relationships indicated by the empirical data, there were no cases where it would have been helpful to include a $dose^2$ or higher order term in the model.) Where the initial fit to data from all four dose groups (three doses + control) was unacceptable, the highest dose group data were deleted, yielding the results in the lower portions of Tables 5 and 6.

It can be seen in the second columns of Tables 5 and 6 that proceeding from the nominal administered doses to the low dose equivalents estimated from the systemic and local forestomach Kms increased the fit of the data to the model for the alimentary system tumor results in both species. The rat data show a marginal (P just over 0.05) fit to the model using the Km estimated from all the adduct observations, but the fit improves to a completely acceptable P = 0.46 when the local forestomach Km is used to estimate the effective delivered doses of active metabolites. For the mouse data, the fit to the full data set (all four dose groups including the control) improves from completely unacceptable values of less than 1 in a million to the barely acceptable P = .059.

In terms of expected low dose risks, the data in Table 5 indicate that removing the high-dose saturation of metabolic activation leads to slightly less than a two-fold upward revision of the central estimate of the slope factor (q1) for the rat data. For the mouse data (Table 6) the indicated q1 is revised upward by slightly more than three-fold after removal of the effects of saturation using the lower estimate of Km from the forestomach DNA adduct data. The final analyses for mice vs. rats in the third lines of each table indicates that mice are about twice as sensitive as rats to the carcinogenic action of TCP. Despite the reservations noted in the document with respect to the high doses used to generate the mouse tumor data, I think that they form a meaningful and important part of the basis for projecting of likely cancer risks from this compound. I think the final estimation of the cancer slope factor for TCP should reflect both the apparently increased sensitivity for the mice and organ-specific or systemic Michaelis-Menten corrections of delivered dose, as illustrated above—combined with similarly modified analyses using the Weibul time to tumor modeling used in the current document.

[*] Haas, C. N. "Dose Response Analysis Using Spreadsheets" Risk Analysis 14:1097-1100 (1994).

Table 5
Results of Fitting a One-Stage Carcinogenesis Model to the Alimentary System Tumor Data for Rats Using Administered Dose vs Two Measures of "Low Dose Equivalent" Estimates of Delivered DNA-Adduct Doses

| Data Set and Dosimeter | P for Fit | MLE q1 | UCL q1 | BMD10 | BMDLo |
|---|---|---|---|---|---|
| Full data set, nominal doses | 4E-04 | 0.199 | Not done | 0.529 | Not done |
| Full data set, systemic Km low dose equiv doses | 0.054 | 0.262 | 0.314 | 0.402 | 0.336 |
| Full data set, forestomach only Km low dose equiv doses | 0.46 | 0.351 | 0.417 | 0.300 | 0.253 |
| | | | | | |
| | | | | | |
| | | | | | |
| 3 lower dose points nominal doses | 0.009 | 0.240 | 0.294 | 0.438 | 0.358 |

Table 6
Results of Fitting a One-Stage Carcinogenesis Model to the Alimentary System Tumor Data for Mice Using Administered Dose vs Two Measures of "Low Dose Equivalent" Estimates of Delivered DNA-Adduct Doses

| Data Set and Dosimeter | P for Fit | MLE q1 | UCL q1 | BMD10 | BMDLo |
|---|---|---|---|---|---|
| Full data set, nominal doses | 8E-10 | 0.216 | Not done | 0.489 | Not done |
| Full data set, systemic Km low dose equiv doses | 3E-07 | 0.262 | Not done | 0.401 | Not done |
| Full data set, forestomach Km low dose equiv doses | 0.059 | 0.664 | 0.823 | 0.159 | 0.128 |
| | | | | | |
| | | | | | |
| 3 lower dose points nominal doses | 3E-06 | 0.288 | 0.294 | 0.365 | 0.358 |
| 3 point systemic Km low dose equiv doses | 2E-05 | 0.324 | Not done | 0.325 | Not done |

***Ralph L. Kodell***

I agree with the combination of etiologically similar tumor types, benign and malignant tumors of the same type, for quantitative purposes.

I disagree with the inclusion of the data on forestomach tumors for cancer quantitation in rats following the administration of 1,2,3-TCP. It is pointed out in the document that the relevance of forestomach tumors in rodents may be of concern because humans do not have a forestomach. The single, major DNA adduct found in many tissues where tumors were observed, including the forestomach, was shown to be S-[1-(hydroxymethyl)-2-($N^7$-guanyl)ethyl]glutathione. However, the mutations found in the forestomach are not consistent with the miscoding properties of this major adduct. Thus, including the forestomach tumors when the postulated MOA is mutagenic seems inconsistent. However, it seems appropriate to include the data on oral cavity papillomas or carcinomas, which were combined in the assessment with the forestomach tumors to form a category called alimentary system total squamous neoplasms. I recommend that these data be re-modeled after removing the forestomach tumors. There are other tumor types (e.g., Zymbal's gland) that also may have questionable relevance to estimating human cancer risk.

I believe that it is appropriate to include data on tumors in multiple organs to estimate the oral cancer slope factor. However, I have some questions and concerns about the quantitative process used to calculate the combined slope factor. I question why the data on mouse tumors were not modeled. It is stated on page 101 that the male and female tumor incidence data were not suitable for deriving low-dose quantitative risk estimates. It is stated that the NTP study design unfortunately missed nearly all of the relevant dose-response range for mice, with both male and female mice having nearly 100% responses at the lowest exposure level, so that there is no information concerning the dose-response relationships at lower exposure levels that could be compared with the rat data. However, it is only the forestomach tumors in mice (Table 4-15) that gave nearly a 100% response at the lowest dose. Several other tumor types gave dose-response information for modeling that could be compared to the rat data. Also, there might be differences in time to tumor development even where raw incidences are similar, and the multistage Weibull model can account for these differences. I believe the mouse data should be modeled. In addition, it is stated on page 101 that NTP noted an apparent dose-related increase in hepatocellular adenomas and carcinomas in male rats, but that the incidences were not individually statistically significantly different from controls. Given that the chronic RfD was set based on increases in liver weights in rats, it seems natural to include hepatocellular adenomas and carcinomas along with the other tumor types in deriving a combined oral cancer slope factor.

Given the postulated mode of action, I believe that linear-low-dose extrapolation is appropriate. It is suggested by the postulated mutagenic mode of carcinogenic action and it is the default option if the mode of action is not understood.

Due mainly to the early termination of the highest dose group in the rat (and mouse) experiment, the multistage-Weibull model was fitted to the tumor data because it has the potential to reflect the influence of competing risks and intercurrent mortality on site-specific tumor rates. I think it was appropriate to use this model separately on the individual tumor types, and then to combine the results for the combined oral slope factor. However, I have some questions and concerns about the modeling. Although NTP stated that neoplasms of the stomach and oral mucosa and mammary tumors were the principal cause of death of most animals dying or killed moribund before the end of the study, all tumors were treated as incidental because it was not clear that a determination could be made for each animal with multiple tumors. I don't follow this reasoning. I believe that those specific tumor types in all dead and killed moribund animals ought to be modeled as fatal tumors. In the case of mammary tumors, this might alleviate some of the modeling problems discussed on page 181.

I think the estimation of a statistically appropriate upper bound on total risk (combined slope factor) is scientifically justified and transparently described. I believe it is an appropriate reflection of the risk of developing any combination of tumor types considered. I think the method is statistically sound. It would be good if a reference to the method were cited. For example, it seems closely related to the method of Gaylor and Chen ("A simple upper limit for the sum of the risks of the components in a mixture," *Risk Analysis* 16, 395-398, 1996). Although the method is statistically sound, I think the terminology might be a little confusing under IV) on page 106 where the term "individual risk estimates" is used. If I understand things correctly, that term means "individual oral slope factors" or, equivalently, "individual estimates of risk per unit of exposure." I suggest that the terminology be changed. Also, I think the column heading "Cancer risk value at BMD" in Table 5-7 is misleading. I suggest changing it to "MLE of slope." It would be informative to show a specific example of how the equation on page 106 was used to deduce the variance for one of the tumor types. For example, for the first row of Table 5-7, the calculation is 3.1 = 2.0 + 1.645 x s.d., which gives s.d. = (3.1 – 2.0)/1.645 = 0.669, so that the variance for that estimated slope factor is $(0.669)^2 = 0.447$.

***Harihara M. Mehendale***

Use of tumors in multiple organs seems reasonable for estimation of the cancer slope factor. However, I have reservation regarding the use of cancer data on the fore-stomach. The rationale and justification for including the fore-stomach and any other areas not applicable for humans needs strengthening.

***Helmut Zarbl***

The use of etiologically similar tumors benign lesion in estimating the upper bound of the OSL is in principle acceptable. Scientists appreciate that cancer is a continuum that involves the progression of premalignant lesion to carcinomas. However, care must be taken when using this approach. The frequency with which premaliganat lesion progress to carcinomas can, in many cases, be quite low. For example less than 1% of Barrett's esophagus lesions progress to carcinomas. In addition, premalignant lesion, for example

skin papillomas, hyperplastic liver nodules, and intraductal proliferations in mammary glands can be reversed by removing the exposure. Thus the inclusion of premalignant lesions can lead to a very high overestimate of risk. The effect of including these lesions should be quantified and discussed for comparison.

