PEER REVIEWER COMMENTS

External Peer Review Meeting on the *Toxicological Review of Hexachloroethane* (CAS No. 67-72-1)

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I. INTRODUCTION

The Integrated Risk Information System (IRIS) is an EPA database 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 (RfD) for noncancer health effects resulting from oral exposure, a reference concentration (RfC) for noncancer health effects resulting from inhalation exposure, 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 a Toxicological Review of Hexachloroethane, which updates an assessment that was posted to the IRIS database in 1987. Hexachloroethane was nominated for IRIS reassessment because it was identified in Superfund sites. The draft document contains an oral reference dose (RfD), a chronic inhalation reference concentration (RfC), and a carcinogenicity 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 for hexachloroethane that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). An existing IRIS assessment for hexachloroethane, which includes a chronic oral reference dose (RfD) and a carcinogenicity assessment, was posted on IRIS in 1987.

The current draft health assessment includes a chronic oral RfD, chronic inhalation reference concentration (RfC), and a carcinogenicity assessment. Below is a set of charge questions that address scientific issues in the assessment of hexachloroethane. Please provide detailed explanations for responses to the charge questions. Please consider the accuracy, objectivity, and transparency of EPA's analyses and conclusions in your review.

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazards?

2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Chemical-Specific Charge Questions:

(A) Chronic Oral Reference Dose (RfD) for Hexachloroethane

1. A 16-week dietary exposure study of hexachloroethane in F344 rats by Gorzinski et al. (1985) was selected as the basis for the derivation of the RfD. Kidney effects were observed in male rats in this study at doses below the range of exposure tested in the available chronic NTP (1989) study. Please comment on the scientific justification for the use of the subchronic Gorzinski et al. (1985) study as the principal study for the derivation of the RfD. Is the rationale for this selection clearly described? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Nephrotoxicity as indicated by atrophy and degeneration of renal tubules in male rats (Gorzinski et al., 1985) was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

3. Benchmark dose (BMD) modeling was applied to the atrophy and degeneration of renal tubules data in male rats to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark

response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of atrophy and degeneration of renal tubules) scientifically justified and clearly described?

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically justified and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

(B) Chronic Inhalation Reference Concentration (RfC) for Hexachloroethane

1. A 6-week inhalation exposure study in rats by Weeks et al. (1979) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Is the rationale for this selection clearly described? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Neurobehavioral effects in Sprague-Dawley rats (Weeks et al., 1979) were selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

3. The NOAEL/LOAEL approach was used to derive the POD for the RfC. Please comment on whether this approach is scientifically justified and clearly described.

4. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs scientifically justified and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

(C) Carcinogenicity of Hexachloroethane

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgrd.html), hexachloroethane is *likely to be carcinogenic to humans* by all routes of exposure. Is the cancer weight of evidence characterization scientifically justified and clearly described?

2. A two-year oral gavage cancer bioassay in F344 rats (NTP, 1989) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantitation is scientifically justified and clearly described. Please identify and provide the rationale for any other studies that should be selected.

3. The renal tubule tumor data in male rats from the NTP (1989) two-year oral gavage cancer bioassay were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

4. EPA concluded that the mode of action for renal tubule tumors observed following oral exposure to hexachloroethane is unknown. An analysis of the mode of action data for renal tumors is presented in the Toxicological Review. Based on this analysis, EPA determined that hexachloroethane-induced renal tumors could not be attributed to the accumulation of α2u-globulin. Please comment on the scientific support for these conclusions. Please comment on whether the analysis is scientifically justified and clearly described.

5. The oral cancer slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk for renal tumors in male rats). Has the modeling approach been appropriately conducted and clearly described?

III. GENERAL IMPRESSIONS

Jack B. Bishop

The document appears to be very comprehensive, with the available scientific literature on hexachloroethane toxicity, for both cancer and noncancer hazards, thoroughly reviewed.

I understand that this is a "standard format" for an IRIS Review; however, the overall length of the toxicological review seems to be a bit excessive, with too much repetition. It would benefit from a reduction of 10-15%.

It would have been helpful to have learned upfront about the limited published literature on HCE and the absence of human data, what the principal studies and toxicity end-points were that were chosen for calculation of the RfD and RfC values, and what these values are. I would also have preferred to see a table up front listing all the studies (with type/species/sex/strain) that EPA considered relevant to this review. Perhaps this could be done in some sort of "Executive Summary."

This was my first time to participate in an IRIS Review; in hindsight, I felt like I should have covered the report in reverse, starting with the dose-response assessment first (Section 5) and examining the details of each study associated with each assessment. The HCE ADME Data in Rats, Mice, Rabbits and Sheep seem to me to be inadequate for extrapolating to human HCE exposure, and we have no reliable data on human exposure(s). Data for assessment of reproductive and developmental effects is inadequate.

I did find the rationale for the various uncertainty factors (UFs) compelling, and appreciated knowing what the EPA sees as data gaps that need to be filled.

Lucio G. Costa

The document provides a comprehensive assessment of the toxicity of hexachloroethane. An oral RfD and an inhalation RFC are derived, and a cancer assessment was conducted. The document is written in the standard IRIS format. It appears that a comprehensive review of the literature has been carried out. All available studies are reviewed and summarized. The rationale for the choice of the studies for determination of the RfD and the RfC, and for the cancer assessment are clearly stated. The document is at times repetitious. This reviewer does not fully agree with some of the conclusions, in particular with the choice of UFs for deriving the RfD and the RfC. Indeed, confidence in the proposed values is justly deemed low by EPA. In addition, the cancer risk assessment may benefit from a clearer and more comprehensive discussion on possible modes of action of hexachloroethane.

Lynne T. Haber

In general, the information is accurately presented, and the document is clearly written and free of typographical or other errors. However, for a chemical with such a small database, the length of the overall Toxicological Review is excessive and tends to work against the ultimate goal of clear communication of risk assessment conclusions and the basis of the conclusions. There are also some errors/incomplete documentation in the uncertainty factor sections, as noted below.

While the structure of the Toxicological Review requires a certain amount of repetition, the authors repeated the same summaries more than is necessary, and missed opportunities to integrate and synthesize the information, to help the reader integrate the data. In particular, Sections 4.6 and 4.7 ask for a synthesis of the data, not a repetition of study design, incidence data, or other details. Rather than a study by study summary with a repetition of the study design and primary data, this section should focus on the bottom lines for each endpoint – What effects are seen? Are there consistencies or apparent inconsistencies (e.g., across species, across routes) in the data? Is there other information (e.g., toxicokinetic differences, differences in binding proteins) that explains the observed differences across species, routes? (similarly for differences between males and females). What information is available about dose-response trends, biomarkers and precursors? While some of this information is presented in the current draft, it is easily lost in the mass of details in this section.

This issue is most obvious in the synthesis sections, but in many other places in the document, improved clarity would be achieved by less repetition of the primary data. Similarly, for supporting sections (e.g., SAR, in vitro and ex vivo tissue studies), the reader would be better served by focusing on how the data relate to better understanding hexachloroethane, rather than a detailed discussion of each study, and the section on MOA (including 4.6.3) would be enhanced by bringing in information from related chemicals and metabolites of HCE (in a synthesized form – not study by study). The section on genotoxicity would also benefit from much more synthesis, rather than a study by study summary, as addressed further in the specific comments.

Ralph L. Kodell

There are quite a number of annoying errors in the presentation and discussion of the toxicology data. There are some discrepancies between text tables and appendices in the cancer assessment. The rationale for not doing BMD modeling of inhalation effects seems insufficient. I disagree with some of the uncertainty factors that have been applied.

The discrepancies in the cancer assessment, even though slight, need to be resolved as they affect the derivation of the oral slope factor. A better rationale for not doing BMD modeling of inhalation effects needs to be provided. The errors in the text and tables need to be corrected; although they make the presentation difficult to follow at times, I do not think that they affect the soundness of the conclusions.

Lawrence H. Lash

The EPA presents a well organized and generally well written summary and analysis of the toxicology profile for hexachloroethane (HCE). The document seems to follow the standard format for these IRIS database reviews, and is comprised of six sections: 1) a brief introduction, 2) a brief section on chemical and physical information about HCE, 3) a summary of toxicokinetic information, 4) a section on hazard identification, 5) a section on dose-response analyses, and 6) a summary of major conclusions for hazard identification and dose-response assessments. The Introduction sets forth the basic process that is followed in assessing and reviewing the literature for HCE and it defines some key terminology that is used in the risk assessment process. Either as part of the Introduction or as an added section, I would suggest inclusion of a brief "Executive Summary" of the report's findings and conclusions. It would be helpful if the readers learned from the start of the report the following key points: 1) the relative paucity of literature on HCE and particularly the very limited data in humans; 2) the choices of principal studies and toxicity end-points for calculation of RfD and RfC values; and 3) the proposed RfD, RfC, and oral slope factor values and how they differ from the previous values. Overall, the document clearly presents the strengths and limitations of the database, the rationale for the various uncertainty factors (UFs), and the data gaps that are needed to minimize uncertainties. In a few places, however, some conclusions or statements are ambiguous and could be misleading. These statements need to be made more carefully. Whereas the analysis of the non-cancer endpoints is generally straightforward and accurate, there are some concerns about the conclusions that are reached for the analysis of cancer risk and calculation of the oral cancer slope factor. Overall, however, the document presents a comprehensive analysis of the existing database for HCE.

Edward A. Lock

Overall the scientific information available, which is rather limited, was presented in a clear and accurate fashion. The use of tables to summarize the findings was very helpful and although I felt when reading it through that certain points were repeated on several occasions, I could not come up with any ideas on how to improve this. I am not a modeling expert and found the information on this aspect in the text and appendices helpful in leading me through the process. The main aspect dealing with the mechanism of renal cancer was clearly spelt out and the limitation for not invoking the α2u-globulin mechanism explained and recommendations made. The issue regarding chemically-induced CPN was discussed in some detail and although I have challenged the interpretation and encouraged further studies to help make a more informed decision, the authors did draw the issues clearly to the reader's attention.

IV. RESPONSE TO CHARGE QUESTIONS

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer hazards?

Jack B. Bishop

The EPA has thoroughly reviewed the available scientific literature on hexachloroethane toxicity, for both cancer and noncancer hazards. However, I found the overall content a bit excessive and redundant. I believe it can stand a reduction of at least 10-15%. Data for assessment of reproductive and developmental effects are inadequate. The clarity, conciseness, and logistic of presentation of the overall review could be improved.

Lucio G. Costa

The document presents and synthesizes all scientific evidence for noncancer and cancer hazards of hexachloroethane in a comprehensive fashion. As for most IRIS documents, there are repetitions, and some parts may be better presented elsewhere in the document. Details on some suggestions are provided in the last section [see Specific Observations].

Lynne T. Haber

In general, the Toxicological Review is logically presented. As noted in the general impressions, excessive repetition and insufficient synthesis means that it is not concise, and these same factors interfere with the overall clarity.

Ralph L. Kodell

The Toxicological Review is logical, but it is not concise. There is considerable repetition from section to section, which seems to be characteristic of EPA's toxicological reviews because of their structure and format. There are quite a few annoying errors in the text and tables in the discussion and summarization of the toxicology data that make it difficult at times to follow the presentation. The rationale for not doing BMD modeling of inhalation effects is not convincing. There are some discrepancies between text tables and appendices in the derivation of the oral slope factor for cancer. Although EPA has clearly presented its rationale for the uncertainty factors that have been applied, I disagree with some of them. If the errors and discrepancies are resolved and a better rationale for not doing BMD modeling of inhalation effects is provided, then I believe that EPA will have clearly presented and synthesized the scientific evidence for noncancer and cancer hazards.

Lawrence H. Lash

The toxicological review is generally well written and logically presented. There is some repetition, particularly between sections 4 and 5. While this is not a major issue, the

presentation in section 5 could be more of an analysis without direct repetition of the data presented in section 4. The limitations of the database are clearly indicated for the different types of studies (i.e., oral vs. inhalation exposure, acute vs. subacute and chronic, cancer bioassays) and for the different endpoints that are examined in each study. Rationale for the choices of principal studies, UF values, and modeling methods are clearly and logically presented. A few conclusions or statements are made that are ambiguous and need to be more precisely presented. Overall, however, the scientific evidence for the major conclusions and the basis for the calculated RfD, RfC, and oral cancer slope values are logically presented.

Edward A. Lock

The review is presented in a logical progression from acute to chronic to risk assessment and the findings are clearly documented. However, I did find as I read through the document that there was quite a lot of repetition of the findings and I wondered if it could not be more concise in places. Overall, there is not a large amount of toxicology data on hexachloroethane and, hence, the same papers are quoted under many headings giving the reader the impression he has read this before, but the slant is different. I have tried to think of ways to change this and not really come up with anything positive, summarizing the findings in tables is good and helps this in part.