The decision to include the data on forestomach tumors is not justified and probably leads to an overestimation of human risk. While the lack of a homologous organ in man is by itself not a sufficient reason to dismiss the forestomach data, there are several other factors that need to be considered. First, this organ is the point of contact for a compound given as a bolus in corn oil. This dose regime clearly leads to elevated exposures relative to exposure in drinking water, where the compound is cleared very effectively. Giving the compound as a bolus also leads the local depletion of xenobiotic metabolism. Gavage can lead to irritation and corn oil is known to synergize with carcinogen exposures. The lack of data to support a genotoxic mechanism in the forestomach (e.g. lack of expected adducts) suggests a non-mutagenic mechanism. When taken together, these factors would argue strongly against inclusion of the forestomach tumor data.

***Lauren Zeise***

A reasonable approach was taken to select the species for analysis. While the mouse may be a bit more sensitive than the rat, and quantitatively the studies appear fairly similar – at about the same dose level as the lowest dose in the mouse study the incidences in the rats are quite high. However one cannot be entirely sure of species similarities because of the high incidence of alimentary tumor in the lowest dose tested in the mouse in both sexes muddies the water. Also, early mortality in the mouse study was observed in all treated animals, including the lowest dose group, so that only limited insight can be gained by comparing findings at non-alimentary sites - a relatively large fraction of animals were not alive for the development of late occurring tumors. On the other hand, the alimentary tumors in the rats are consistent with a linear dose response relationship and offer little additional insight on the dose response in the low dose region. Thus the argument that the rat offers a lot more over the mouse and is a lot more suitable than the mouse is not completely compelling. Performing the analysis on the mouse data to at a minimum provide some perspective on this uncertainty regarding the differences in sensitivity and the extent potency may be underestimated.

As discussed above in response to General Charge Question 3, judging from the studies of similar compounds, confounding by gavage is unlikely to explain the large cancer effect seen. A systematic and quantitative review of structurally related compounds using existing assessments can provide some insight on this issue. Perusing the TD50 tables for DBCP quickly, the findings for inhalation and gavage are quantitatively comparable on a mg/kg basis. The Reed et al. analysis and other documents provide quantitative characterizations for the diet study. Absent strong evidence that the forestomach findings from the gavage study over predicts activities local to the site of compound administration, the forestomach should not be excluded from the assessment. Even if over prediction is found, rather than exclude the site, an adjustment can be made.

The justification provided for the application of linear low dose extrapolation is sound and described in a transparent and straightforward fashion. The application of the multistage Weibull model is appropriate given the intercurrent mortality in the male and female rat experiments. The approach to separate modeling of each tabulated tumor type/site is appropriate and adequately justified. Differing features of the pharmacokinetics can be another cause of site specific dose response relationships, but the explanation provided is general enough to capture this as well.

One issue worthy of more consideration is the treatment of all tumors as incidental. The time dependent modeling requires one of two possible extreme assumptions, neither of which can be true – that the tumors are very rapidly lethal, or that the tumors never kill the animal. Certainly given the large mortality resulting from the forestomach carcinoma, the assumption cannot be true for this lesion. On the other hand, the degree of lethality must also be considered. In any event, some sensitivity analysis to check the importance of this assumption on the results appears in order.

A reasonable strategy was applied in selecting the order of the models, conducting the analysis, performing the multi-site analysis. The modeling was well thought out and described. As an alternative to the approach of estimating the potency and bounds from the BMD, BMDL and BMDR, estimates of the distribution of $q_1(t-t_0)^z$ at t=lifetime could be obtained for each tumor site/type and the distributions statistically combined using Monte Carlo analysis. This would be somewhat more accurate, especially in cases with substantial contributions from higher order terms in the multistage analysis, where the MLE on $q_1$ may be near zero or unstable. Coming up with a combined estimate for the multiple sites is consistent with the recommendations of the 1994 National Research Council's Science and Judgment report.

Another issue for consideration is the development of an inhalation unit risk for the compound, if not in the current assessment in the relative near term as a separate exercise. As illustrated in the table in the response to charge question 1, structurally similar compounds (e.g., DBCP, EDB) have similar tumor findings and studies by inhalation routes. Consideration of differences in oral and inhalation potencies for these compounds can provide the basis for development of an inhalation unit risk. These compounds like 1,2,3-TCP produce tumors local to and distant from the site of compound administration, but for these compounds this has been seen for the inhalation route as well, unlike TCP which has not been so tested. As a start to inform the selection of an inhalation unit risk, comparisons of unit risks across route could be done for EDB and DBCP. Both compounds like 1,2,3-TCP form the highly reactive episulfonium ion. To move toward a unit risk for the inhalation route, comparisons of potencies by gavage, inhalation, and for DBCP the diet, can be made. This could be approaches with TD50s published in the Cancer Potency Database (http://potency.berkeley.edu/chemnameindex.html). These values are time corrected by assuming cancer increases with the third power of age. A linear dose response is used to generate them, but this should not introduce too much error and it will enable a systematic look at cross route differences.

The combination of similar benign and malignant tumors arising from the same cell type for quantitative analysis is a longstanding practice in cancer risk assessment and there are no compelling reasons to depart from the standard practice for this case.

The discussion on page 112 provides very good justification for including the forestomach in unit risk calculations and the discussion will not be repeated here. In addition, 1,2,3-TCP produces tumors local to the site of first contact as well as tumors at distant sites, and affects different cell types. Structurally similar compounds produce lung and nasal cancer when exposure occurs via inhalation, and forestomach and other alimentary cancers with oral exposures. Thus it is reasonable to assume that site specific cancers will occur in humans, and the site of first contact is a concern, even though humans do not have forestomachs.

## V. SPECIFIC OBSERVATIONS

***James V. Bruckner***

p.4, pgr. 3, lines 5-8: I agree that the % absorbed orally would have been greater than 75 – 84%. The ending of the last sentence is confusing. It can be clarified by stating that the feces likely contained unabsorbed TCP and TCP metabolites.

p. 5, pgr. 1: It should be stated in the text that Mahmood et al. (1991) administered TCP by corn oil gavage. Highly-lipophilic chemicals like TCP partition into oils, and are not absorbed systemically to a significant extent until the oil is emulsified and digested. This substantially delays their absorption.

p. 5, pgr. 2: More information about the time-courses of $^{14}$C-TCP/metabolite distribution in blood and key tissues (e.g., liver, kidney, fat) should be given. Include the terminal elimination half-lives, if they were stated by Volp et al. (1984).

p. 8, pgr. 1, lines 8-12: It is not clear to this reviewer why the findings of Weber and Sipes (1990) support the metabolic pathways proposed by Mahmood et al. (1991).

p. 9, pgr. 3: More detail should be given about the toxicokinetic findings of Volp et al. (1984). It should be stated that the researchers quantified the tissue deposition of both parent (unchanged) TCP and radiolabel (primarily TCP metabolites at all mid to later time-points) over time following iv injection of male F-344 rats with 3.6 mg $^{14}$C-TCP/kg. Some 95% of this dosage was metabolized. The parent compound was deposited largely in adipose tissue, as would be anticipated for such a lipophilic compound. The PBPK model accurately predicted the time-courses of iv-injected TCP in blood and tissues, although this would be anticipated, since adjustments were made in some of the model input parameters, based on the empirical data.

pp. 10 & 11: The liver and kidney weight changes are clearly presented in the text and in Tables 4-1 and 4-2. It can be stated in the text that increases in relative liver and kidney weights in male and female rats are dose-dependent, but increases in absolute organ weights are not, other than the absolute liver weights of the females.

p. 12, last line: Was 125 mg/kg the LOAEL for histopathological changes in the liver and kidney? This should be stated. What was the extent of the hepatic necrosis in the one male rat in the 32 and the 63 mg/kg/day groups?

p. 15, pgr. 2, lines 3 & 4: It is stated here that NTP considered the dose-response increases in liver and kidney weights to be consistent with the histopathological changes in these organs. It is stated in the last two lines on page 11 that NTP (1983) did not consider the changes in relative organ weights to be associated with organ toxicity. As only the absolute liver weight of female rats appeared to be dose-dependent, how can the initial statement be valid?

p. 15, pgr. 2, lines 5 & 6: It does not seem reasonable to establish a LOAEL of 32 mg/kg for hepatocellular necrosis on the basis of a morphological change in 1 of 10 male rats. There were

no increases in serum enzymes in this group. Apparently, only SDH was elevated (slightly) in the 63 mg/kg males.

p. 19, pgr. 2, lines 3 & 4: Is it possible to more specific about increased liver weights being consistent with histopathological results (e.g., the most substantial increases in liver weight and histopathological changes occurred in the two highest dosage groups of male and female mice)?

p. 20, pgr. 1: Can the statistical significance of any changes in absolute organ weights be determined in Merrick et al. (1991)? The decreases in body weight gain, in of themselves, result in increases in relative organ weights.

p. 20, pgr. 4, lines 4-6: There is not a dose-related increase in lymph node hyperplasia in the male rats.

p. 21, pgr. 1, lines 1 & 2: It is problematic to establish a LOAEL of 1.5 mg/kg for myocardial necrosis on the basis of morphological changes in 1 or 2 of 10 animals in the 1.5, 7.4 and 15 mg/kg groups. Is there any information on the incidence/severity of this lesion in S-D historical controls?

p. 21, pgr. 2, line 3: Emulphor assures a stable aqueous emulsion of TCP, rather than solubilizing the lipophilic chemical in drinking water.

p. 22, pgr. 1, lines 12 & 13: It is worthwhile to point out that the NOAEL and LOAEL values from the drinking water study of Villeneuve et al. (1985) are substantially higher than for the preceding gavage studies. As TCP is readily absorbed from the GI tract, bolus dosing results in relatively high blood and target organ TCP levels. Ingestion of the same total dose over a period of hours will result in lower TCP levels and less pronounced cytotoxicity. The findings of La et al. (1996), as described in the second paragraph on page 51 of the current document, illustrate this phenomenon.

p. 23, lines 3-5: Were hematology, clinical chemistry and organ weight data obtained from survivors at 15 months? These results should be described in the text of the document.