Two general points regarding the non-cancer and cancer hazards as follows: for the non-cancer endpoint, the administration of hexachloroethane in the diet leads to loss due to sublimation and in the Gorzinski paper, although they attempt to take this into account, the actual dose the rats receive is still not very precise as you are balancing loss due to vaporization, daily food consumption and exposure via inhalation?? So I wondered why the more recent NTP (1989) 90-day study, where the dose was by gavage and hence the exposure somewhat more precise, was not used?

For the cancer hazard, it is still possible, in my opinion, that the renal cancer could fit with current ideas on chemically-induced renal cancer that is a combination of male rat-specific α_2u-globulin nephropathy and exacerbation of chronic progressive nephropathy interacting to produce in male rats, but not female rats, a small increase in the incidence of renal cancer (see later comments). I also felt that the discussion on some aspects of the renal injury was presented in rather a negative way, e.g., page 79 - Other possible modes of action: “There is insufficient evidence to support an α_2u-globulin-related mode of action for renal tumors following HCE exposure.” Rather than saying hexachloroethane met 6 out of 7 of the criteria needed to class it as working by an α_2u-globulin mechanism but could not definitely be put in this class as the protein in the hyaline droplets has not been examined for α_2u-globulin.

General Charge Questions:

2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review.

Jack B. Bishop

I believe an immunohistochemical assessment of kidneys from the NTP 90-day study animals for the presence of a2µ-globulin would help close the data gap regarding mechanism responsible for the male rat kidney lesions; however, I don't know that it would significantly alter the assessments' overall conclusions.

Data for assessing hexachloroethane potential for effects on reproduction and development were inadequate. Additional studies are needed.

Lucio G. Costa

No additional studies are known.

Lynne T. Haber

I do not know of any other relevant studies.

Ralph L. Kodell

I do not know of any additional studies.

Lawrence H. Lash

The document states that all references up to February, 2010 were retrieved for development of this toxicological review. A search of the PubMed database on 10 August, 2010 revealed one additional reference that could provide relevant, additional information on the toxicological effects of HCE. It should be noted, however, that this more recently published study investigated a variety of HCE-based pyrotechnic smokes rather than pure HCE. As with all such studies, attribution of effects to a single component in the mixture is difficult. Thus, it is likely that this study would not provide any new, significant insight into HCE-induced toxicity. The reference is included here, however, for the sake of completeness.

Additional study:

Hemmilä M, Hihkiö M, Kasanen JP, Turunen M, Järvelä M, Suhonen S, Pasanen AL, Norppa H. (2010) Cytotoxicity and genotoxicity in vitro and irritation potency in vivo of two red phosphorus-based pyrotechnic smokes. *Mutat. Res*. **701(2)**, 137-144 [PMID: 20601099] Epub 2010 Jun 18.

Edward A. Lock

Much is made of the fact that the α2u-globulin mechanism cannot contribute to the increase in renal cancer in male rats, as only hyaline (protein) droplets were reported in the renal tubules, it not having been established that they contain the protein α_2u-globulin. **I strongly recommend that somebody goes back to the rat NTP 90 day study and confirms or refutes an increase in this protein using immunocytochemistry in the kidneys of male rats**. I recommend this particular study as α_2u-globulin declines with age, as the levels of testosterone drop, and will not be present in the kidneys of male rats from the 2 year studies. Once this is known then it would seem likely that the increase in renal cancer in male rats could be explained by a combination of α2u-globulin-mechanism and hexachloroethane-induced exacerbation of spontaneous chronic progressive nephropathy (CPN). **Both of these modes of action are not considered relevant to humans**. I attach a recent review I wrote in collaboration with Dr Gordon Hard on this issue; current thinking supported by studies confirms that chemicals can exacerbate the progression of chronic progressive nephropathy in both male and female rat kidneys. If the CPN pathology is severe enough it can lead to an increase in renal tubular adenoma/carcinoma which is normally only expressed in male rat kidney as the CPN is more progressive in this sex of rat and in some cases this, plus a combination of α2u-globulin accumulation, explains the male rat specificity. Neither of these responses are seen in humans, as discussed in our paper, and hence the renal tumors are considered to be male-rat specific (Lock and Hard, 2010).

Chemical-Specific Charge Questions:

(A) Chronic Oral Reference Dose (RfD) for Hexachloroethane

1. A 16-week dietary exposure study of hexachloroethane in F344 rats by Gorzinski et al. (1985) was selected as the basis for the derivation of the RfD. Kidney effects were observed in male rats in this study at doses below the range of exposure tested in the available chronic NTP (1989) study. Please comment on the scientific justification for the use of the subchronic Gorzinski et al. (1985) study as the principal study for the derivation of the RfD. Is the rationale for this selection clearly described? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Jack B. Bishop

Of the available chronic and subchronic studies of hexachloroethane (NTP 1989 103-week F344 rat gavage study; NCI 1978 78-week Osborne-Mendel rat gavage study; NCI 1978 91-week B6C3F1 mouse gavage study; NTP 1989 13-week F344 rat feed study; Gorzinski et al. 1985 16-week rat feed-study), the Gorzinski et al. 1985 study would appear to be the most appropriate for determining the RfD since it was the only study in which a NOAEL was established for renal tubular effects in male rats. The EPAs rationale for this selection was clearly described on pp. 84-90 of the review document.

Lucio G. Costa

The sub-chronic study of Gorzinski et al. (1985) was chosen over a subchronic NTP study (NTP, 1989). The original Gorzinski study was reviewed and found to have limitations in the amount and detail of results reported. However, results show dose-related effects on renal morphology in male rats, with a NOEL of 1 mg/kg/day. Results in the NTP study were similar. However, the lowest dose tested (343 mg/kg/day) proved to be a LOEL. The Gorzinski study would thus appear the better choice between sub-chronic studies. There is also a chronic NTP study (NTP, 1989) with hexachloroethane in rats. However, even this study provided no NOEL value but only a LOEL of 7 mg/kg/day for male animals. Though this study could be modeled to derive a BMD, EPA chose a BMD value derived from the Gorzinski study, as it was lower. I have no major issue with this decision, which is acceptably described on pages 84-90 of the document.

Lynne T. Haber

This is a reasonable choice in the absence of information about why effects were observed at lower doses in the subchronic study than is estimated by the BMD modeling for the chronic studies, taking into account the extrapolation needed for the chronic study. However, this apparent inconsistency needs to be addressed more explicitly in the discussion of the choice of principal study, and is an important uncertainty.

An alternative would be the BMDLs for kidney effects (increased severity of tubular nephropathy; linear mineralization) in male rats (NTP, 1989). These endpoints have

similar BMDLs of 2.6 and 3.2, respectively. Using the chronic study would have the advantage of being for the study duration of interest, but has the disadvantage of requiring extrapolation below the lowest dose. The Toxicological Review should discuss these opposing considerations in choosing the principal study, rather than simply defaulting to the lowest POD.

Ralph L. Kodell

Toxic effects in the kidney were the most consistent adverse effects seen across the subchronic and chronic studies reviewed. Based on EPA's review, it appears that the two primary competing critical effects are atrophy and degeneration of renal tubules observed in a 16-week study by Gorzinski et al. (1985) at incidences of 10%, 20%, 70% and 100% in male F344 rats at doses of 0, 1, 15, and 62 mg/kg-day; and moderate to marked nephropathy observed in a 103-week study by NTP (1989) at incidences of 36%, 48% and 60% in male F344 rats at doses of 0, 7 and 14 mg/kg-day. EPA stated that the ability of the NTP (1989) study to inform the kidney effects observed at the lowest dose tested in the Gorzinski et al. (1985) study is limited because the lowest dose tested in the NTP chronic exposure study represented a LOAEL. I do not disagree with the choice of the Gorzinski study as the principal study. However, I believe that a better rationale for choosing the Gorzinski study over the NTP study is simply that it produced significant kidney toxicity at only 16 weeks and produced the lowest BMD and BMDL values. Moreover, I think the NTP study does provide information that can be used in selecting an uncertainty factor for subchronic-to-chronic exposure. As I will discuss below under charge question A4, I think that a UF of 10 is too large, and that a smaller factor of 3 should be considered.

Lawrence H. Lash

The Gorzinski et al. (1985) study was appropriately chosen as the principal study for derivation of the RfD. Comparisons were made with the NCI (1978) chronic mouse study and the NTP (1989) chronic rat study based on the consistency of the responses, the dose response of the observed nephropathy, the level of nephropathy in controls, and the number of HCE doses and animals tested. The document also explained the ability to calculate NOAEL and LOAEL values based on the data from each study. Rationale for the kidney as being the primary target organ (i.e., most sensitive) was clearly explained. Although hepatic effects were also consistently observed, the document clearly explained the differences in sensitivity between the liver and kidneys as target organs. The only other candidate for the principal study for derivation of the RfD is the NTP (1989) chronic rat study. The document explains the limitations of that study based largely on the study design and characteristics of the database. Specifically, the document states "The ability of the chronic NTP (1989) study to inform the effects observed at the lowest dose tested in the Gorzinski et al. (1985) study is limited because the lowest dose tested in the chronic exposure study represented a LOAEL."

Edward A. Lock

I did raise some concern regarding the Gorzinski et al., (1985) study with regard to actual exposure due to feeding in the diet and suggested the NTP (1989) study, but I subsequently found that the NOAEL was not established. Hence, with the data available, I support the use of the 16-week dietary exposure study. In an ideal world, one would like to conduct further studies over 16 weeks in F-344 rats, giving hexachloroethane by gavage at doses below 34mg/kg/day.

(A) Chronic Oral Reference Dose (RfD) for Hexachloroethane

2. Nephrotoxicity as indicated by atrophy and degeneration of renal tubules in male rats (Gorzinski et al., 1985) was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Jack B. Bishop

Selection of atrophy and degeneration of renal tubules in male rats as the critical effect for the RfD seems most appropriate and scientifically justified. It was an effect that has been consistently observed, it was sensitive and it was dose dependent.

Lucio G. Costa

Atrophy and degeneration of renal tubules in male rats appears to be the critical effect in all studies upon oral administration of hexachloroethane. This is a primary effect, while effects in liver are secondary.

Lynne T. Haber

The choice is reasonable, taking into account the points noted in the response to the previous question.

Ralph L. Kodell

I believe that the selection of atrophy and degeneration of renal tubules in male rats (Gorzinski et al., 1985) is scientifically justified. Among all candidate PODs, this endpoint leads to the second smallest BMD and BMDL values. Only slight hypertrophy and/or dilation of proximal convoluted renal tubules lead to slightly smaller BMD and BMDL values. However, EPA stated (p. 90) that tubular nephropathy in the NTP 103-week study was characterized as atrophy and degeneration of renal tubules, which endpoint has been consistently observed following HCE exposure in several studies. Thus, the justification for choosing it as the critical effect is clearly described and seems justified. However, I believe that the moderate to marked nephropathy observed in the NTP chronic study (page 25 and pages B-17, B-18) is equally relevant because it is reflects the dose-response for essentially the same endpoint following *chronic* exposure. I believe that atrophy and degeneration of renal tubules in male rats at 16 weeks is justified as the critical effect, but that the dose-response for tubular nephropathy at 103 weeks should be taken into account in selecting a subchronic-to-chronic uncertainty factor as I will discuss below.

Lawrence H. Lash

Selection of atrophy and degeneration of renal tubules in male rats, as observed in the Gorzinski et al. (1985) study was appropriately selected as the critical effect for derivation of the RfD. This selection was based on consistency of the effect being observed, the effect being the most sensitive one observed, and the existence of dependence on HCE exposure dose. Thus, the choice of this effect is clearly justified and is preferable to other potential choices, such as hepatic necrosis.

Edward A. Lock

It is clear that hexachloroethane causes renal injury, degeneration and regeneration in proximal renal tubules in male rat kidneys, which have a strong resemblance to that seen with chemicals that cause the accumulation of the male-rat specific protein α2u-globulin. A confounding factor in the identification of renal tubule atrophy and degeneration is spontaneous CPN which can also cause renal tubule degeneration (Wolf and Mann, 2005). Therefore, it is now standard practice to score the extent of spontaneous CPN in 90-day and 2-year studies. This was not conducted on the Gorzinski et al., (1985) study and, hence, I question if it is possible to separate the degeneration due to spontaneous CPN from that due to the chemical in this study. I am not a pathologist and hence would defer this to an expert renal pathologist to clarify this point. I think this endpoint is justified, my concern is just related to the possibility that the chemically related renal injury could occur at higher doses than those used in the calculations.

(A) Chronic Oral Reference Dose (RfD) for Hexachloroethane

3. Benchmark dose (BMD) modeling was applied to the atrophy and degeneration of renal tubules data in male rats to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted and clearly described? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% increase in the incidence of atrophy and degeneration of renal tubules) scientifically justified and clearly described?

Jack B. Bishop

The BMD modeling was appropriately conducted and clearly described. Selection of a 10% increase in renal tubular atrophy and degeneration as the BMR for deriving the POD is justified and clearly described.

Lucio G. Costa

The BMD modeling appears to have been carried out appropriately and is clearly described. The benchmark response chosen also appears appropriate.

Lynne T. Haber

The approach is consistent with EPA guidance and is clearly described.

Ralph L. Kodell

The BMD modeling has been appropriately conducted and clearly described. The selection of a 10% extra risk as the BMR for deriving the POD follows EPA's standard procedure. In this document it is said to represent a minimally biologically significant response level.

Lawrence H. Lash

Choice of the BMR as representing a minimally biologically significant response level to derive the POD is clearly explained and seems to be scientifically justified. The document applies the modeling approach to several datasets and obtains a 100-fold range of values for the POD. The document then provides rationale for choosing one particular POD value, based on sensitivity and concordance of the specific renal pathology (viz., atrophy and degeneration of renal tubules) across multiple studies. Thus, the POD value used for derivation of the RfD (i.e., 0.728 mg/kg-day) does indeed seem to be the most scientifically appropriate value.

Edward A. Lock

See comments above which are relevant such that you could end up with a higher POD, possibly above 1 mg/kg/day, which would influence the RfD. I think a 10% increase can

be justified as you need some scope in differentiating between the spontaneous and chemically-induced injury; and this is clearly described in the text.

(A) Chronic Oral Reference Dose (RfD) for Hexachloroethane

4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfD. Are the UFs scientifically justified and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

Jack B. Bishop

Applications of UFs of 10 for the UF_A inter- and UF_H intra-species uncertainties are both justified. I concur that no UF_L is needed as the Gorzinski study provided a NOAEL. There are deficiencies in the data base, such as the lack of a test for multi-generation reproductive toxicity; so I would assign a UF_D of 3. The NTP 1989 chronic study did not provide a NOAEL and, although the endpoints in this chronic study were slightly different and perhaps less sensitive than those of the subchronic, the results are pretty much in line. Nevertheless, it seems to me the UFs for subchronic to chronic study uncertainty should be somewhere between 2 and 4, but not 10. Thus, I would go with a composite UF of ~1000 resulting in an RfD between 0.0007 and 0.001.

Lucio G. Costa

A total UF of 3000 was applied to the value of BMDL10 of 0.728 mg/kg/day, which resulted in an RfD of 0.0002 mg/kg/day. Two standard 10 UF were chosen for interspecies and intraspecies differences. This appears appropriate, as no information is available to modify these factors. A 3 UF was applied to account for deficiencies in the data base. This also appears to be appropriate. An additional subchronic to chronic UF of 10 was used, as the Gorzinski et al. (1985) study was a 16-week study. The use of this additional UF is debatable. The Gorzinski study was chosen over other studies as it provided a NOEL, though it is a less than an ideal study. The chronic NTP study provided a LOEL of 7 mg/kg/day. By applying three 10 UF (for interspecies and intraspecies differences and for LOEL to NOEL, in addition to a 3 UF for insufficient data base, the resulting RfD would be (with a 3000 UF) 0.002 mg/kg/day. Thus, it is the opinion of this reviewer that a 300 UF applied to the BMDL10 derived from the Gorzinski et al. (1985) study would suffice, for a resulting RfD of 0.002 mg/kg/day. Nevertheless, a 1000 UF, as suggested by other panel members, would be acceptable.

Lynne T. Haber

The discussion of the UFs includes some nice analysis and thinking about the data beyond defaults. However, I would recommend additional enhancements and some changes in specific UFs:

UFA and UFH: I agree with the bottom line that the data are not sufficient to develop a chemical-specific value. However, the criteria for using a chemical specific value are (primarily) whether the chemical is sufficiently well understood to identify the active form, and whether data on an appropriate dose metric are available from adequate studies

in the species for the critical effect and in humans. It is not necessary for the toxicokinetics to be "fully characterized." See:

IPCS (International Programme on Chemical Safety) (2005). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment. (http://whqlibdoc.who.int/publications/2005/9241546786_eng.pdf)
(EPA has similar guidance under development – using the term data derived extrapolation factors.)

UFS: Based on an overall weight of evidence evaluation of the data, I believe a factor of 3 is more appropriate for this factor. This takes into account the following: As noted, this factor addresses the issue of progression. A chronic study is available with a somewhat higher BMDL (NTP 1989), and a higher LOAEL (no NOAEL). There is, however, uncertainty in the chronic study, due to the large extrapolation from the response at the LOAEL. Both the chronic and subchronic studies are in F344 rats, so the difference is not due to differences between strains. While there is uncertainty in the NOAEL in the chronic study, the finding of a higher BMDL in the chronic study than in the subchronic study suggests that any progression is small. Conversely, because the endpoints noted in the subchronic study are more sensitive than those in the chronic study, and the background is lower, there is the potential that the BMDL for the chronic study is too high. However, I would not expect it to be too high by a factor of 37 (calculated by taking the BMDL from the subchronic study, dividing by 10, and taking the ratio with the chronic BMDL: 2.6/0.07 = 37). Using a factor of 3 for the subchronic study is more reasonable, and accounts for any uncertainties in the chronic data.

UFL: OK.

UFD: It would be useful to remind the reader about some general rules of thumb for the database uncertainty factor. As the authors know, this uncertainty factor addresses the question of whether the correct critical effect has been identified, and whether a new study using a different design or different species would identify a different critical effect or lower point of departure (aside from differences addressed with UFL and UFS). A "complete" database indicating no need for a database uncertainty factor (unless the existing studies indicate that specialized studies are needed to evaluate endpoints such as neurotoxicity or immunotoxicity) includes subchronic or chronic systemic toxicity studies evaluating the appropriate range of endpoints in 2 species, developmental toxicity studies in 2 species, and a multigeneration reproductive toxicity study. For HCE, the database includes multiple systemic toxicity studies in rats, a cancer study in mice that included some evaluation of noncancer endpoints, several developmental toxicity studies in rats (but not a second species), and no studies of functional effects on reproductive toxicity. (Because of the importance of the NCI mouse study in partially covering the issue of systemic toxicity in a second species, the NOAEL/LOAEL for this study (reported on p. 31) should be included in Table 4-19.) Thus, the database is missing the 2-generation reproductive toxicity study, a developmental toxicity study in a second species, and has a weak second species for systemic toxicity. The missing data are

ameliorated by information on related chemicals and metabolites, all of which indicate that the liver and kidney are the key targets, and are more sensitive than reproductive and developmental endpoints. The absence of reproductive tract histopathology in the chronic and subchronic systemic toxicity studies also supports this conclusion. Thus, I agree with a UFD of 3, based on these considerations, and the general conclusion that, for chemicals with a complete database, the systemic toxicity and reproductive toxicity studies are more likely to drive the assessment that developmental toxicity studies (see Dourson, M.L., L. Knauf and J. Swartout. 1992. On reference dose (RfD) and its underlying toxicity database. Tox. Ind. Health 8(3): 171-189). I agree that a factor of 1 is probably not appropriate, in light of the deficiencies in the systemic toxicity data and the lack of a functional reproductive toxicity assay, although a factor of 1 might be supported if further information is available that supports a conclusion that the rat is the most sensitive species for this class of compounds, and that the kidney is the most sensitive target. However, the authors should give a more complete description of the missing studies, and other relevant considerations, as discussed in this comment.

Ralph L. Kodell

A default UF_A of 10 for interspecies uncertainty seems justified, there being relatively little HCE toxicokinetics information in animals and none in humans.

A default UF_H of 10 for intraspecies uncertainty seems justified in the absence of information on the variability of response to HCE in humans. There is no evidence to suggest a need for an additional factor to account for sensitive subpopulations (e.g., children).

I do not believe that a UF_S value of 10, the default value, is needed for subchronic-to-chronic exposure. I disagree with the rationale that because the lowest dose tested in the NTP study was considered a LOAEL, the NTP (1989) chronic study cannot inform the dose-response for kidney effects in the 16-week study of Gorzinski et al. (1985). I disagree with the rationale that there are no data to exclude the possibility that chronic exposure could increase the severity of the observed kidney effects or could result in similar effects at lower doses. EPA stated (p. 90) that tubular nephropathy in the NTP 103-week study was characterized as atrophy and degeneration of renal tubules, which is the critical effect from Gorzinski et al. (1985), and which has been consistently observed following HCE exposure in several studies. The fact that the BMD and BMDL for kidney tubular nephropathy from the NTP study (3.81 and 2.60) were actually *larger* than the corresponding values for the Gorzinski study (1.34 and 0.728) (Table 5-2) argues against the likelihood that chronic exposure would increase the severity of the observed kidney effects or would result in similar effects at *lower* doses. If a factor greater than 1 is needed at all, it should not be 10. I recommend a UF_S value of 3.

A UF_L value for LOAEL to NOAEL extrapolation was not applied. The rationale given is that a 10% increase in the incidence of renal tubule atrophy and degeneration is considered a minimally biologically significant change. To me a 10% BMD (BMDL) is more reflective of a LOAEL than a NOAEL. I suppose that UF_L =1 is supported by

common practice, but I would prefer a UF_L value of 3 because I think a 10% BMR is more than minimal and that a 10% BMD ought to be reduced like a LOAEL is reduced.

I do not like using a database uncertainty factor, UF_D. In general, I think that you either have enough data to set an RfD or you don't. In this case I think there are sufficient data to set an RfD, even though a multigenerational reproductive study is lacking in the HCE toxicity database. The toxic effects that were observed in the available developmental toxicity studies were observed at higher doses than the doses that induced renal toxicity in the subchronic and chronic studies. I recommend not applying a UF_D, or equivalently, setting UF_D=1.

I believe that a composite uncertainty factor of 10x10x3x3x1 ~ 1000 should be used instead of 3000.

Lawrence H. Lash

A composite UF of 3,000 was used in derivation of the RfD. This was based on application of 4 of 5 possible UF values. Rationale for use of each value was clearly and concisely explained, and appears to be scientifically justified. I would agree with this value and thus recommend retention of a composite UF of 3,000.

(1) A default UF of 10 was applied for interspecies extrapolation. This choice is based on the incomplete toxicokinetics for HCE and the general lack of data in humans. This is completely appropriate as virtually no information is available from exposed humans.

(2) A default UF of 10 was used to account for extrapolation of subchronic-to-chronic exposure. This is rightly based on the 16-week exposure used in the principal study and the lack of an appropriate and alternative chronic study. Although it may be argued that this UF value could be reduced to 3 because the 1989 NTP study does provide some information on chronic exposure, I would not recommend this because of the limitations in that study, which are clearly described in the document. Although it is doubtful that additional studies would markedly change the POD value, I do not believe that there is enough justification to downgrade this UF with only one study being considered.

(3) A default intraspecies UF of 10 was applied to account for potentially sensitive human populations. This is entirely appropriate because there is a complete lack of data on the potential impact of interindividual differences in metabolism or influences of other factors (e.g., age, gender, pre-existing conditions, etc.) on HCE metabolism, disposition, or toxic response.

(4) An UF of 3 was applied to account for deficiencies in the HCE toxicity database, including the lack of a multigenerational reproductive study. This is standard practice and appears justified. Again, although additional data would not be likely to markedly change the POD value, some caution is needed in the interest of public health, particularly considering the lack of breadth of the HCE database.

(5) No UF value for a LOAEL to NOAEL extrapolation was applied because this aspect is considered in the BMDL modeling that was used to calculate the POD. This argument is clearly and concisely presented.

Edward A. Lock

The uncertainly factors UF_A and $UF_{H,}$ I guess, are pretty standard practice, and with so little data on aspects of toxicity covering reproduction etc., UF_D at 3 is acceptable. It is the UF_S that is somewhat debatable around the issue of renal tubule nephropathy and the progression of the lesion with time; is the moderate to marked tubular nephropathy at 2 years (NTP 1989) more marked than the degeneration seen at 16 weeks?

(B) Chronic Inhalation Reference Concentration (RfC) for Hexachloroethane

1. A 6-week inhalation exposure study in rats by Weeks et al. (1979) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Is the rationale for this selection clearly described? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Jack B. Bishop

The rationale for selecting the study by Weeks et al. for derivation of the RfC is clearly described and scientifically justified. It is the only repeat-exposure study available and I could not identify any other suitable study.