Tables 4-9b and 4-10b: The 3 mg/kg absolute liver and kidney weight values for male rats do not appear to be statistically significantly elevated over their controls.

p. 27, last 3 lines and p. 28, first 3 lines: See comment above.

p. 33, pgr. 1, lines 16 & 17: Should the word “Absolute” be inserted at the beginning of these two sentences (i.e., before the words “Brain” and “Ovary”, as well as “spleen” and “testis”?

p. 35, lines 5 & 6: Was a morphometric procedure used to reach the diagnosis of hepatocellular hypertrophy?

p. 36, last 3 lines: It is somewhat confusing to read about the protocols and effect levels of Johansen et al. (1988) on page 33, only to encounter two lower exposure concentrations in Table

4-18. One way to avoid the confusion would be to add a short introductory paragraph, at the top of page 33, briefly describing the 3 phases of the investigation.

p. 37, pgr. 5: Same recommendation for introducing the first and second phases of the investigation by Miller et al. (1987a, b).

p. 39, pgr. 1, lines 7 & 8: Although no histopathological changes were evident in the testes, the decreases in weight may have been reflected by decreased sperm counts.

p. 41, top: It would be helpful to add a paragraph summarizing the more important NOAEL and LOAEL values from the work of Miller et al. (1987a, b).

p. 49, lines 1-3: Some of the reported findings of Weber and Sipes (1990) are confounding. One would anticipate GSH depletion to result in decreased production of episulfonium ions and decreased protein binding. It is also surprising that induction of CYP2B1/2 would markedly reduce TCP metabolism/binding in rats. The increased protein binding following inhibition of CYPs with SKF 525-A may result from a shift to the GSH pathway. Both the oxidative and GSH pathways generate cytotoxic, mutagenic metabolites from DBCP (Omichinski et al., 1988a,b; Soderlund et al., 1995).

p. 50, Table 4-24: Inclusion of this table was an excellent idea. It allows the reader to readily compare tumor incidence with DNA adduct levels in each target tissue for different TCP doses.

p. 60, pgr. 3, lines 4-7: It would be useful to include the organs in which DBCP was found to be carcinogenic in the NCI (1978) bioassay. This will reinforce the fact that DBCP is also a multi-site carcinogen.

p. 60, pgr. 4: ATSDR's (1992) *Toxicological Profile for DBCP* could also be cited as a general source of information about the halocarbon's metabolism, modes of action, cytotoxicity, carcinogenicity, etc.

p. 61, pgr. 2: It should also be pointed out that TCP is rapidly and extensively absorbed from the GI tract. Although no information is apparently available on absorption from the lungs, numerous studies of closely-related volatile organic chemicals (VOCs) demonstrate that VOCs are absorbed even more quickly from the lungs (Bruckner et al., 2008).

Reactive VOC metabolites produced in the microsomes, cytoplasm or mitochondria primarily bind to enzymatic and structural proteins *in situ* (in the immediate proximity of their formation). This is a general, though logical mode of cytotoxic (i.e., noncancer) action for this class of chemicals (i.e., halocarbons).

p. 63, lines 1-4: The document's authors should note that the degree, or extent of hepatocellular injury was quite modest, even with the highest TCP dosages at which dysplasia and neoplasia occurred.

p. 63, pgr. 3, lines 4-9: It would be instructive to clarify that mild clinical chemical and histopathological findings paralleled one another. The more striking and toxicologically-significant findings were signs of chronic irritation, hyperplasia, dysplasia and neoplasia in a variety of tissues.

p. 63, pgr. 3, lines 3 & 4: Development of tolerance is another explanation for the diminution of adverse effects upon chronic dosing with TCP. NTP (1993), for example, saw an elevation in serum ALT in male rats at week 8, but not at the termination of the 17-week study. Seven female mice died within 2 weeks of receiving 250 mg TCP/kg/day, though only one additional animal in this group succumbed by week 17. Bruckner et al. (1989) reported development of resistance to the hepatotoxicity of 1,2-dichloropropane during 10 days of oral bolus dosing of rats.

p. 64, pgr. 1, line 7: It is surprising that no adverse effects on male reproductive performance or fertility were observed. Gavage of male rats with 500 or 750 mg/kg of 1,2-dichloropropane daily for 13 weeks produced degenerative changes of the testicular epithelium and epididymal spermatogonia (Bruckner et al., 1989). DBCP and EDB, of course, are also classic spermatotoxins.

p. 64, pgr. 3: The test species should be identified.

p. 66, pgr. 2: There is no mention here or in the full accounts of the NTP (1993) findings of tumor incidences in 3 or 10 mg/kg mice or rats at the termination of the 2-year bioassay. These results should be compared with those at the 15-month sacrifice.

p. 66, next to last line: The words “multisite, multispecies” should be inserted at the end of this line to better characterize the carcinogenicity of DBCP.

p. 67, pgr. 1, lines 1 & 2: Other modes of action likely contribute to TCP’s carcinogenicity. All exposure levels apparently caused irritation and chronic inflammation of tissues at the initial sites of contact in the pulmonary and GI tracts. Focal areas of necrosis and regenerative hyperplasia were also usually seen.

p. 67, next to last line: The word “metabolite(s)” should be inserted between “1,2,3-trichloropropane” and “with.”

p. 68, pgr. 2: Weber and Sipes’ (1990) finding that BSO-induced GSH depletion causes a decrease in hepatic DNA binding contradicts what would be anticipated. Lower GSH levels would be expected to reduce formation of GSH conjugates and the ensuing episulfonium ions. Unfortunately, there has been little subsequent research other than their 1992 paper to answer the questions raised by their 1990 publication. DBCP metabolic activation has received much more attention. Both oxidative and GSH conjugation metabolites contribute to DBCP’s adverse effects.

p. 68, pgr. 3, line 10: Again, tumor data from NTP’s (1993) 2-year sacrifice were not described in the current document.

p. 69, pgr. 1, line 6: The S9 fraction contains both cytosolic and microsomal enzymes, so both P450-catalyzed oxidation and GSH S-transferase-catalyzed conjugation of TCP occurs. Provision of an excess of NADPH "drives" the metabolism in favor of oxidative reactions. This induced/ "souped up" metabolic system is typically far in excess of what occurs *in vivo.*

p. 71, pgr. 4, lines 1 & 2: It should be mentioned here again that there was no increase in binding after the second TCP dose, but a doubling of the original value with the third dose. This amounts to something less than additivity.

p. 71, pgr. 4, line 6: It would be helpful to readers to express all *in vivo* doses in mg/kg.

p. 72, pgr. 1, lines 2 & 3: It is stated here that metabolism of TCP to its metabolites may be a key event in the mutagenic mode of action. This is too weak a statement. The documents' authors previously stated in lines 21 & 22 of the first full paragraph that "…the metabolism of TCP is necessary to activate the chemical's mutagenic potential."

p. 72, pgr. 3: This reviewer seriously doubts that the parent compound reacts with or binds to cellular proteins or DNA. In-depth studies of many structurally-related VOCs consistently demonstrate that the original compounds must undergo metabolic activation to reactive metabolites (Bruckner et al., 2008).

p. 73, pgr. 1, lines 11-15: It is important to point out here that other modes, or mechanisms of action may also be involved. As mentioned previously, the continued presence of inflammatory and growth mediators in areas of chronic inflammation and ongoing cell division may be biologically significant. 1,1,2-Trichloroethylene, for example, is generally believed to produce renal tumors in rats and humans by a mixed (cellular mutations and proliferation) mechanism of action (Bruning and Bolt, 2000).

p. 73, pgr. 2, lines 3-6: It is related here that DBCP induced DNA damage at concentrations lower than those required for cytotoxicity. Carcinogenicity and cytotoxicity were elicited by the same doses of TCP in the NTP and NCI bioassays.

p. 74, line 1: Remove the word "may." There is a clear body of evidence detailed below that reactive metabolites of TCP are responsible for DNA binding and mutagenicity.

p. 74, pgr. 2, line 2: Insert the word "certain" between "for" and "nucleic."