Lucio G. Costa

The subchronic study (six weeks) by Weeks et al. (1979) appears to be the only study available for deriving an inhalation RfD. Thus, if such value has to be determined, there is no other choice. The study presents data on exposure of rats, dogs, guinea pigs and quails to hexachloroethane; however, several details are lacking from the paper. A concentration of 465 mg/m^3 was appropriately considered the NOEL for most species.

Lynne T. Haber

While the study appears to be the best and most appropriate available, it appears to barely meet the guidelines for study adequacy, and more details need to be provided to document that it was sufficient as a principal study. The previous EPA evaluation was based on essentially the same database, and apparently did not consider the data adequate, in light of the absence of a current RfC. In light of this difference, the documentation should address why it was considered appropriate to calculate an RfC from the Weeks et al. (1979) study when a previous assessment did not calculate an RfC. Specifically, the RfC guidelines specify that inhalation studies adequately evaluating the respiratory tract are needed; test guidelines note the evaluation of the lungs, trachea, larynx, and multiple sections of the nose. No information is provided in the write-up on the thoroughness of the histopathology of the respiratory tract, aside from the reports of mycoplasma-related lesions in the nose, trachea and lung, and it is not entirely clear whether these were found on gross examination or histopathological examination. In addition, a significant limitation of the study is that there was no evaluation of effects immediately post-exposure; with a 12-week recovery time, effects may have been missed, although it appear unlikely that histopathology at the low concentration would have been missed, in light of the absence of histopathology at the high concentration.

The limitations to the principal study and overall database discussed here and in my responses to the other charge questions related to the RfC should also be noted in Section 5.3, in the discussion of the uncertainties in the RfC.

Ralph L. Kodell

The limited human studies of HCE in workers are inadequate and there are no chronic studies. The 6-week inhalation study of Weeks et al. (1979) is the only repeated exposure study available; it evaluated several species at three concentrations of HCE for a variety of toxicological endpoints. The rationale for choosing the study by Weeks et al. (1979) as the principal study for derivation of the RfC is clearly described and scientifically justified. I cannot identify any other studies that should be considered.

Lawrence H. Lash

Although the oral exposure database has some deficiencies, the inhalation exposure database is even more limited. Thus, there are few viable choices for the principal study to be used as the basis for derivation of the RfC. The Weeks et al. (1979) study has several strengths. First, this is a subchronic inhalation study that was conducted in 4 species. Second, 3 doses plus a control were administered. Third, with some exception (e.g., the quail), a consistent neurotoxicity response was observed across species. As noted in the document, the subchronic inhalation study by Weeks et al. (1979) was the only repeated exposure study available, so was selected as the principal study for the derivation of the RfC. Although limitations in the study exist, including how and what type of information were reported, this is really the only plausible choice. One striking aspect about this study that the document does not address is the high dose of HCE at which the neurotoxicity is observed. It is surprising that the document does not question the relevance of neurobehavioral effects that occur, albeit consistently across multiple species, at a dose of 2,517 mg/m^3. Some discussion of this is certainly warranted. Although one can consider that it is important to establish a threshold for all adverse effects, it may be that certain modes of action, such as neurotoxicity, do not even come into play until one reaches very high doses. Thus, the issue of relevance should be discussed.

Edward A. Lock

There seems to be no choice as the Weeks et al. study is the only one available and, hence, is justified. The rationale for selecting this study is provided and justified.

(B) Chronic Inhalation Reference Concentration (RfC) for Hexachloroethane

2. Neurobehavioral effects in Sprague-Dawley rats (Weeks et al., 1979) were selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

Jack B. Bishop

Selection of neurobehavioral effects in Sprague-Dawley rats (Weeks et al., 1979) as the critical effect for determining the RfC appears to be scientifically justified and clearly described.

Lucio G. Costa

The original Weeks et al. (1979) study was reviewed. The authors state that upon repeated inhalation exposures to hexachloroethane, no effects were seen in rats, dogs and guinea pigs at the two lower concentrations. At the highest concentration, it is reported that dogs "developed tremors, were ataxic, hypersalivated, showed severe head bobbing, facial muscular fasciculations, and held their eyelid closed. One dog convulsed." These signs may be reflective of a nervous system involvement. No signs of this nature were observed in guinea pigs. In rats, "all animals showed tremors, ruffled pelt and red exudate around the eye." As these effects were the major adverse effects seen, it would be appropriate to consider them, and determine the NOEL accordingly. In support of this, tremors were also seen in rat dams upon exposure to the same high concentration of hexachloroethane, and upon administration of 500 mg/kg orally (Weeks et al. 1979). No effects were seen in rats tested for spontaneous motor activity or active avoidance at any concentration. Overall, this limited data indicate that signs possibly reflecting CNS excitation were observed in dogs and rats at the highest concentration of hexachloroethane. As such, these can be considered as the critical effect for the RfC.

Lynne T. Haber

Choice of the critical effect is challenging in light of the limited number of studies of appropriate duration, the long post-exposure period before sacrifice, and the lack of any clear exposure-related lesions. The document makes an adequate argument for neurobehavioral effects as a critical effect, particularly in light of the support from clinical signs in other studies. However, I would recommend several enhancements/clarifications. First, in light of the limited data, it would be useful to draw on structure-activity relationships for related chemicals, to support the conclusion of neurotoxicity as the critical effect. Structure-activity relationships may also shed some light on expected dose-response relationships. In the discussion of the principal study (or maybe in the synthesis of noncancer effects), it would be useful to highlight more the striking apparent difference in target between the oral and inhalation studies, with the former affecting liver and kidney, and the latter affecting the nervous system, and discuss whether this difference is likely to be real and possible reasons. (While there may have

been mild effects on the liver and kidney that recovered by the time of sacrifice in the inhalation studies, only the Weeks study reported neurological effects following oral exposure. Was this a route-related difference, or difference in the reporting of clinical signs between Weeks and other studies? If route-related, it could be due to the impact of first-pass metabolism, indicating that the neurological effects are due to the parent compound, while metabolite(s) contribute more to the liver and kidney effects.) The importance of addressing such route-specific differences is also part of the reason that it is useful to address the synthesis sections (4.6 and 4.7) by endpoint, rather than by route, so that information on similarities and differences between routes for each endpoint can be considered.

With regard to the choice of critical effect, it would be useful to additionally highlight that the respiratory effects were not chosen because they were attributed to the mycoplasma infection.

Ralph L. Kodell

The EPA accepted the study authors' attribution of increased respiratory lesions in rats to a potentiation of an endemic underlying mycoplasia infection rather than a direct result of HCE exposure, and hence did not select this as the critical endpoint. Statistically significant neurotoxic effects were seen in rats (subchronic and reproductive studies) and dogs (subchronic study) at the highest dose. Even though the behavioral toxicity study in rats did not show significant behavioral toxicity, the tested endpoints were elevated compared to the controls. Reduced body weight was observed at the highest dose in rats (subchronic and reproductive studies) and guinea pigs (subchronic study). The EPA selected neurobehavioral effects as the critical effect because of its consistent observation across studies in rats and dogs. This is scientifically justified, although the same reasoning could be used to select reduced body weight observed across studies in rats and guinea pigs. However, given that both effects have the same NOAEL and LOAEL, it shouldn't make any difference which effect is chosen for deriving the RfC, because BMD modeling was not done. I could not find the incidence data on tremors in dogs and pregnant rats. I believe these data ought to be reported somewhere, perhaps in Table 4-20.

Lawrence H. Lash

The document explains how such neurobehavioral effects were consistently observed and were observed in multiple species. Other effects that were observed could readily be explained as being linked to other causes and not dependent on the HCE exposure. The only concern that I have, as expressed above, is that the doses of HCE at which effects were observed are very high. The issue of dose relevance with respect to human exposures was not really discussed nor were the implications of effects only occurring at such high doses. Otherwise, the choice of neurobehavioral effects as the critical effect upon which to base derivation of the RfC seems scientifically justified and the rationale behind this choice was clearly explained in the document. It is rather curious, however,

that no nephrotoxicity was observed after inhalation exposure considering how prominent this effect is after oral exposure. Some comment on this might be warranted.

Edward A. Lock

Based on the data available, it does appear that neurotoxicity is justified and clearly described. The fact that no renal lesions were observed by this exposure route is interesting and is presumably due to toxicokinetics and absorption, and a strain difference in the rat, Sprague-Dawley versus F344?

(B) Chronic Inhalation Reference Concentration (RfC) for Hexachloroethane

3. The NOAEL/LOAEL approach was used to derive the POD for the RfC. Please comment on whether this approach is scientifically justified and clearly described.

Jack B. Bishop

It appears to me that no other option other than the NOAEL/LOAEL was available to derive the POD for the RfC. The approach used was clearly described and, in view of the limited options, scientifically justified.

Lucio G. Costa

This approach is acceptable. Since inhalation exposure to hexachloroethane in the Weeks et al. (1979) study was intermittent, the NOEL was adjusted to obtain a POD of 83 mg/m^3.

Lynne T. Haber

Yes. The data are insufficient to use any other approach. However, the reason for not doing BMD modeling is not accurately characterized. The reason for not doing the modeling is because no incidence data were available at exposures other than the high concentration. It *would* be possible to do the modeling with 100% response at the high concentration if there were data on the response at intermediate concentrations.

Ralph L. Kodell

The reason stated for not applying BMD modeling was that it was precluded because 100% of the high-exposure animals displayed neurological effects. I do not think that is a valid reason without some qualifications. Was BMD modeling attempted? If so, was it not possible to fit any models successfully, perhaps because of the 100% response at the highest dose? Simply having a 100% response at the highest dose does not preclude BMD modeling. Unfortunately, the incidence data for neurotoxic effects were not presented. It is only stated that the response at 2517 mg/m^3 was 100%. Response percentages at 0, 145 and 465 mg/m^3 were not reported. Although the NOAEL/LOAEL approach is scientifically justified when BMD modeling cannot be done satisfactorily, I do not believe it has been clearly described why BMD modeling could not be done in this case. *[At the panel meeting, EPA indicated that the individual responses at each dose level were not available in the study of Weeks et al. (1979). I believe that should be stated as the reason why BMD modeling was not done. This deficiency also needs to be made clear in the presentation and discussion of the toxicology data.]*

Lawrence H. Lash

Rationale for choosing a NOAEL approach rather than BMD modeling for derivation of the POD for the RfC is clearly and succinctly explained. The document states that the

BMD modeling approach cannot be used because 100% of the animals exhibited neurotoxicity at the highest dose administered. Hence, the NOAEL (= 465 mg/m^3) was used as the starting point for deriving the POD. Because the exposures in the critical inhalation study were intermittent, this value was adjusted for continuous exposure, resulting in a POD of 83 mg/m^3. A further adjustment was made to derive a human equivalent concentration (HEC), although the calculations resulted in no change in the POD. In describing how the HEC was derived, the document explains the category of gas into which HCE should fall (page 98, paragraph 1). I found this description and the resulting conclusion that HCE is likely a Category 2 gas to be confusing. A more thorough explanation would be helpful.

Edward A. Lock

I am not an expert in this area, but it seems a logical approach and is explained in the document.

(B) Chronic Inhalation Reference Concentration (RfC) for Hexachloroethane

4. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs scientifically justified and clearly described? If changes to the selected UFs are proposed, please identify and provide a rationale.

Jack B. Bishop

The UFs of 10 for UF_H, 10 for UF_S, and1 for UF_L are each clearly described and justified. However, I don't believe interspecies toxiokinetic uncertainty is adequately covered and think the UF_A should be 10. There is sufficient diversity in toxicity studies to support a UF_D of 3. So, I agree with a composite UF of 3000 but would derive it in a slightly different manner.

Lucio G. Costa

A total UF of 3000 applied: 3 UF for interspecies differences, 10 UF for intraspecies differences, 10 UF for subchronic to chronic, and 10 UF for database insufficiency, for a resulting RfD of 0.028 mg/m^3. This reviewer feels that, though a chronic inhalation study is not available, a developmental study is, and the last UF for database insufficiency may be lowered to 3 for a total UF of 900 (or 1000) and a resulting RfD of ~ 0.08 mg/m^3.

Lynne T. Haber

I will address the uncertainty factor discussions one by one.

UFA: This text is generally correct and well-written. However, the reduction to a factor of 3 when the RfC dosimetry is applied is because the dosimetry addresses part of the toxicokinetics. Toxicodynamics refers to the *effect* of the chemical on the body (or – using the IPCS definition – it refers to the interactions once the chemical enters the target tissue). Either way, I don't see how the dosimetry addresses toxicodynamics. (This comment also applies to text on pp. 101-102.) It would also be useful to add some additional explanation/justification for reducing the factor to 3 when the RGDR based on the blood:air partition coefficient defaulted to 1 based on the absence of data. This discussion would be enhanced by comparison with b:a partition coefficients for rats vs. humans for other related chemicals, and a discussion that the factor is usually close to 1, or defaults to 1 because the animal partition coefficient is larger than the human value (and so the RfC guidance recommends using a ratio of 1, rather than a value larger than 1).

UFH: This text is fine.

UFS: This text is fine. It would be useful to note also that this was done in the absence of any longer-term studies (because such studies could either be used as the principal study or provide perspective on the potential for progression).

UFL: This text is fine.

UFD: Going to the 5 key studies noted above in the UFD discussion for the RfD – for inhalation, there is systemic toxicity data available from 3 mammalian species, developmental toxicity in one species, and no reproductive toxicity data. The intent of the statement that the "absence of teratogenic effects does not abrogate concern given the paucity of the inhalation database" is not clear. It would be more useful to specifically address the key study types and remaining concerns. Based on a cursory review of the studies available, one might initially suggest that a factor of 3 is sufficient, since the only missing studies are the multigeneration reproductive toxicity study and developmental toxicity in a second species (and developmental toxicity is usually not the driver, particularly for the small chlorinated alkanes). However, an argument can be made for a factor of 10 – the observed neurotoxic effects were crude and severe (tremors), and there was no evaluation of more sensitive neurological effects at the NOAEL. Limitations to the systemic toxicity studies as noted above, including the absence of any immediate post-exposure sacrifice and histopathology, can also be noted. However, moderating the concern about recovery from histopathology lesions (which might make 465 mg/m^3 an effect level) is the observation that there was no histopathology at 2517 mg/m^3, a 5-fold higher exposure, so it seems less likely that histopathology at 465 mg/m^3 would have been seen even under a standard study design.

These same issues apply to the text on p. 102 discussing data gaps.

Ralph L. Kodell

I do not think a UF_A interspecies value of 3 is justified. The rationale is that toxicokinetics differences have already been accounted for by using the ratio of animal and human blood:gas partition coefficients, so all that is needed is a factor of 3 to account for toxicodynamics. But, because neither blood:gas partition coefficient is known, a ratio of 1 was used. I do not see how this can be considered an adjustment for interspecies toxicokinetics uncertainty. I believe a UF_A value of 10 should be used. *[At the panel meeting, a panel member pointed out that using a ratio of 1 for interspecies toxicokinetics differences in the absence of data follows the guidelines and is the conventional approach. Although that is the conventional interspecies adjustment, I still do not follow how that adjustment reduces or eliminates toxicokinetics uncertainty. It seems that using an interspecies adjustment ratio of 1 in the absence of information does not reduce uncertainty about using that ratio. More explanation would be helpful.]*

The default value of UF_H =10 for intraspecies uncertainty seems justified in the absence of data on human-to human differences in susceptibility.

A default value of UF_S = 10 for subchronic-to-chronic extrapolation seems justified.

Provided that the NOAEL/LOAEL approach is the correct approach (i.e., that BMD modeling cannot be done), then I agree that UF_L = 1, as there is no extrapolation involved.

I dislike database uncertainty factors. It's true that there is only a single study on which to base an RfC for HCE. However, that single study included several species, a general toxicology study, a reproductive study and a neurobehavioral study. The lack of a developmental neurotoxicity study is a concern, and to some degree, the lack of a multigenerational reproductive toxicity study. However, I think a UF_D value of 10 is excessive. If a UF_D must be applied, I suggest $UF_D = 3$.

I believe that a composite uncertainty factor of 3000, as proposed, is justified, but I would arrive at it differently: 10x10x10x1x3 = 3000.

Lawrence H. Lash

As with the RfD, a composite UF value of 3,000 was applied to the POD for derivation of the RfC. This is somewhat surprising considering the even larger data gaps present in the inhalation database as compared to the oral exposure database. Thus, one might have expected a larger composite UF for derivation of the RfC as compared to that applied to derivation of the RfD. Of the 5 possible UFs, 4 were applied:

(1) An UF of 3 for animal-to-human interspecies extrapolation was applied. The rationale for not applying a default value of 10 was that the toxicokinetic component of uncertainty was accounted for in derivation of the HEC. Because of concerns with the rationale for how the HEC was calculated (see above), I did not find the choice of an UF of 3 to be convincing. A default UF of 10 is suggested; this change would change the composite UF to 10,000 rather than 3,000. Alternatively, an UF of 3 could be retained if better justification is provided.

(2) An intraspecies UF of 10 was applied because of the complete lack of information about human variability with regard to HCE metabolism and toxicity. As with the intraspecies UF value for the RfD, a default value seems appropriate and justified here.

(3) A subchronic-to-chronic UF of 10 was applied to account for use of a POD that is based on a subchronic exposure. Considering that no chronic inhalation studies are available and that this is standard practice, use of the default UF value is appropriate and justified.

(4) Because the RfC derivation used an actual NOAEL, no UF was applied for a LOAEL-to-NOAEL conversion. Again, this is standard practice and is in line with the approach used for derivation of the RfD with the oral exposure database.

(5) A full default UF of 10 was applied for data deficiencies. Again, considering the paucity of inhalation exposure data, this is appropriate and justified.

In comparing the present derivation of an RfC value to the previous IRIS summary done in 1987, it is surprising that the current document does not make more of the decision to derive an RfC here but to not do so in the previous risk assessment. This is because both the previous and current risk assessments were based on the same inhalation exposure

data (i.e., Weeks et al. (1979)). A little more discussion of why the current assessment felt it appropriate to derive an RfC and why the previous one did not feel it was appropriate is needed here.

Edward A. Lock

The UFs applied are justified in the light of the amount of data available by this route of exposure and the rationale is clearly spelt out.

(C) Carcinogenicity of Hexachloroethane

1. Under the EPA's 2005 "Guidelines for Carcinogen Risk Assessment" (www.epa.gov/iris/backgrd.html), hexachloroethane is "likely to be carcinogenic to humans" by all routes of exposure. Is the cancer weight of evidence characterization scientifically justified and clearly described?

Jack B. Bishop

Yes, the observation of renal adenomas and carcinomas in male rats and liver tumors in male and female mice following oral exposure to hexachloroethane makes the weight of evidence characterization scientifically justified and it was clearly described.

Lucio G. Costa

Based on the overall weight of evidence, classification of HCE as "likely to be carcinogenic to humans" appears excessive. However, after a review of the available data, and a discussion on the descriptors present in the EPA's guidelines, this classification appears inevitable. The compound would specifically fall at the low end of this group. See also Comment to Question 4.

Lynne T. Haber

I agree with the overall weight of evidence descriptor. However, the summary of the overall weight of evidence should capture the uncertainties, as well as the bottom line conclusions. As the document discusses elsewhere, there is substantial data supporting the hypothesis that the kidney tumors in the male rats are related to α2u-globulin nephropathy (or that the nephropathy contributes a substantial portion of the tumor load), but data on one endpoint are missing, so the data are insufficient to show that this hypothesized mode of action applies for the male rat kidney tumors. This point and associated uncertainties should be noted in the summary of the overall weight of evidence, to give a complete picture of the issues. Similarly, in addition to noting that liver tumors in mice and adrenal tumors in rats were observed, the summary should note that there are uncertainties regarding human relevance of these tumor types. Thus, it would also be appropriate to note that while the most appropriate choice of descriptor is "likely to be carcinogenic to humans," the weight of evidence is on the low end of the spectrum for this descriptor, due to issues for all three observed liver types.

Ralph L. Kodell

There were dose-dependent statistically significant increases in combined renal tubule adenomas and carcinomas in male rats (NTP, 1989) and in hepatocellular carcinomas in male and female mice (NCI, 1978) by the oral route of exposure to HCE. There are no chronic inhalation or dermal exposure studies. Because tumors that developed due to oral exposure occurred at sites remote from the absorption site, the EPA guidelines allow the assumption that an internal dose will be achieved regardless of route. Thus, the weight of

evidence characterization is scientifically justified and clearly described. *[There was much discussion at the panel meeting about the characterization "likely to be carcinogenic." The discussion was mostly concerned with the relevance of the carcinogenic endpoints observed. Although the panel eventually agreed on the "likely" characterization, it appeared to attach more relevance to the hepatocellular tumors in mice than to the renal tumors in rats. EPA will probably need to revise its rationale considerably, including the detailed discussion of mechanisms. I must rely on the expertise and recommendations of other panel members in that regard.]*

Lawrence H. Lash

This is probably the major area of the current document for which differences of opinion will likely exist. The document states that although there are no cancer studies of HCE in humans, the existence of renal adenomas and carcinomas and pheochromocytomas and malignant pheochromocytomas in male F344/N rats, hepatocellular carcinomas in male and female B6C3F1 mice, and the indications from an Osborne-Mendel rat liver foci assay that HCE can act as a promoter, support a conclusion that HCE is "likely to be carcinogenic in humans." As will be further discussed below, the potential role of α2u-accumulation as the underlying mechanism for renal tumors in male rats is unclear. Because of this, the potential relevance of male rat kidney tumor data for human health risk assessment comes into question. The document takes the default position that because renal tumors also occur in female rats and because they cannot unequivocally conclude that α2u-accumulation plays a role, the renal tumors are relevant to humans. While I do not completely disagree with this, I believe that the conclusion is overstated. A problem lies in the descriptors used by the EPA. While there are two potential descriptors, "likely" and "suggestive," I do not believe that either are completely appropriate. According to the U.S. EPA cancer guidelines, the evidence for HCE being carcinogenic in humans is clearly more than "suggestive." Concerns about the relevance of rat kidney tumors and mouse liver tumors, however, makes a descriptor of "likely" seem overstated. Additionally, the relevance of pheochromocytomas is subject to considerable uncertainty as well, and this seems to be minimized by the current document. Finally, although the rat liver foci assay suggests that HCE can act as a tumor promoter but not an initiator, this evidence is fairly weak. In virtually every other test of potential genotoxicity, HCE was negative. Although the document clearly states this, this fact does not seem to influence the final conclusion as strongly as it should. Hence, although I think calling HCE a "likely carcinogen in humans" would seem to be overstated, consideration of the U.S. EPA cancer guidelines makes this the only plausible choice, although this reviewer is not entirely satisfied with such a choice.

Edward A. Lock

With reference to the renal cancer seen with hexachloroethane, it is my opinion that the mechanism is probably related to chemically-induced exacerbation of CPN plus a role for α2u-globulin nephropathy to account for the increased incidence of renal tumors in male rats only. It is clear that spontaneous CPN is increased by the chemical in both male and female rat kidneys with almost all male rats, control and treated, affected with the

severity being more marked in the treated. Hard et al., in a number of cancer bioassays quoted in Lock and Hard (2010), has reported that tumors in the kidney are almost always associated with the highest grade of CPN, using his grading scale in grade 7 to 8 or in the EPA scale grade 4. I think it is important to ascertain in this study the incidence of end stage renal failure or the next highest grade (high severe) in the control versus treated rats and the presence of foci of atypical hyperplasia and if the adenomas were within areas of CPN. This would add support to the exacerbation of CPN. In female rats, exacerbation of CPN is also observed, with an incidence of 44% on the control and 80-90% in the treated, but the severity is much lower than in males. This finding is consistent with the exacerbation of CPN mode of action with no renal tumors observed in females, the severity of CPN being lower than in males despite the much higher doses given to the females. Plus the α2u-globulin nephropathy does not occur in this sex, so the insult is less. The role for α2u-globulin nephropathy is based on many of the pathological hallmarks of this nephropathy which are clearly documented in Table 4-21 of the report and hexachloroethane appears to meet all the criteria except for the identification of the protein in the droplets, which in my opinion needs to be undertaken to help with the risk assessment with regard to renal cancer. It is also worth noting that pentachloroethane causes the α2u-globulin nephropathy (Goldsworthy et al., 1988). Mineralization of the papilla following chronic exposure is one of the more important findings that showed an increased incidence in male rats. There was a high incidence in female rats exposed to the low dose of hexachloroethane, however the control and high dose hexachloroethane where the same, questioning the lack of dose response relationship.

The two processes are not mutually exclusive and, in this case, I feel that a combination of indirect cytotoxicity via a α2u-globulin mechanism and exacerbation of CPN could both contribute to the small increase in renal tumors in male rats only. These processes are intimately associated throughout the course of the α2u-globulin nephropathy disease progression. With some chemicals the α2u-globulin mechanism is the major driving force, while with others it can be important early and then it appears exacerbation of CPN takes over as the main mode of action (Lock and Hard, 2010).