p. 76, pgr.2, lines 3 & 4: There is a substantial database on the toxicokinetics of halocarbons and other VOCs in rodents versus humans. It is routinely assumed that 100% of ingested halocarbons are systemically absorbed by mammalian species. The extent of P450-mediated metabolism is usually in the following order: mice>>rats>humans. First-pass hepatic elimination of well-metabolized, orally-administered halocarbons is therefore greater in rodents than in humans. Systemic absorption of inhaled VOCs is largely governed by a subject's respiratory/alveolar ventilation rate, blood:air partition coefficient, cardiac output and VOC metabolic rate (Astrand et al., 1975; Bruckner et al., 2008). Each of these is substantially higher in mice and rats than in humans (Gargas et al., 1989; Brown et al., 1997). Thus, the internal dose

of TCP received upon equivalent inhalation exposures will be substantially greater in mice than rats, and in rats than humans. A study in which rats and humans were subjected to an equivalent inhalation exposure to perchloroethylene (PERC) showed that the rats received a 7.7-fold greater internal dose of PERC and metaboligically activated a substantially greater amount of the halocarbon (Volkel et al., 1998). Levels of covalent protein adducts were much lower in the blood of humans than rats exposed to PERC under identical exposure conditions (Pahler et al. 1999). Glutathione S-transferases catalyze the formation of reactive, cytotoxic episulfonium ions from DBCP in hepatocytes, testicular epithelium and other cell types. Rat testicular cells are more efficient than human cells in metabolically activating DBCP (Bjorge et al., 1996). These researchers found much lower covalent binding and DNA single strand breaks in the human cells. It should also be noted that human livers express much higher levels of epoxide hydrolase than do mouse livers. Epoxide hydrolase detoxifies epoxides such episulfonium ions.

p. 77, pgr. 4, line 4: It may be advisable here to give a brief explanation of the logic behind assuming that early-life exposures to chemicals such as TCP may result in an increased lifetime cancer risk.

p. 78, pgr.2, lines 5-7: Enhanced CYP450 activities may give rise to increased amounts of DNA-reactive, as well as cytotoxic metabolites. The mutagen, 1,3-dichloroacetone, is formed via a CYP450-catalyzed oxidative pathway (Weber and Sipes, 1992). Episulfonium ions are also hypothesized to be formed as a result of glutathione conjugation of an oxidative metabolite (Figure 3-1). Increases in the capacities of these pathways should result in increased cancer risk from moderate to high doses of well-metabolized halocarbons such as TCP. This is not the case for trace to low levels of such compounds present in environmental media. As most people have amounts of CYP450s and GSH S-transferases far in excess of those required to metabolize all of trace levels of halocarbons, even more enzyme due to polymorphisms or inducing agents is of no consequence (Kedderis, 1997; Lipscomb et al., 2003).

p. 80, pgr. 3, line 8: Define the abbreviation BMR the initial time it appears in the text.
Does a BMR of 10% imply that a 10% increase over controls in liver weight has been selected as a point of departure?

p. 90, pgr. 2, lines 1 & 2: It is still not clear why an empirical NOAEL is not an actual NOAEL.

p. 91, pgr. 2, lines 1 & 2: Does inhaled TCP come into contact with bronchus-associated lymphoid tissue? How much of a cellular barrier prevents direct contact?

p. 93, pgr. 1, lines 2-4: Again, there were no dose-dependent increases in absolute or relative liver weights in male or female rats inhaling TCP over a 13-week period. Many of the groups' liver weights were significantly increased over controls, but not in a dose-dependent manner.

p. 97, last 2 lines & p. 98, first 2 lines: It is quite likely that certain tumors would be caused by lower doses of TCP than were administered in the NTP (1993) bioassay. It should also be noted, as previously mentioned in this review, that ingestion of the total administered dosages in drinking water (i.e., in divided doses) will result in lower covalent binding, cytotoxicity, and very likely carcinogenicity. Administration of the entire dosage at one time also results in direct

contact of a relatively high concentration of TCP with tissues of the respiratory and GI tracts. This undoubtedly produces more pronounced irritation, inflammation, cell death and eventually an increased tumor incidence at portals of entry.

p. 98, last pgr.: Did the document's authors contrast the incidence of benign and malignant tumors of each tissue at the interim and final sacrifices, in order to make a determination about the progression of TCP-induced benign tumors to malignant tumors?

p. 111, pgr. 2: It is clear that TCP itself is not cytoxic, unless there is a high enough concentration to cause irritation and inflammation at portals of entry into the body. A sufficiently high brain concentration of TCP and most other unmetaobolized VOCs will inhibit neuronal function and produce CNS depression. The parent compound is not mutagenic. Weber and Sipes (1992) demonstrated the formation of 1,3-dichloroacetone (DCA), a direct acting mutagen, via CYP450-mediated oxidation of TCP. CYP450 induction enhanced DCA formation and covalent binding to proteins, whereas CYP450 inhibitors had the opposite effect. These investigators and Mahmood et al. (1991) detected metabolites of the glutathione (GSH) conjugation pathway, which may be involved in formation of episulfonium ions. Several *in vitro* studies of TCP cited in the current document show pronounced mutagenicity when provision is made for high CYP450 activity. Mutagenicity is usually absent without induced rat liver microsomes, cytosol and a NADPH generating system.

p. 111, pgr. 2, lines 3-6: The orally-administered dose reasonably approximates the absorbed (internal) dose of the parent compound. The most appropriate target organ dose would be the amount of bioactive metabolite(s) reaching that organ. This will likely be a combination of short-lived metabolites formed by the oxidative and GSH conjugation pathways. Unfortunately, we do not yet know the identity of some of these proximate toxicants. Thus, one logical alternative would be to use the total metabolized dose as the dosimeter.

p. 112, pgr. 2, lines 3-5: Tumors of the rat forestomach should not be included/utilized to derive a slope factor for the reason stated here. The mouse tumor data, however, should be considered.

p. 115, pgr. 3, lines 7 & 8: Again, it is not scientifically defensible to state that increased liver weight is on a "continuum" with necrosis. Substantially higher oral doses (e.g., 125 mg TCP/kg) are required to kill (any) hepatocytes than are needed (3-8 mg/kg) to produce hypertrophy.

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***Richard J. Bull***

Most of the issues have been addressed above in the general comments and responses to charge questions.

***Dale Hattis***

p. 4:

"Estimates for the percent absorption of the oral dose can be made by summing the mean values for the radiolabel recovered in the urine and exhaled as $CO_2$ (Table 3-1). By this approach, estimates of the absorbed oral load are 75% in male rats, 68% in female rats, and 84% in male mice. The percent recovered from feces was not used in this calculation because it is likely to contain both an absorbed and non-absorbed fraction. However, the true extent of intestinal absorption is likely to have been greater than the presented 75-84%, because a portion of the radiolabel that appeared in feces, which was not included in the above absorption estimates, would also have been absorbed."

Table 3-1 also includes radiolabel recovered from liver, blood, kidney, skin and adipose tissue. This should be noted in a revision of the first sentence quoted above. However it does not appear to include sampling from the rest of the carcass (or a homogenate prepared from the residual carcass), which would include the digestive system (exclusive of feces), brain and other organs. Thus the quoted total absorption figures are likely somewhat low because of this omission. The amount of bias from this could and probably should be estimated by comparing the likely weight of the residual carcass to the weight of the organs that were included in the measurements from generic data on the proportions of rat and mouse bodies represented by different organs.

p. 8:

"Evidence for the involvement of CYP in 1,2,3-trichloropropane metabolism is provided by the in vitro formation of 1,3-dichloroacetone when isolated rat or human hepatic microsomes were incubated with 1,2,3-trichloropropane (Weber and Sipes, 1992). The formation of 1,3-dichloroacetone, an intermediate in the formation of ACPC and CPC, occurred only in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and was enhanced by the addition of such CYP inducers as phenobarbital and dexamethasone. Conversely, formation of 1,3-dichloroacetone was blocked by the CYP inhibitors SKF-525A and 1-aminobenzotriazol. In support of the Mahmood et al. (1991) scheme for the metabolic transformation of 1,2,3-trichloropropane, the findings of Weber and Sipes (1990) provide inferential evidence for the involvement of glutathione in 1,2,3-trichloropropane metabolism by the demonstration that experimental glutathione depletion was associated with increased 1,2,3-trichloropropane binding to hepatic protein and decreased binding to DNA."

Glutathione depletion alone is not unambiguous evidence of direct glutathione metabolism of 1,2,3-trichloropropane because the reactive metabolites resulting from CYP metabolism could subsequently react with glutathione and result in glutathione depletion. However the case is made more plausible by the known role of glutathione in making the same type of reactive episulfonium metabolites as postulated here from the related compounds ethylene dichloride, ethylene dibromide, dibromochloropropane, perchloroethylene, and trichloroethylene. This appreciable literature on related compounds should at least be mentioned in support. It is further supported later by the Weber and Sipes (1990) findings reported on page 49 of the document.

p. 8:

"Mahmood et al. (1991) and Volp et al. (1984) demonstrated that urine is the primary route of 1,2,3-trichloropropane excretion in rats and mice."