With hexachloroethane it is my opinion, based on the data, that both mechanisms are involved and the CPN is the main mode of action. I accept that in three cases the adenomas had progressed to carcinoma and in one case metastasis had occurred. However, it is clear that if the mechanism is as suggested then the increase in tumor incidence has no relevance to humans (Hard et al., 2009; Lock and Hard, 2010).

With regard to the liver tumors in mice, the mechanism does appear to be unknown. It is unlikely to be due to peroxisome proliferation, as the structurally related analogue pentachloroethane, a metabolite of hexachloroethane, does not cause peroxisome proliferation in mouse liver (Goldsworthy and Popp, 1987).

I cannot comment on the pheochromocytomas other than to say the response was not dose-related and, hence, question the relevance of the increase at the low dose of hexachloroethane.

(C) Carcinogenicity of Hexachloroethane

2. A two-year oral gavage cancer bioassay in F344 rats (NTP, 1989) was selected for the derivation of an oral slope factor. Please comment on whether the selection of this study for quantitation is scientifically justified and clearly described. Please identify and provide the rationale for any other studies that should be selected.

Jack B. Bishop

Selection of the NTP 1989 data on renal tubule adenomas and carcinomas in male rats for quantification of cancer risk is clearly described and scientifically justified on the basis of this male rat data providing the larger oral slope factor, indicating male rats have greater sensitivity to hexachloroethane than male and female mice.

Lucio G. Costa

Choice of this study appears appropriate.

Lynne T. Haber

The male rat kidney tumors are a reasonable basis for the quantitation, recognizing the uncertainties regarding human relevance. The text on p. 106 under the table regarding the impact of α2u-globulin would more accurately be characterized as the data are "*insufficient* to support the conclusion that…" rather than they do not support the conclusion. EPA correctly concluded that the data are insufficient to meet the criteria in the 1991 guidance. The kidney effects seen in female rats and in mice do indicate that an additional MOA may be operating, but this difference in MOA could explain the difference in potency between the male rats and other strains/species.

Table 5-6 presents an oral slope factor for hepatocellular carcinomas in female mice, but I did not see the supporting output in the appendix. I was particularly interested in how the modeling was done, in light of the highly non-monotonic nature of the data. In light of those uncertainties and the very low calculated slope factor for this endpoint, I would recommend not including that endpoint in Table 5-6.

Ralph L. Kodell

No human studies of the possible carcinogenicity of HCE are available. Two studies were considered by EPA for derivation of an oral slope factor, NCI (1978) and NTP (1989). BMD modeling was done for combined tubule adenomas and carcinomas and for combined pheochromocytomas and malignant pheochromocytomas in rats (NTP, 1989). However, the latter endpoint could not be modeled successfully to achieve a good model fit. BMD modeling was also done for hepatocellular carcinomas in male and female mice (NCI, 1978). Because the oral slope factor was largest for combined renal tubule adenomas and carcinomas in male rats, i.e., the rats were more sensitive than the mice, EPA chose the NTP (1989) bioassay for deriving an oral slope factor to quantify cancer

risk. This is scientifically justified and has been clearly described. *[At the panel meeting, a panel member argued convincingly that the renal tubule adenomas and carcinomas in male rats were not relevant to humans, because neither of two possible mechanisms that have been established in rats is operative in humans. I was persuaded that the renal tumors should not be used to derive an oral slope factor. There was also limited discussion by panel members of the relevance to humans of mouse hepatocellular carcinomas in mice. I believe the somewhat low incidence observed in control mice and the strong observed dose-response relationship in males supports the use of these tumor data. Having heard no objections by panel members to the use of these data, I believe that EPA should base the oral slope factor on hepatocellular carcinomas in male mice from the NCI study (NCI, 1978).]*

Lawrence H. Lash

The document presents a thoughtful analysis and comparison of the NTP (1989) chronic rat cancer study and the NCI (1978) chronic mouse cancer study. Limitations were noted in the NCI mouse study in terms of the dosing schedule. BMD modeling was performed on the aggregate data for the renal adenomas and carcinomas in male rats and for the hepatocellular carcinomas in both male and female mice. A linear dose extrapolation method was used to calculate the oral cancer slope factor. Table 5-7 concisely presents a summary of the various uncertainties in the cancer risk assessment; this is an excellent table. Part of the basis for choosing the NTP (1989) study was that male rats were found to be more sensitive to HCE-induced carcinogenesis than either male or female mice. Another rationale for choosing this study was that because there is no information on which species is most applicable to humans, a default position of using the most sensitive species was taken. This conclusion is logical, appropriate, and scientifically justified. Although the relevance of rat kidney tumors for humans is subject to some question because of the α2u response that is most likely occurring, and although no tumors were observed in female rats, nephrotoxicity has been observed in female rats. The adverse renal response in female rats indicates that an additional mechanism that is independent of α2u occurs, likely in both males and females. Such a mechanism, under appropriate conditions, could lead to tumors in females. For example, some studies on trichloroethylene-induced renal cancer have suggested that one potential mode of action involves repeated cycles of cell injury and repair, which could ultimately lead to dysregulation of cell proliferation and neoplasias. An analogous process could be occurring with HCE, although additional study is clearly needed to support this as a potential mode of action.

Edward A. Lock

The reason for selecting the NTP (1989) for derivation of the oral slope factor is clear and I support the use of this study for that purpose. I do have concerns, however, with regard to the shape of the dose response curve being linear low-dose extrapolation, as the mode of action is not known. I accept that the basis for the mouse liver tumors is unknown. The reason for not accepting the mode of action in the kidney is clearly stated, but could change if α2u-globulin measurements are made and the concept of CPN potentiating

renal tumors is accepted, as discussed above. If, for example, the renal tumors were considered not relevant to humans, I guess the analysis would shift to mouse liver tumors giving an answer similar to that stated at present for the kidney?

(C) Carcinogenicity of Hexachloroethane

3. The renal tubule tumor data in male rats from the NTP (1989) two-year oral gavage cancer bioassay were selected to serve as the basis for the quantitative cancer assessment. Please comment on whether this selection is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected to serve as the basis for the quantitative cancer assessment.

Jack B. Bishop

The selection of the renal tubule tumor data in male rats from the NTP two-year cancer bioassay study (NTP 1989) as the basis for the quantitative cancer assessment is scientifically justified and clearly described.

Lucio G. Costa

The choice of renal tubule data in male rats from this study appears appropriate as the basis for the quantitative risk assessment.

Lynne T. Haber

The selection is scientifically justified based on the available data.

Ralph L. Kodell

EPA considered several cancer endpoints for quantitative cancer assessment, two in male rats (NTP, 1989) and one in male and female mice (NCI, 1978), as indicated under question 2 above. The renal tubule tumor data in male rats from the NTP (1989) bioassay yielded the largest oral slope factor via BMD modeling, indicating greater sensitivity of the rats compared to the mice. Thus, the renal tubule tumor data in male rats were selected for quantitative cancer assessment. This is scientifically justified and has been clearly described. *[As noted above in my response to question 2, I no longer consider the renal tubule tumor data in male rats to be relevant to humans, and I favor instead the use of the hepatocellular carcinoma data in male mice for the quantitative risk assessment.]*

Lawrence H. Lash

Rationale for choosing the renal tubule tumor data from male rats as the basis for the quantitative cancer assessment is indeed the most logical, based on the quality of the study, concerns with the dosing regimen used in the mouse study, and the sensitivity of male rats to the HCE-induced tumors. Some of the concerns with the mouse study, including the absence of any evidence of hepatocellular carcinomas in humans, essentially eliminates those tumor data from consideration as the basis for a quantitative cancer assessment. Hence, the choice of the renal tubule tumor data in male rats from the NTP (1989) study is the only possible one from the limited database.

Edward A. Lock

I wonder if this study is the correct one to use for quantitative cancer assessment, mainly, as discussed above, I am not convinced that the renal tumors are the best endpoint as it is possible to make a case that the findings may have little or no relevance to humans. If that position is accepted then the NCI study showing liver tumors in mice would be the best study.

(C) Carcinogenicity of Hexachloroethane

4. EPA concluded that the mode of action for renal tubule tumors observed following oral exposure to hexachloroethane is unknown. An analysis of the mode of action data for renal tumors is presented in the Toxicological Review. Based on this analysis, EPA determined that hexachloroethane-induced renal tumors could not be attributed to the accumulation of α2u-globulin. Please comment on the scientific support for these conclusions. Please comment on whether the analysis is scientifically justified and clearly described.

Jack B. Bishop

EPA has clearly described why they conclude there is insufficient evidence to attribute the renal adenomas and carcinomas observed in male rats administered hexachloroethane to an *α2u-globulin* MOA. While the supplemental reference material of Lock and Hard (2010) makes a compelling case for this MOA, the paper by Doi et al. (2007) makes an equally compelling case that, while this MOA may contribute to the renal tumor response, the critical components of nephropathy associated with the development of tumors remains unknown. The EPA's analysis and conclusion is scientifically justified.

Lucio G. Costa

The document discusses evidence of α2u-globulin formation and of chronic progressive nephropathy (CPN) as possible modes of action for the observed renal tubular tumors, and concludes that neither can provide support for a rat-specific effect. This reviewer points the attention to the case of another chemical, tetrahydrofuran, which appears to cause similar tumors. In this other case, the issue has been raised that advanced CPN and low-grade α2u-globulin nephropathy may contribute to renal proliferative lesions (see Chhabra et al. Toxicol. Sci. 41: 183-188, 1998, and Bruner et al. Regul. Pharmacol. Toxicol. 2010, in press). As neither condition has a pathologic counterpart in humans, the conclusion that hexachloroethane may be a likely human carcinogen may want to be further discussed, eventually distinguishing similarities and differences between these two chemicals. The contribution of the mice hepatic tumors (NCI, 1978) and the pheochrocytomas (NTP, 1989) to the overall cancer risk assessment of HCE needs to be better and clearly explained, in the context of the descriptors in the EPA's Carcinogenesis guidelines.

Lynne T. Haber

First, I'd like to commend the author of Section 4.7.3.1. This is the best section of the Toxicological Review, and clearly and concisely provides information on the underlying biology for the α2u-globulin MOA, as well as succinctly presenting the guidance on evaluating the MOA and addressing how the available data on HCE relate to the criteria in the guidance. However, even in this section, once the author starts to address the modified Hill criteria, the focus is too much on a study by study evaluation and summary,

rather than focusing on the bottom lines across studies and any limitations to those bottom lines.

According to the 1991 guidance on α2u-globulin, it is correct to conclude that the renal tumors cannot be attributed to this mechanism. According to that guidance, affirmative responses in each of 3 categories are required to show that α2u-globulin could be a factor: (1) increased number and size of hyaline droplets; (2) the accumulating protein in the hyaline droplets is α2u-globulin, and (3) additional aspects of the pathological sequence are present. EPA does a nice job of walking through the guidance with regard to α2u-globulin, and correctly concludes that attribution of the tumors to α2u-globulin is not possible in the absence of identification of the protein in the droplets as α2u-globulin. The evaluation according to the modified Hill criteria is generally well-done. However, the dose-response section begins by addressing the correct issue ("accumulation of α2u-globulin… must occur at lower doses than subsequent α2u-globulin-related effect"), but then loses that focus, and becomes simply an evaluation of the dose response for each endpoint. A table showing the doses at which each of the key events in the progression occurs will help in evaluation of the concordance. Based on quick review of the tables, it appears that the data on the key events (to the extent that data are available) are concordant, showing key events occurring at the same or lower doses as tumors. Similarly for temporality, the data show that hyaline droplets and other key events occur at time points before tumors are observed. Thus, it is not correct to say that a temporal relationship cannot be established from the reported data.

Finally, on p. 79, the text notes that several recent reviews reviewed the hypothesized MOA of α2u-globulin accumulation as a key event for renal carcinogenicity of HCE, but it's not clear if the conclusions of those reviews are provided. Please clearly state what each of those reviews concluded. (A quick review of the Doi study suggests that the paper excluded HCE from its analysis, but the paper is relevant to understanding the general MOA.)