It is clear from the subsequent sentences that the urinary elimination is primarily in the form of metabolites rather than the parent chemical. I would alter the quoted sentence to make this clear. This has some importance because all of the metabolites appear to be reactive and therefore could be involved in both toxicity and carcinogenicity.

"Volp et al. (1984) examined the time-dependent distribution of [1,3-$^{14}$C]-1,2,3-trichloropropane in male Fischer rats (three rats per time point) following i.v. injection of 3.6 mg/kg. The data from this study demonstrated rapid excretion of the radiolabel; after 24 hours 30% of the initial radiolabel had been exhaled, 40% had been released in the urine, and 18% in feces. Unchanged 1,2,3-trichloropropane was not detected in the urine."

For the same reason described in my previous comment, I think this sentence should refer to trichloropropane and its metabolites, rather than just trichloropropane, lest the reader make the mistake that the author is referring only to the unchanged parent molecule.

p. 15:

"The critical effect is hepatocellular necrosis in male rats, with a NOAEL of 16 mg/kg-day and a LOAEL of 32 mg/kg-day.

In my view the several other effects seen at 16 mg/kg-day and lower make it questionable that this dose should be considered a NOAEL.

In general I find the discussion in this section a bit tedious as it mainly duplicates information contained in the tables.

p. 22:

"NTP (1993) conducted a 2-year study of the toxicity and carcinogenicity of 1,2,3-trichloropropane in F-344/N rats, the data of which was also published in Irwin et al. (1995)."

Sorry to nit-pick, but data are always plural, so the proper verb here is "were".

p. 27

Throughout this section results are summarized in terms of the rather obsolete NOAEL/LOAEL framework, rather than being more quantitatively characterized by benchmark dose analyses. I think this should be changed.

p. 48:

"In the time-course study, maximum trichloropropane-equivalent covalent binding to hepatic proteins (approximately 600 pmol/ mg) was observed 4 hours after trichloropropane administration and was approximately 2.5-fold greater than at 1 hour post-administration. Maximal covalent binding to hepatic DNA (approximately 250 pmol/ mg) was observed 24 hours after administration. By 72 hours the amount of radioactivity bound to both protein and DNA had returned to levels below those measured 1 hour post administration. At the point of maximal binding, the amount of [$^{14}$C]-1,2,3-trichloropropane-derived radioactivity bound to hepatic proteins was more than double the amount bound to hepatic DNA."

My impression is that reactive molecules often have much greater binding to protein than to DNA. There should be no implication that the potential significance of DNA binding for mutagenic modes of action is thereby diminished.

"Administration of three consecutive doses each of 30 mg/kg 1,2,3-trichloropropane, separated by 24 hours, produced a linear increase in the amount of [$^{14}$C]-1,2,3-trichloropropane-derived radioactivity bound to hepatic proteins. Repeated dosing did not affect the amount of the chemical equivalent bound to DNA until the third dose at which point the amount of bound radioactivity doubled."

I think this dubious pattern is likely of no significance. Most likely there is considerable noise in the DNA binding measurements.

"In the metabolic study, induction of CYP450 (CYP) isozymes with phenobarbital pretreatment significantly reduced chemical binding to hepatic protein and DNA by 70 and 64%, respectively, when compared with controls. However, induction of CYP450 isozymes with #-naphthoflavone pretreatment did not significantly alter binding to either macromolecule. Depletion of reduced glutathione (GSH) by BSO pretreatment increased binding to hepatic proteins by 342% and decreased binding to DNA by 44% when compared with controls, with the increased covalent binding due to decreased GSH conjugation of a TCP metabolite."

This set of findings is of great significance—indicating that a GSH conjugate—probably via an episulfonium ion is likely most responsible for DNA binding whereas a particular CYP induced by phenobarbital is likely most responsible for the protein binding. This type of implication should be pointed out to the reader sooner, rather than later.

"The S-[1-(hydroxymethyl)-2-($N_7$-guanyl)ethyl]glutathione adduct indentified by La et al. (1995) is an $N_7$-guanyl adduct shown in Figure 4-1. This adduct is unusual in that it crosslinks a physiological oligopeptide, reduced glutathione, to DNA by a chemical carcinogen (Ozawa and Guengerich, 1983)."

The adduct might have been considered unusual in 1983, but certainly by now the similar examples of similar DNA reactive glutathione adducts formed from ethylene dibromide, ethylene dichloride, dibromochlorpropane and other related small molecular weight alkanes and alkenes provide ample precedent.

p. 66:

"The hypothesized mode of action for 1,2,3-trichloropropane induced carcinogenicity is through a mutagenic mode of action. Specifically, the data suggest that bioactivated 1,2,3-trichloropropane may bind directly to DNA resulting in a mutagenic event that may lead to tumorigenicity in animals. However, although there are in vitro data indicating that 1,2,3-trichloropropane may be genotoxic, there is a lack of in vivo information linking a mutagenic mode of action to the observed carcinogenicity in animal bioassays."

The last sentence seems to assume a greater burden of proof for a mutagenic mode of action ("in vivo information" than is or should be needed for a reasonably firm tentative conclusion. I would stress, in addition, the overall consistency of the trichlororopane findings with those for analogous substances (ethylene dibromide, ethlylene dichloride, and dibromochloropropane) that give rise to similar glutathione conjugates that generate mutagenic episulfonium ion activated metabolites. I would also note that the strong likelihood of a mutagenic mode of action meads that the Age Dependent Adjustment Factor(s) for early life exposure should definitely be applied for early-life exposures to this compound, in my opinion.

p. 70:

"Ito et al. (1996) analyzed the forestomach tumors in the B6C3F1 mice from the NTP, 1993 bioassay for *ras* gene mutations. Ten of the 16 forestomach tumors contained highly specific H-*ras* or K-*ras* activating mutations, of which 6 tumors had H-*ras* mutations at codon 61 and 4 with K-*ras* mutations at codon 13. These mutations are not consistent with the miscoding properties of S-[1-(hydroxymethyl)-2-($N_7$-guanyl)ethyl]glutathione, the major DNA adduct of 1,2,3-trichloropropane, and indicates that another mode of action may be involved. La and Swenberg (1997) observed an increase in the concentration of the endogenous DNA adducts, 8-hydroxydeoxyguanosine, 1,$N_6$-ethenodeoxyadenosine, and 3,$N_4$-ethenodeoxycytidine, in rats following 1,2,3-trichloropropane exposure for one week.

I think this is a red herring. We may not know everything about how the adducts give rise to the specific pro-carcinogenic mutations found; but this should not be used to imply some mysterious and unidentified other mode of action.

p. 71:

The in vitro studies were positive for genotoxicity or mutagenicity at concentrations ranging from 0.001 to 1000 µg/plate, and indicate that point mutations are the most consistent type of genetic alteration induced by 1,2,3-trichloropropane and occur at lower concentrations than the chromosomal damage.

I would rather say, "…are detectable above background at lower concentrations than the chromosome damage." Just because you cannot detect elevations below certain concentrations does not mean that the genetic changes do not "occur"—indeed relevant theory indicates that they really should occur at all finite exposures (see Hattis, D., "Pharmacokinetic Principles for Dose Rate Extrapolation of Carcinogenic Risk from Genetically Active Agents," Risk Analysis,

Vol. 10, pp. 303-316, 1990; Hattis, D. and Goble, R. L. "Uncertainties in Risk Assessment for Carcinogenesis: A Road Map Toward Practical Improvements" White paper for the U.S. Environmental Protection Agency, May, 2007.)

p. 73:

"Mutagenicity as a mode of action for carcinogenicity in humans is generally accepted and is a biologically plausible mechanism for tumor induction. The formation of DNA adducts in organs that also displayed an increase in the tumor incidence in rats and mice indicates coherence of the effects and is evidence supporting a mutagenic mode of action (Table 4-24). Binding of 1,2,3-trichloropropane metabolites to DNA is currently the most likely theory for the mode of action of the tumor formation. However, the formation of DNA adducts of 1,2,3-trichloropropane in tissues other than those where tumors formed (La et al., 1995) is an area of uncertainty associated with the suggested mutagenic mode of action. DNA adduct formation for some tumor types may be necessary but not sufficient for the induction of tumors and is not an uncommon occurrence as DNA adducts of known direct-acting carcinogens (e.g., benzo[a] pyrene) have been observed in tissues where tumors were not found. The formation of DNA adducts in non-tumor forming tissues and organs may signify that DNA adducts by themselves are insufficient to cause tumors or that the increased mortality in the rats and increased tumor incidence in other organs precluded tumor formation in the non-tumor forming organs."