Ralph L. Kodell

The conclusion stated on page 80 is that there is insufficient evidence to conclude that the renal adenomas and carcinomas observed in male rats administered HCE (NTP, 1989) are related to an α_{2u}-globulin mode of action. EPA presented a clear argument to justify this conclusion. *[A the panel meeting, it was argued persuasively by a panel member that even if an α_{2u}-globulin mode of action could be ruled out, the only other established mode of action for these tumors in rats would also not be relevant to humans. So, the argument and conclusion by EPA regarding α_{2u}-globulin may be moot.]*

Lawrence H. Lash

As briefly noted above, the discussion of the potential mechanistic role of α2u-accumulation in the renal toxicity and renal tumors induced by HCE raises a possible point of contention. The central problem with the HCE renal toxicity and renal cancer database is its lack of completeness with regard to key measurements that should have

been made to more fully assess and validate the mechanistic importance of α2u-accumulation. Because the hyaline droplets were not analyzed by immunohistochemistry to confirm the presence of α2u, a key criterion in assessing the importance of α2u in the underlying mechanism was not and cannot be established. Based on this database deficiency, the document concluded that α2u-accumulation could not be the underlying mechanism. The important implication of this conclusion is that the renal toxicity and cancer data cannot be considered as male rat-specific processes. These data were, therefore, considered to be relevant to humans. In support of this conclusion, the document noted the occurrence of nephrotoxicity in female rats, who do not exhibit the α2u-response. With regard to renal tumors, however, these were only observed in male rats. Thus, while I appreciate the rationale for taking a default approach that non-cancer renal effects cannot be fully ascribed to the α2u-process (which would make them irrelevant for human health risk assessment), the same cannot necessarily be said for the cancer endpoint. Whereas I do not ultimately disagree with the conclusion to use the renal data for human risk assessment, my concerns lie with the rationale and explanation for the conclusion. Rather than state that they conclude that α2u is not involved, the document should be clearer about concluding that while α2u may explain ***in part*** the renal effects, other modes of action also exist that would not necessarily exclude the database from risk assessment as being male rat-specific responses.

Edward A. Lock

The basis for not selecting the α2u-globulin nephropathy is, in my scientific judgment, not strong as 6 of the 7 criteria laid down in the EPA document on this topic have been met with the only outstanding one, actual demonstration of the protein in the hyaline droplets, which is easy to do and has not been conducted. I accept that there can be other causes which can lead to hyaline droplet formation in the proximal renal tubules; however, the weight of evidence from structurally related analogues such as pentachloroethane and metabolites such as tetrachloroethylene (Goldsworthy et al., 1988) would suggest to me that it is likely to be α2u-globulin. If this is correct then this hypothesis can be invoked. The other concern is related to the fact that hexachloroethane exacerbates CPN (NTP, 1989; Hard et al., 1993) and, as discussed above and in Lock and Hard, (2010), this can lead to an increased incidence of renal cancer if the extent of the CPN is severe. This also needs to be resolved by determining if all the rats with renal tumors had severe or high severe end stage renal failure, which supports the exacerbation of CPN mode of action.

(C) Carcinogenicity of Hexachloroethane

5. The oral cancer slope factor was calculated by linear extrapolation from the POD (i.e., the lower 95% confidence limit on the dose associated with 10% extra risk for renal tumors in male rats). Has the modeling approach been appropriately conducted and clearly described?

Jack B. Bishop

EPA's modeling approach has been appropriately conducted and clearly described.

Lucio G. Costa

The approach used is standard and appears appropriate.

Lynne T. Haber

Yes.

Ralph L. Kodell

EPA used linear extrapolation from the $BMDL_{10}$ in the absence of information on mode of action to suggest otherwise. The modeling appears to have been appropriately conducted. However, there are slight discrepancies between the BMD_{10} and $BMDL_{10}$ values reported in Table 5-6, page 106, and those that resulted from the BMD modeling reported in Appendix B. For renal tubule tumors in male rats, the BMD and BMDL values in Table 5-6 are 3.73 and 2.44, respectively, while the corresponding values on page B-54 are 3.74496 and 2.45283. Similarly, for hepatocellular carcinomas in male mice, the respective values in Table 5-6 are 37.03 and 14.44, compared to 38.0933 and 13.8018 on page B-57. These discrepancies need to be resolved, as they have a slight effect on the candidate oral slope factors. The BMD modeling results for hepatocellular carcinomas in female mice are reported in Table 5-6, but not in Appendix B. Results of the attempted BMD modeling for pheochromocytoma/malignant pheochromocytomas in male rats are shown in Appendix B, but because a good fit could not be achieved the results were not used further and thus are not reported in Table 5-6. For the modeling of hepatocellular carcinomas in male and female mice, EPA used the matched vehicle control data (Table 5-5), which I believe is the most appropriate control data to use. However, because this is different from NCI's use of pooled vehicle control data in its analysis (page 30 and footnote to Table 4-10), I believe this difference ought to be stated. Some clarifications are needed. *[At the panel meeting, EPA indicated that the BMD modeling of hepatocellular carcinomas in female mice was unsuccessful. Thus, the results presented in Table 5-6 should be removed. If EPA agrees not to use renal tubule adenomas and carcinomas in male rats for deriving an oral slope factor, then those BMD modeling results should also be removed.]*

Lawrence H. Lash

The modeling approach used, involving linear extrapolation from the POD, is a standard or default approach that is taken in the absence of a more complete understanding of the mechanism of action. The document explains how this approach is taken because of the absence of dose-response data at low doses. Although I have some concern about the specific wording of some of the justification statements (see specific comments below), the overall conclusion reached to use the linear extrapolation method is appropriate.

Edward A. Lock

The modeling approach has been clearly defined. The question for debate is around the relevance of the renal tumors, as discussed above, which will impact the POD, linear cancer slope etc.

V. SPECIFIC OBSERVATIONS

Jack B. Bishop

General: There are numerous "inconsistencies" throughout the review document. One example is the use of "corn oil gavage" when describing some studies and just "gavage" is used on others, when corn oil is obviously the vehicle in both cases.

p. x: PBPK is duplicated.

p. 12, second paragraph, line 4: Could the 3 possible stabilization reactions be illustrated in Fig. 3-1?

p. 16, second paragraph, line 5: Suggest replacing "fur deposition" with "deposition of HCE on the fur of the rats."

p. 29, last paragraph, lines 3 and 9: Replace "granulose" with "granulosa."

p. 84, last sentence: I have a hard time understanding the emphasis of this sentence. Are you saying that you believe the HCE exposure exacerbates normal CPN because of the increased severity of the observed nephropathy and the dose-dependent increases in the incidence of mineralization? If so, please restructure the sentence accordingly.

Lucio G. Costa

p. 23: Data discussed on top of this page may also be presented in a table.

p. 29-30: Tables 4-8 and 4-9 - Specify rat strain.

p. 31: Top section - It should be clearly stated that no renal tumors were observed in mice.

p.37: Table 4-13 - Vehicle instead of Diluent?

p. 44: First few sentences - Are these relevant?

p. 45: Title (4.5) - Specify that mode of action is referred to as carcinogenesis?

p. 52: Data by Lattanzi et al. (1998) may be moved back to Section 4.5.1.

p. 65: Section 4.7.2 - Repetitive. See 4.2.1.2.

p. 84: Section 5.1.1- Repetitive. Should focus on why Gorzinski et al. (1985) was chosen over other studies.

p. 96: Second paragraph - Not clear how results of the study by Weeks and Thomasino (1978) may support notion of neurotoxicity. Consider, however, hypoxia.

p. 108: Table 5-7, Section four - Seems to contradict p. 110 (dose metric).

Lynne T. Haber

P. 5: While the format of the Toxicological Review mandates separating the absorption, distribution, metabolism, and excretion text, it helps the readability of the text and the reader's understanding to allow some overlap between the sections. For example, the second paragraph of 3.1 and several other places throughout Chapter 3 address the Mitoma study and the measurement of "excreta," but the text never addresses (or it is well buried) whether Mitoma reported urinary and fecal radioactivity separately, or only as total excreta. If only total excreta are reported, this should be noted explicitly, so that it is clear that the authors, not just EPA, combined the two. In Section 3.1, this is relevant because fecal excretion could include biliary excretion, which would mean a higher percent absorption, but this is not addressed.

P. 7: The discussion of the differences in kidney concentrations in male and female rats was well-done.

P. 17, third line: The conclusion that the effects from smoke bomb exposure are "not likely" a result of HCE seems overly strong. In a brief literature search, I could not find any evidence of liver effects from zinc chloride or zinc oxychloride. It seems to me more likely that the respiratory effects from the smoke bombs are due to the zinc compounds, while the liver effects are attributable to hexachloroethane. These data provide qualitative support that HCE can affect the liver, and are a useful part of the hazard characterization, though dose-response information are clearly not available. In light of this utility, a bit more information on the liver effects observed would be useful.

P. 31, second paragraph: The authors are correct in noting that the increases in hepatocellular carcinoma in females were not dose-dependent. The second clause of the second to last sentence in the paragraph supports the first clause, and so the sentence should not begin with "although."

P. 31, footnote a: The discussion of the pooled controls is puzzling. If the groups were pooled, why is the total only 60? Did the control groups for the individual studies only contain 20/group? Was this due to initially small groups, or low survival? It would be useful to add some additional information, and, if the control groups were very small, to note something about sample size for the individual control groups, to explain why the pooled group is so small.

P. 31-32: The Weeks study is complex and challenging to present clearly, but additional information will help the reader, and help support the final conclusions. For example, the third line mentions nonpregnant females, but there has not yet been any mention in the write-up of a developmental toxicity component. Deaths in the 4th week are mentioned, but there is no identification of the affected exposure levels or whether a cause of death was identified. More importantly, the description of the histopathology evaluation is very cursory, as noted above in the specific charge questions. Finally, it is important to

emphasize additionally that no sacrifice and histopathology were conducted until 12 weeks post-exposure, allowing for a substantial post-exposure recovery time, and meaning that additional effects may have been missed.

Section 4.3: The appropriate unit for statistical analyses of developmental toxicity studies is the litter, not the fetus (since the mother is the exposed unit; see EPA's developmental toxicity risk assessment guidelines). Sometimes authors inappropriately conducted statistical analyses by fetus, rather than by litter, and it is not possible to report litter-based statistics. However, in such cases, the study summary should note this as a study limitation.

P. 38 and P. 41: The Forward states that the Toxicological Review provides support for the hazard and dose-response assessment for the chemical, and is not intended as a comprehensive toxicological treatise. In light of that mandate, what is the purpose of including the sheep (and quail) studies? If the authors judge them truly relevant to the conclusions of the document, some context is needed. Similarly (p. 41), if the authors judge the sheep studies as a useful addition to the hazard characterization, they should be included as supporting studies, rather than the primary information listed first, as in Section 4.4.3. Dog studies are generally considered more relevant than sheep studies, and the rat studies clearly show neurological effects.

P. 41, last paragraph: While it is correctly stated here and elsewhere in the document that it is not known whether certain effects are due to the parent or metabolites, more context is possible. Many of the effects reported are known effects of many of the metabolites. Thus, while it cannot be ruled out that the parent also causes these effects, and it may not be possible to determine how much of the effect is due to the metabolite, it is useful to note that the metabolites may be contributing at least some of the toxic endpoints. This sort of integration would be useful to address in the synthesis sections, and may shed some light on the potential impact of differences in metabolism due to polymorphisms or other differences.

P. 42, first paragraph of 4.4.3.1: Please use consistent units to aid in comparison across chemicals.

P. 43: Tremors clearly are a neurotoxic endpoint. However, based on my understanding of the Kulig paper (cited in the initial description of the Weeks study), lack of grooming *can be* an indicator of neurotoxicity; it is not *necessarily* an indicator of neurotoxicity. Ruffled pelt can also be a nonspecific indictor of general malaise. It is important not to over-interpret the data, especially since this is a co-critical effect.

P. 45 ff.: Almost all of the key data for the genotoxicity studies are presented in the nice summary in Table 4-15. If the comments were expanded to also note study limitations (and doses – this could be done by changing test system to an intermediate row header, rather than a full column), this would eliminate the need for most of the individual study write-ups, which just repeat the information in the table. These changes would allow the author to focus on the synthesis, consistencies and inconsistencies in the data. For

example, the nice summary on p. 45 provides most of the information desired in a synthesis of the data, but a key question is raised by the write-up on p. 45 and not answered there or in the more detailed discussion. According to the write-up, Jackson et al. considered the data to be "insufficient for evaluation." Why? Based on the write-ups, it appears that there is a full spectrum of genotoxicity tests as well as supplemental tests (e.g., adduct formation). Did Jackson et al. judge some of the studies inadequately conducted? What studies did they consider to be missing or inadequate? This is important information for the overall conclusion that I did not see addressed.

P. 53: The statement that the data support the conclusion of HCE binding to DNA is enzymatically catalyzed is over-simplified. The data support the conclusion that CYP450 catalyzes the metabolism of HCE to forms that bind DNA. In addition, the information on DNA, RNA, and protein binding is reported without context here and in several other places in the document. These types of binding can have very different implications, which should be noted. DNA binding can lead to mutations (and ultimately to cancer), according to a process hypothesized to follow one-hit kinetics. Protein binding can lead to cytotoxicity, other cell changes, or to cancer via nonmutagenic MOAs. RNA binding can interfere with transcriptional regulation, which ultimately lead to cancer, but not with one-hit kinetics. The Toxicological Review needs to make those distinctions.