Again, I would emphasize the detectability issue. It may well be that tumors are formed in non-target tissues, but not at levels that are detectable above background in the particular bioassays that have been done.

p. 73:

"Data are available that indicate that the bolus exposure to 1,2,3-trichloropropane may overwhelm cellular glutathione levels in the forestomach and induce lipid peroxidation (La and Swenberg, 1997; Ito et al., 1996). This lipid peroxidation leads to an increase in the etheno DNA adducts 1,$N_6$-ethenodeoxyadenosine and 3,$N_4$-ethenodeoxycytidine and the hydroxyl radical-derived 8-hydroxydeoxyguanosine (La and Swenberg, 1997; Ito et al., 1996). "

The language about the bolus exposure overwhelming glutathione-based defense mechanisms suggests a threshold theory for cellular defense mechanisms that is fundamentally flawed in its implication of threshold dose response for somatic mutation and associated carcinogenic risk from purely pharmacokinetic considerations. I deal with this extensively in a recent white paper for EPA (Hattis, D. and Goble, R. L. "Uncertainties in Risk Assessment for Carcinogenesis: A Road Map Toward Practical Improvements" White paper for the U.S. Environmental Protection Agency, May, 2007), an excerpt of which is included below:

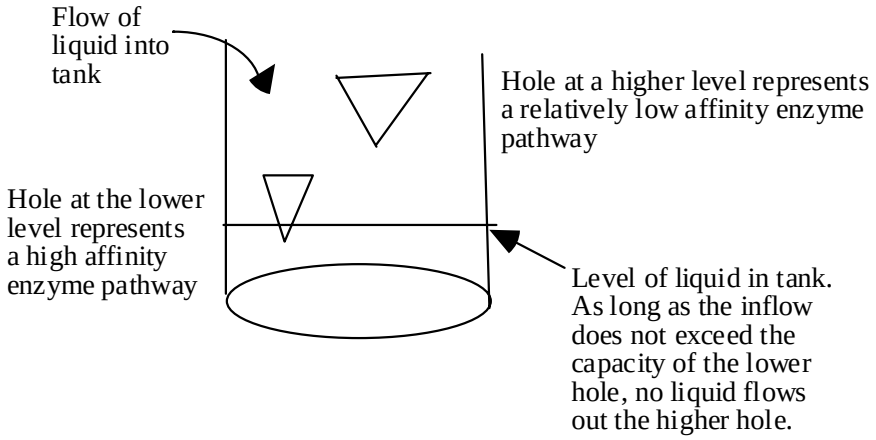
"In the 1970s and early 1980s it was recognized that basic bimolecular reaction kinetics require a fundamental linearity between the concentration of DNA reactants and relevant sites on DNA. However it was also recognized that there were many opportunities for at least high-dose nonlinearities both before and after DNA reaction in the sequence of events from intake of a

DNA reactive agent (or a metabolic precursor) into the body to the ultimate manifestation of tumors (Hattis 1990).

In the 1970s some looked to pharmacokinetics as a potential source of threshold dose response relationships that might intervene between toxicant intake and the delivery of DNA reactive molecules to the nucleus of relevant cells. Figure 2 is an illustration similar to one that was published in Science (attributed to researchers at Dow Chemical) that attempted to make this pharmacokinetic-based threshold idea plausible. In the diagram, liquid (representing a continuous dosage of a toxicant) flows into a tank with two triangular holes. The level of liquid rises in the tank until some begins to flow out of the lower of the two holes (representing a high-affinity metabolic pathway producing a "safe" metabolite). A further rise occurs until the amount of liquid flowing out of the tank equals the amount flowing in. If the inflow is small enough that it can be completely balanced by flow out of the lower hole, then the liquid will not rise to the level of the higher hole (representing the lower affinity enzyme producing the dangerous metabolite). Thus the analogy predicts a threshold of inflow into the tank, below which all of the metabolism is via the "safe" high affinity pathway.

Figure 2

**Argument for the Plausibility that Thresholds Might Arise From the Competition Between Metabolic Pathways Producing Safe and Dangerous (DNA Reactive) Metabolites**



Unfortunately, this representation of the competition between higher and lower affinity metabolic pathways is not compatible with conventional Michaelis-Menten enzyme kinetics (Hattis, 1990; Slikker et al. 2004). Using the basic Michaelis/Menten equation, the rate of the activating reaction (producing the dangerous metabolite, D) is:

$$\frac{dD}{dt} = \frac{V_{max}[C]}{K_m + [C]}$$

where [C] is the concentration of substrate (the form of the toxicant that is absorbed from the environment), Vmax is the maximum rate of the reaction that produces the dangerous metabolite, and Km (the Michaelis constant) is the substrate concentration at which the reaction proceeds at half of its maximum velocity (Vmax). Similarly the rate of the competitive detoxifying reaction (producing the safe metabolite, S) is:

$$\frac{dS}{dt} = \frac{V_{max}'[C]}{K_m' + [C]}$$

The [C]'s in the denominator of both equations can be neglected at low doses when they become small relative to the $K_m$'s. At low doses we can therefore find the ratio of the substrate [C] that goes by the dangerous and safe metabolic pathways by simply dividing the two equations:

$$\frac{\text{rate of D production}}{\text{rate of S production}} = \frac{V_{max}[C]/K_m}{V_{max}'[C]/K_m'}$$

and because the numerator [C]'s now cancel, it can be seen that we are left with a ratio of four constants. This means that below the dose region where there is appreciable saturation of the enzymes producing either the safe or the dangerous metabolite, the fraction of the substrate taken by each pathway approaches a constant, independent of dose. There are no dose rate effects in this low dose region, there can be no thresholds, and indeed the system must operate linearly at the limit of low dosage, albeit with a different distribution of metabolism between “safe” and “dangerous” pathways than would be observed at higher doses. At the limit of high dose, the ratio of production of the dangerous to the safe metabolites is governed only by ratio of the two Vmax values; whereas at lower doses the Km’s become progressively more involved. If the higher affinity (lower Km) pathway produces the dangerous metabolite, then the fraction of material metabolized by the dangerous pathway will be greater than at the highest saturating doses, resulting in a convex-upward dose response relationship for DNA damage (e.g. the pattern seen for vinyl chloride). On the other hand, if the safe pathway has the lower Km then the portion of the chemical processed by the safe pathway will be greater at lower doses than is seen at higher doses. In the abstract of a paper (Gehring et al 1978) describing a process model for carcinogenesis from electrophilic agents, Perry Gehring, [then a leader of the toxicology group at

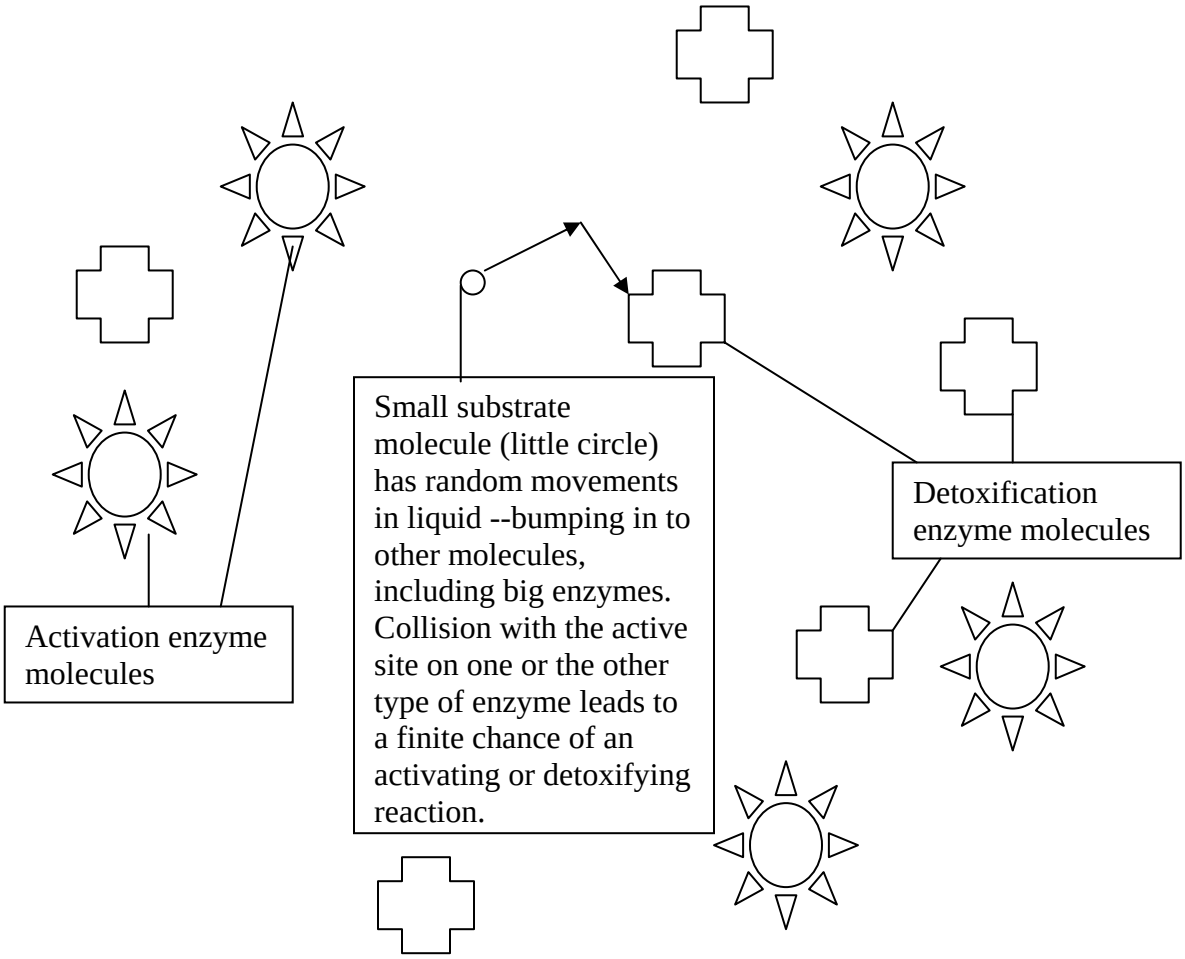
Dow Chemical (Gehring 1978)] acknowledges that there should be an expectation for some "albeit negligible" carcinogenic risk from genetically acting chemicals at low doses.

It is well to emphasize that the basic Michaelis-Menten equation applied above is not simply an empirical formula. It is well grounded in fundamental mechanistic considerations of receptor association and dissociation kinetics with reasonably wide applicability (Hoel, 1985), The maximal velocity, Vmax, arises because there are a limited number of enzyme molecules available to catalyze the reaction, and each enzyme molecule is necessarily constrained to operate at a finite maximal rate (which varies according to substrate) in converting substrate into its product. The fact that the reaction proceeds linearly at low doses (with a rate constant of Vmax/Km) arises from the fact that the reaction is limited by the rate of diffusion of the substrate molecules into the active site of the enzyme—a rate that must be linear with substrate concentration at the limit of low doses. In the light of this Figure 3 offers a more accurate molecular-scale vision of the competition between enzyme-mediated activating and detoxifying processes. Each small substrate molecule has a "random walk" through a cellular compartment as it rebounds from collisions with other molecules. At the limit of low dosage, when there are few or no other similar substrate molecules around, the substrate molecule must have a finite chance of encountering the active site of each type of enzyme (or, similarly, a transport molecule taking it to a different compartment). Therefore each type of enzyme or macromolecular transporter must have finite opportunity to process the substrate molecule at the limit of low dosage.

The basic Michaelis-Menten enzyme equation form applies with equal force to active transport processes (in which specialized molecules utilize energy to pump specific molecules or ions into our out of cells), and to DNA repair processes. Thus the fundamental expectation for low dose linearity and high dose saturation applies similarly to these other components of the causal chain between external exposure and the generation of somatic mutations that are components of carcinogenesis. At the limit of low substrate concentration the Michaelis-Menten enzyme/transport reaction rates are limited by the rate of diffusion of substrate molecules into the active sites of the enzymes/transport molecules; and those diffusion processes, given a specific temperature, are linear functions of substrate concentrations. At the limit of high concentration (where the substrate concentration is very much larger than Km), the reaction must approach a finite maximal rate (which, of course, varies according to the substrate) because there are a limited number of enzyme molecules and each one must have a limited capacity to process substrate."

Figure 3

**A Molecular Vision of the Low-Dose Competition for Substrate between Activating and Detoxifying Enzyme Molecules**




p. 77:

"According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (*Supplemental Guidance*) (U.S. EPA, 2005b), children exposed to carcinogens with a mutagenic mode of action are assumed to have increased early-life susceptibility. The *Supplemental Guidance* (US EPA, 2005b) recommends the application of age-dependent adjustment factors (ADAFs) for carcinogens that act through a mutagenic mode of action and are assumed to convey early-life susceptibility. Given the weight of the available evidence, 1,2,3-trichloropropane may be acting through a mutagenic mode of carcinogenic action; however, the database is lacking in vivo evidence that mutagenic events occur following 1,2,3-trichloropropane exposure. For these reasons, the application of ADAFs when assessing risks associated with early-life exposure is not recommended. "

I disagree. In my view the evidence is more than sufficient to support a mutagenic mode of action as by far the most likely possibility; and the ADAFs really should be recommended to be applied in this case.

p. 106:

"An estimate of the 95% upper bound on the summed oral slope factor was calculated by assuming a normal distribution for the individual risk estimates, and deriving the variance of the risk estimate for each tumor site from its 95% upper confidence limit (UCL) according to the formula…"

My reconstruction of the likelihood distribution for the cancer slope factors supports the use of a normal distribution to represent the uncertainties in this case, although I suspect that a lognormal distribution may be indicated in cases where there are upward turning nonlinearities at high doses.

***Ralph L. Kodell***

1. Section 4 discusses increases in "right" kidney weights for rats and mice, but section 5 discusses only kidney weights. What is the significance of "right" and shouldn't it be used consistently?

2. Page 38, lines 3.5: The sentence is inconsistent with the previous sentence. It is redundant anyway, and should be deleted.

3. Page 45, line 18: It needs to be stated explicitly that 17 mated females out of 20 was the CONTROL response.

4. Page 45, lines 27-28: To what does "either generation" refer? Wasn't it a single-generation study?

5. Page 85, line 3: Insert "of which" between "some" and "were."

6. Page 109, Table 5-7: I was not able to get the values in this table to match the values in Appendix C-1. For example, page 172 gives the MSW modeling results for male rat squamous papillomas and carcinomas. This corresponds to the first row of Table 5-7. In the table, the BMD for $R=10^{-2}$ is $4.9x10^{-3}$. But, if I use the MSW parameters on page 172 at t=104, I get an extra risk of $2.04x10^{-3}$, not $10^{-2}$. Also, in conflict with the table's value of $10^{-2}$, the appendix has an extra risk of $10^{-5}$ at the mle dose, $4.88x10^{-3}$. Does the discrepancy have something to do with the fact that time is shown as 70 in the appendix, instead of 104? If so, why is time 70 used, instead of the customary 104 weeks? I don't think that's the problem, because by my calculation based on the parameters in the appendix, an extra risk of $10^{-5}$ at dose=$4.9x10^{-3}$ corresponds to time = 37.

7. Pages 155 and 158: I didn't check to see how the AIC is defined in the BMD software, but I am surprised to see negative values. Are these correct?

***Harihara M. Mehendale***

No specific comments provided.

***Helmut Zarbl***

- For subchronic and chronic studies, please indicate age at beginning of study.
- Table 4-8, are the 'a' and 'b' footnotes reversed for females? Page 66, last sentence before Section 4.7.2 , too strong , use "suggest" rather than "likely"
- Table 5.1, what are the units for values?
- Page 103, line "Therefore…." This sentence does not make sense. Clarify what is meant.
- Page 123, the last sentence is not true based on the data provided. Rephrase.

***Lauren Zeise***

p. 11. An explanation is needed that relative weight measures presented for the various organs are the organs weights relative to the bodyweight of the animals. NTP uses the term "organ weight to body weight ratios" in its table headers. This is somewhat clearer. That mean relative weights are calculated on a group basis is also needed. That is they are calculated by taking the mean organ weight for the group and dividing that by the mean bodyweight for the group. The result will not be the same as a calculation on an individual animal basis – that is taking for each animal its organ weight and dividing it by its bodyweight and then averaging these to get the mean. Doing this calculation on a group rather than individual animal basis is not ideal, but unfortunately this is the way the data in the NTP report presents the information.

Tables 4-1, 4-2, 4-4 and 4-5 all report NTP is the source for the change in mean relative and absolute weight statistics, yet these values cannot be found in the NTP report. It would be preferable and clearer to report the statistics given in the NTP report and then, the change with respect to the control (as the Toxicological Review does for Tables 4-9). If the values for change in these tables were calculated by EPA rather than NTP these columns should be annotated as such. The statistical significance tests appear to be from NTP.

p. 11, last two lines. Some clarification is needed regarding why changes in relative organ weights were not considered to be indicators of organ toxicity. The report needs to be clear that this applies to brain and heart only. For example NTP (1993, p. 28) states "This

dose-related trend of increased liver and kidney weights in rats receiving 1,2,3-trichloropropane was consistent with the clinical pathology and histopathology findings." p. 15, first full paragraph. An explanation about why the depression in erthyocyte mass in rats - evidenced by decreased hematocrit, hemoglobin, and erythrocyte counts - were not considered to be "biologically significant" is needed.

p. 15, second paragraph. NOAEL and LOAELs are reported for hepatocellular and renal tubular necrosis. A more integrative discussion is needed that addresses the supporting findings from clinical chemistry. For example at first glance the male rat LOAEL for liver necrosis of 32 mg/kg-d appears to be based on a finding in one of ten animals in that as well as higher dose groups, but other markers of hepatocellular damage are noted - depressed synthesis of pseudocholinesterase and other markers. This should be better laid out. With regard to renal effects the regenerative hyperplasia seem at 8 weeks in the top 3 male and female rat dose groups is also noteworthy and if not also considered a critical effect some discussion as to why should also be provided.