Table 4-19: The doses for the Gorzinski et al. 1985 study in the dose column do not match the doses under NOAEL/LOAEL.

P. 63, last lines: The biochemistry endpoints measured by Weeks are evaluations of hepatic structural damage (e.g., AST and ALT reflect cell leakage into the serum), not measurements of liver function. I do not recall seeing any evaluation of liver function (e.g., serum bilirubin).

Section 4.8.1: If metabolism by the CYP450 is flow-limited, changes in enzyme amounts tend to have minimal impact on the tissue dose of active chemical. (See work by Lipscomb and colleagues on TCE related to this issue.) This point also applies to the discussion on p. 112.

Section 5.1.5: It would be useful to note that the database uncertainty factor was adopted after the previous HCE RfD was developed, so the addition of the factor is a change in method, not a change in understanding of the data.

P. 98: The discussion of gas categories is generally good, but it would be useful to additionally recognize that the consideration of water solubility relates to the question of whether we would expect interaction with respiratory tissue. I agree with the conclusion that the HEC is calculated using the methods for category 3 gases, using the human and animal blood:air partition coefficient (not the blood partition coefficient, as is stated in the last paragraph before 5.2.3.)

P. 101, first paragraph: Note also that other potential PODs had higher BMDLs – not just higher NOAELs/LOAELs.

P. 102, end of first full paragraph: The statement that "human variation may be larger or smaller" is correct, but misleading to those unfamiliar with the methods. As documented in various EPA publications (e.g., the 2002 staff paper), a number of studies have been conducted on human variability, and the default factor of 10 covers a high percentile of chemicals. Furthermore, the factor of 10 is not intended to cover the entire range of human variability from the most sensitive to the least sensitive. Instead, it extrapolates from a human NOAEL equivalent (using classical rough equivalents) or from a human median parameter (using the CSAF/DDEF approach) to the sensitive individuals.

Table 5-7: The table correctly states that the potential impact of the specified approaches could increase or decrease the oral slope factor. However, as shown, the presentation misses that, for many of the approaches, the plurality of the evidence is that the effect is in one general direction (generally the conservative approach is taken). This is also missed in the justification – that the choices made are typically science-based health-protective approaches. Furthermore, the basis and support for the BW3/4 scaling (i.e., much of basal metabolism scales with BW3/4) is not noted under the justification for that approach.

P. 112: It would be useful to note that the previous cancer assessment was developed prior to the publication of the NTP study, and so the change is due to the availability of new data, not different evaluation of the same data.

Ralph L. Kodell

Page 57, Table 4-19: There are a number of errors in this table.

- Row 3: The high dose is 293 – not 62.
- Row 4: The doses are 0, 34, 67, 134, 268, 536 – not 0, 113, 536.
- Row 4: The male LOAEL is 34 – not 113.
- Row 4: The female NOAEL and LOAEL are not shown; they are 67 and 134.
- Row 5: The dose are 0, 1, 15, 62 – not 0, 360, 62.
- Row 5: The male LOAEL is 15 – not 360.
- Row 5: The female LOAEL is not shown; it is 62.
- Row 6: The low dose is 113 – not 7.
- Row 6: The study is NCI (1978) – not NTP (1989).
- Row 8: The doses are 0, 7, 14 for males and 0, 57, 114 for females – not 0, 7, 500.

Page 58, line 25: The NOAEL is reported to be 7, which is incorrect. The NOAEL is 1 (Table 4-2, page 21).

Page 64, line 4 from bottom: The tumors were renal, not hepatocellular.

Page 65, line 1: It was male mice, not male rats, that demonstrated a statistically…

Page 89, Table 5-2, row 4: The endpoint is *incidence* of moderate to marked tubular nephropathy – not *increased severity* of tubular nephropathy.

Page 89, line 5 from bottom: The given range of PODs, approximately 60 – 0.6 mg/kg-day is incorrect based on Table 5-2. It should be 41.89 to 0.71. Appendix Table B-1 shows an additional effect not included in Table 5-2 (hepatocellular necrosis in female rats), which had a $BLDL_{10}$ (POD) of 60.18 and would give the reported upper limit of 60. There is no tabled $BMDL_{10}$ equal to 0.6.

Page B-53: The sub-heading should include the word renal to describe the tumors.

Lawrence H. Lash

1. The document uses "CYP450" as an abbreviation for "cytochrome P450." This is incorrect. Typically, one sees "CYP" used in the primary literature as an abbreviation for cytochrome P450 in general. When discussing a specific cytochrome P450 enzyme, one would use an abbreviation such as CYP2E1.

2. Page 4, lines 2-3: The document states that HCE production was "between 2 and 20 million pounds." This is quite a wide range. Why is the document not more precise? If the production data are uncertain, unclear, or incomplete, some statement to this effect should be made.

3. Page 4: Most of the uses for HCE that are described are in the past. The document should be clearer about current uses and current potentials for human exposure.

4. Page 8, section 3.3: Individual CYPs should be referred to as "enzymes" rather than "isoforms."

5. Page 8 and elsewhere: In discussing the metabolism of HCE, the document makes it fairly clear that the database is quite incomplete, particularly in humans. However, there is a fair amount of discussion about trichloroethylene (TCE) and perchloroethylene (Perc). Inasmuch as the toxicology databases for these two chemicals are far more extensive than that for HCE, the document should be clearer about their relevance to HCE. Although one can readily appreciate that that the database deficiencies may make it impossible to provide a reasonably accurate assessment of this, the document needs to clearly state this. The current manner of presentation leaves the reader wondering how important effects that have been ascribed to TCE and Perc may be for understanding those of HCE. An important consideration is that the rates at which TCE, Perc, and other common metabolites may be generated from HCE may be rather low, thereby diminishing the importance of these HCE metabolites in the mode of action.

6. Page 11, bottom paragraph: Some of the rationale behind Figure 3-1 on page 9 is presented here. This is a bit confusing and should probably be presented along with the figure. This would make it clearer to the reader which aspects of the metabolic pathway are well established and which are presumed based on limited data.

7. Page 13, para. 1: More thorough evaluation of the CYP inhibition data is needed. Many of the studies that are summarized here and elsewhere in this subsection used non-selective or only broadly selective CYP inhibitors. Accordingly, conclusions about the involvement of specific CYP enzymes in HCE metabolism need to be made more carefully as the data from such inhibitor studies include some degree of ambiguity.

8. Page 16, section 3.5: The rationale for the use of styrene PBPK modeling to obtain metabolic estimates and kinetic parameters for HCE is unclear. Some evaluation in the document of the appropriateness or strength of these data would be helpful and would seem to be needed.

9. Page 17, section 4.1: Although it is made clear that the HCE database for humans is very limited in scope, the document cites some studies that clearly involved mixed exposures, particularly those in which the specific amounts of HCE were unknown or at best poorly characterized. This needs to be made clearer when such data are presented. Otherwise, such data from mixed exposures may be improperly interpreted.

10. Page 18, last sentence of 2nd full paragraph: After describing some human exposure data, the document states, "These results demonstrate that a considerable increase in plasma HCE can occur after a relatively brief occupational exposure, even though workers used fairly sophisticated personal protective equipment." I think that this conclusion is too strong, considering the high degree of variability and very limited number of samples. Interpretations of such data should be made more carefully.

11. Page 19, para. 2, next-to-last sentence: In summarizing some of the clinical data, the document states, "The interpretation of small differences in clinical parameters, within the normal range, is uncertain." In this case, I believe that the statement is too weak. It seems to this reviewer that too often when reviewing clinical or epidemiological literature, too much latitude is given to the data and principles of statistical significance are ignored or are at best only loosely applied. In this case, my interpretation would be that if there are only small differences (Were these statistically significant?) but the values are all considered to be within the "normal range," then the only possible conclusion is that no effect was observed.

12. Page 41, section 4.4.3, para. 1: The document states that the rats in the particular study "exhibited slight, but not statistically significant, behavioral effects." Such a statement is inappropriate; if there was no statistical significance, then no effect (slight or otherwise) can be concluded to have occurred. A similar statement is made on page 43, para. 2, line 1.

13. Page 53, para. 1 of text: The nature of the controls needs to be clarified.

14. Page 68, para. 1: As noted above, I do not agree with some of the wording of the interpretation of the role of α2u. In this case, the document states, "In the absence of information demonstrating the involvement of α2u-globulin processes, male rat renal toxicity/tumors are considered relevant for risk assessment purposes." Although I agree

with the risk assessment taking the default position that the renal toxicity and renal tumors should be considered at this stage as relevant to humans, it is inaccurate to imply that there is no evidence linking α2u-accumulation to the responses. It would be more appropriate to state: "…in the absence of definitive evidence…" The facts are that there is evidence, but it is not complete, and there is evidence suggesting that an additional mode of action is involved as well. The statement on page 70, para. 2, which uses the phrase, "in the absence of sufficient evidence…" is certainly more appropriate.

15. Page 76, last sentence of top para.: Again, rather than state that effects may not be due to α2u, it would seem more accurate to state that either some effects are not due to α2u or that an additional mode of action is also involved. Similarly, the last sentence of the top paragraph on page 79 should also be modified. The last sentence of the top paragraph on page 80 does properly express this concept. Thus, there seems to be some inconsistency in interpretations throughout the document. Also, the statements about α2u in the first full paragraph of text on page 106 need similar revisions. Statements on page 110, para. 4 about α2u are also misleading. By stating "if α2u were involved, the renal tumor data would not have been used," indirectly makes the conclusion that there is no involvement of α2u. To summarize this reviewer's opinion about how the α2u data should be interpreted, it would seem most appropriate to state: (1) there is evidence of α2u-involvement, although it has not been conclusively demonstrated (i.e., data gap); (2) there is evidence that other modes of action are involved in producing renal toxicity in male and female rats; such modes of action may be involved in addition to or instead of an α2u-dependent mechanism; and (3) that the risk assessment will take the conservative or default approach of considering renal toxicity and renal tumors as relevant to humans in the absence of sufficient or definitive evidence of α2u-involvement.

16. Page 98, para. 1: As noted above, the rationale presented for how HCE gas is categorized does not make complete sense and seems contrived.

Edward A. Lock

Overall, the report is put together well and I did not spot any spelling mistakes or typing errors. The findings are reported factually, as they are known, and although I have taken issue with the position adopted in the report, it is clearly justified in the text, so congratulations to the compilers. The report does, however, need shortening and focusing.

P. 77, 1st sentence in 3rd para: "In addition, the data in female rats and mice of both sexes…….. do not support an α_2u-globulin-associated mode of action." Delete as does not make sense! You cannot expect female rats or mice of either sex to support the α_2u-globulin mechanism as they do not have the protein! I would say they are consistent with the α_2u-globulin-mechanism, as there were no renal tumors in these animals.

Attached are some key references for consideration for inclusion and I enclose a copy of our recent book chapter for your information and consideration. The Hard et al. (1993)

reference is in the list, I just put it here to point out that that this refers to hexachloroethane potentiating CPN.

References

Goldsworthy TL, Popp JA (1987). Chlorinated hydrocarbon-induced peroxisomal enzyme activity in relation to species and organ carcinogenicity. *Toxicol Appl Pharmacol.* **88**,225-33.

Goldsworthy TL, Lyght O, Burnett VL, Popp JA.(1988) Potential role of alpha-2 mu-globulin, protein droplet accumulation, and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene, and pentachloroethane. *Toxicol Appl Pharmacol.* **96**, 367-79.

Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE, Valcovic LR (1993). Hazard evaluation of chemicals that cause accumulation of alpha 2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. *Environ Health Perspect.* **99**, 313-49.

Hard GC, Johnson KJ, Cohen SM.(2009) A comparison of rat chronic progressive nephropathy with human renal disease-implications for human risk assessment. *Crit Rev Toxicol.* **39**, :332-46.

Lock EA and Hard GC. (2010) $Alpha_{2u}$-globulin nephropathy and chronic progressive nephropathy as modes of action for renal tubule tumor induction in rats, and their possible interaction. In Cancer Risk Assessment:chemical carcinogenesis, hazard evaluation and risk quantitation. Eds Hsu C-H and Stedeford T, John Wiley& Sons, New Jersey, pp 482-500.

Wolf DC, Mann PC (2005) Confounders in interpreting pathology for safety and risk assessment. *Toxicol Appl Pharmacol.* **202**, 302-8.