A statement regarding the significance of findings of decreases in relative and absolute heart and brain weight in the mice (reported on page 16) is needed.

pp. 19-22. The reader should be informed about why NOAELs and LOAELs are being identified for the subchronic non-cancer endpoints in the NTP mice studies and the Villeneuve et al. drinking water study in rats, since subchronic RfDs are not calculated. Also, the discussion is confusing, with multiple critical effects for each sex identified, and inadequate integration and justification of the selection. If the text is left here, better justification is needed.

pp. 33-40. For making crude comparisons of effect levels it would be of interest to see exposures in the inhalation studies expressed also as mg/kg-d doses.

p. 43, table 4-23 and p. 42, first paragraph. Suggest double checking the significance values for fertility for the 17/19 vs. 38/38 groups, if that is the comparison that was done. (for the fourth litter at 60 mg/kg and 3rd litter at 120 mg/kg).

p. 45, first full paragraph. Would double check the statistics – 10/50 vs. 17/20 – by Fisher's exact this is a significant difference.

p. 45. Unclear what is meant by lower mating performance for males – no baseline group is given to compare the control group – It is stated that all groups including the control have low performance, without any norm given. With the small numbers of animals and poor showing in the control animals this test is far from ideal and some criticism of it seems in order.

p. 46, bottom paragraph. Need to clarify add "1,2,3-trichloropropane treatment groups" to the sentence "incidence of choliangiocarcinomas was significant increased in all exposed males and females after 13 months"

p.47. Section 4.5 solely focuses on the MOA for carcinogenesis, and not other toxicities addressed in the report. The header for the section should reflect this. A few orienting sentences laying out what this section is doing and a motivation for presentation of topics would be helpful. Since genotoxicity studies – discussed in section 4.5.2 - can also be considered "Mode of Action Studies" the header for section 4.5.1 some other header for 4.5.1 would be preferred. Perhaps something like "Studies of Covalent DNA Binding and Modulation of Adduct Formation" The separation between these two subsections is not that tidy and there is repetition of information. It is unclear whether the La and Swenberg study on indirect formation of DNA endogenous adducts is best placed as it currently is on page 59 or should be moved up, or whether the two sections should simply be combined.

p. 48, sentence starting "Depletion of reduced glutathione…" seems contradictory

p. 50. To help the reader understand the importance of the findings the control group tumor incidence should also be given in table 4-24.

p.51. The generalization is made that a greater amount of DNA adduct was extracted from animals treated with gavage compared to drinking water, but in tumor forming tissue the result was only significant for one site. Some comment in this regard is needed. Also the differences are only by less than a factor of 2 for the liver and that should be noted.

p. 52. In first sentence on bacterial assays suggest adding "in various Salmonella strains" after "mutagenic"

p.59. Regard the site concordance for tumors observed in the NTP study and adducts – fourth sentence in the second paragraph should be corrected – the lung, pancreas and kidney were not sites of tumor formation in the male mouse although the pancreas and kidney were in male and female rats.

p.60, first sentence in structure activity relationships. "Clear" should be inserted in front of "evidence." (The evidence for carcinogenicity of DBCP in whole animal models is overwhelming.) Also, later in the paragraph, it is worth pointing out that tumor type was squamous cell carcinoma, as with 1,2,3-TCP, and that the finding was in male and females of both species, as with 1,2,3-TCP. Furthermore, mammary gland adenocarcinoma in female rats is seen with 1,2,3-TCP and it was also seen with DBCP in both the oral NCI and inhalation NTP studies. This should also be noted. Also while in the chronic inhalation study DBCP caused tumors of the nasal turbinates and lung, 1,2,3-TCP has not been studied by chronically by inhalation but subchronic inhalation studies clearly indicate that the lung and nasal turbinates are target sites for 1,2,3-TCP toxicity. This could be better spelled out in the Toxicological Review.

There are some errors in the structure-activity write-up. The last sentence on the page indicates that DBCP caused liver cancer in rats and mice, but this is not the case, at least in the NTP and NCI studies. The last sentence in the first paragraph in the subsection

indicates that inhalation DBCP caused bronchial tumors in rats and mice of both sexes, but this is only correct for mice.
p.61, top. The connection between the DBCP finding of aberrant spermatogonial and bone marrow cells and 1,2,3 TCP was not clear and should be better described.

p.61, second sentence under oral exposures. As noted above, the NTP bioassays are a study series rather than a single study. p. 65 last sentence also refers to the NTP carcinogenicity studies as a single bioassay.

p.63. Could add at the bottom of the page that the decreased hematopoetic measures could not be explained by blood loss due to tumor for the subchronic.

p. 65 sentence fragment at the bottom of the page that begins with "Statistically significant…" The NTP found the tumors listed provide clear evidence of carcinogenicity in the four studies (male and female rats and mice), so the statement can be made somewhat stronger.

p. 66, top of page. Uterine tumors in mice were also increased and found to be related to 1,2,3-TCP treatment. These should be added to the list.

p.66, last line – to point 3) could modify "carcinogenicity" with "similar site-specific" since the finding of a similar site-specific pattern of tumors is also compelling.

p. 67. The section 4.7.3 header "Mode of Action Information" is similar to the header for section 4.5.1 "Mode of Action Studies" Perhaps a header like "Mode of Action Analysis"

p. 67. The discussion regarding overall genotoxicity should not give weight to studies reported in abstract which cannot be examined. These studies should also be removed from Table 4-26.

p.73. Other chemical carcinogens form adducts in a variety of tissues but are observed to induce cancer in only a few. This could be noted.

p.73, 3rd sentence. This is only a partially described MOA. Paraphrasing charge question D.3 below, a fuller description would be something like "The proposed mode of action includes bioactivation of 1,2,3-trichloropropane leading to DNA adduct formation followed by the induction of mutations in cancer-related or onco- genes, and eventually resulting in cancer."

p. 73. The biological plausibility and coherence paragraph focuses almost entirely on DNA adduct formation, ignoring the large body of in vitro evidence on mutation and supporting evidence from structurally related compounds for a mutagenic mode of action.

p.73. The discussion of DBCP in the other possible modes of action section supports mutagenesis as the MOA and is therefore misplaced. It should be moved up as it supports the hypothesized MOA.

p.77 In the discussion of potential for developmental and reproductive toxicity structural similarity to DBCP could be raised.

p. 78. There are other obvious gender differences exhibited in the cancer data, including the induction of mammary adenocarcinoma in females (also induced by DBCP in both oral and inhalation studies) and of course the uterine effects.

p. 78. The section 4.8.3 on other susceptibles should be expanded to provide more detail and perhaps some group characteristics on individuals that should be considered.

p. 79. All the non-cancer findings noted by NTP should be included in the list of adverse effects in the 2nd paragraph.

p.97. In the listing of sites, uterine tumors are missing

p. 102 Middle paragraph. The pharmacokinetic data presented early in the document indicate the compound is well absorbed. This is true as well for other structurally similar halogenated aliphatic compounds. Speculation regarding the effects of the forestomach lesions increasing absorption of the compound is unnecessary given the expectation that the compound is completely absorbed, or at least nearly so.

p. 102. When first introducing the multistage Weibull it may be best to describe it as a Multistage in dose Weibull in time model and then simplify it to a multistage-Weibull.

p.115, third paragraph. The kidney is also an important site of toxicity in subchronic studies and this should be noted as well. Significant non-cancer findings have also been observed in subchronic studies in bile duct, lung, and nasal turbinates and this should be noted as well.

p. 116, bottom. A dose related increasing trend in Zymbal's gland tumors was seen in both male and female rats.

p. 122, bottom. In contrast to NTP's characterizations of its studies, the text refers to the NTP studies as a single study. While the studies' design and results are transmitted in a single technical report, they actually represent a series of studies in two sexes and two species. For example, NTP refers to its efforts for the compound as "2-year studies."

**Comments more editorial in nature:**

Toxicokinetic section, page 4: Should note the observed half life in rodents reported in the literature. The NTP report cites a paper by Gingell et al. 1987 indicating half life of …

p. 9, second paragraph. The text should be clear about the several urinary metabolites found that are not captured in Figure 3-1. ACPC was observed, but the same acronym as used in the figure is not used in this paragraph, as it should be.

p.9, last paragraph, last sentence. Wasn't clear if this was an empirical observation or finding of the model. If it is a finding of the model it would be better presented in the modeling section. If it is an empirical observation a citation is needed.

pp. 11-16. Data and p-values presented in tables are reiterated in text. This is unnecessary.

p. 23. Suggest adding a table footnote that the survival rate and probability of survival is for the two year time point.
pp.23-26. The repetition in the text of values reported in the tables is unnecessary and the report would be easier to read if the text mostly referred to the tabulated values without repeating them.

p. 23. NTP did not report organ weight change results for the 2 year studies in rats. This should be noted to let the reader in on why the 2 year results don't appear in the Toxicological Review.

p. 20, top line. "effect" not "affect"

p. 26, second paragraph, second line. Suggest adding "by NTP" after "not considered"

p. 45. although it is implied it would be clearer to indicate the control group of 17/20.

p. 48. Considering what the results of the Weber and Sipes study means would be helped by addition of a table with perhaps four column: the treatment, what it did to enzyme levels, an indication of the increase or decrease in adduct DNA formation, and interpretation of finding in terms of active metabolic pathway.

p. 49. A little more detail is needed on what is meant by "a single, major DNA adduct was formed irrespective of the tissue type."

p.52. The kidney is a target organ for 1,2,3-TCP carcinogenicity in the male rat.

p. 123. Suggest adding that based on the tumors findings the NTP reported "clear evidence" of carcinogenicity in both genders of rats and mice.

p. 123. Regarding the caveat that considering benign tumors as indicative of carcinogenic potential may lead to overestimation, another caveat indicating that certain benign tumors in and of themselves can also lead to significant adverse health consequences could also be noted.