

**FINAL**  
**REVIEWER COMMENTS**

**External Peer Review Meeting on the  
*Toxicological Review of Trichloroacetic Acid*  
(CAS No. 76-03-9)**

**Prepared for:**

Diana Wong, Ph.D., DABT  
National Center for Environmental Assessment  
U.S. Environmental Protection Agency  
1200 Pennsylvania Ave, N.W. (8601P)  
Washington, DC 20460

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**Peer Reviewers:**

Penelope A. Fenner-Crisp, Ph.D., DABT  
David W. Gaylor, Ph.D.  
Ronald L. Melnick, Ph.D.  
Martha M. Moore, Ph.D.  
Michael A. Pereira, Ph.D.  
Ivan Rusyn, M.D., Ph.D.  
Andrew G. Salmon, Dr. Phil.  
Anthony R. Scialli, M.D.  
Alan H. Stern, Dr.P.H., DABT

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**TABLE OF CONTENTS**

I. INTRODUCTION..... 1

II. CHARGE TO THE REVIEWERS..... 3

III. GENERAL IMPRESSIONS ..... 5

IV. RESPONSE TO CHARGE QUESTIONS ..... 10

    General Charge Questions ..... 10

    Chemical Specific Charge Questions..... 16

        (A) Oral Reference Dose (RfD) for Trichloroacetic Acid ..... 16

        (B) Inhalation Reference Concentration (RfC) for Trichloroacetic Acid..... 31

        (C) Carcinogenicity of Trichloroacetic Acid ..... 34

V. SPECIFIC OBSERVATIONS ..... 57

## **I. INTRODUCTION**

The Integrated Risk Information System (IRIS) is an EPA database of 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 Trichloroacetic Acid. The current IRIS assessment was developed in the early 1990s, and does not have an oral RfD, inhalation RfC, and quantitative cancer assessment. Trichloroacetic acid was nominated for IRIS reassessment by the Office of Water. There was a need to evaluate trichloroacetic acid for the Stage 2 Disinfectants and Disinfection Byproducts Rule. The draft document slated for the external peer review contains a chronic reference dose (RfD) and a cancer slope factor (CSF) for trichloroacetic acid.

### **Peer Reviewers:**

#### **Penelope A. Fenner-Crisp, Ph.D., DABT**

Independent Consultant  
North Garden, VA 22969

#### **David W. Gaylor, Ph.D.**

Gaylor and Associates, LLC  
Eureka Springs, AR 72631

#### **Ronald L. Melnick, Ph.D.**

Ron Melnick Consulting, LLC  
Chapel Hill, NC 27514

#### **Martha M. Moore, Ph.D.**

National Center for Toxicological Research (NCTR)  
Food and Drug Administration  
Jefferson, AR 72079

**Michael A. Pereira, Ph.D.**

Ohio State University Comprehensive Cancer Center  
Columbus, OH 43210

**Ivan Rusyn, M.D., Ph.D.**

University of North Carolina  
Chapel Hill, NC 27599

**Andrew G. Salmon, D.Phil.**

Office of Environmental Health Hazard Assessment (OEHHA)  
California EPA  
Oakland, CA 94612

**Anthony R. Scialli, M.D.**

Tetra Tech Sciences  
Arlington, VA 22201

**Alan H. Stern, Dr.P.H., DABT (*Chair*)**

Independent Consultant  
Metuchen, NJ 08840

## **II. CHARGE TO THE REVIEWERS**

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of trichloroacetic acid that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). An existing assessment on the IRIS database for the health effects associated with trichloroacetic acid exposure does not provide an oral reference dose (RfD) or inhalation reference concentration (RfC), or quantification for carcinogenicity.

The current draft health assessment includes an (RfD) and a carcinogenicity assessment. Below is a set of charge questions that address scientific issues in the assessment of trichloroacetic acid. Please provide detailed explanations for responses to the charge questions.

### **General Charge Questions:**

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazard?
2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of trichloroacetic acid.

### **Chemical-Specific Charge Questions:**

#### **(A) Oral Reference Dose (RfD) for Trichloroacetic Acid**

1. A 60-week drinking water study in mice (DeAngelo et al., 2008) was selected as the basis for derivation of the RfD for trichloroacetic acid. Please comment on whether the selection of DeAngelo et al. (2008) as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.
2. Liver toxicity (hepatocellular necrosis) was selected as the critical effect for the determination of the point of departure (POD). Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.
3. Benchmark dose (BMD) modeling was conducted on the liver and testicular effects in male mice exposed to trichloroacetic acid in the drinking water study by DeAngelo et al. (2008) in order to determine the POD. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., 10% extra risk of hepatocellular necrosis) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the

BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

4. Please comment on the rationale for the selection of the uncertainty factors applied to the POD for the derivation of the RfD. If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s).

**(B) Inhalation Reference Concentration (RfC) for Trichloroacetic Acid**

1. An RfC was not derived for trichloroacetic acid. Has the scientific justification for not deriving an RfC been clearly described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study.

**(C) Carcinogenicity of Trichloroacetic Acid**

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), the Agency concluded that trichloroacetic acid is likely to be carcinogenic to humans by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the weight of evidence characterization scientifically justified?
2. Have the studies supporting the discussion of the mode(s) of carcinogenic action been clearly described?
3. EPA has concluded that the available data do not support any specific mode of action. In addition, EPA has determined that the data are not supportive of PPARalpha agonist-induced peroxisome proliferation as the sole mode of action leading to tumor formation. Please comment on whether these determinations are scientifically justified.
4. A 104-week drinking water study in mice (DeAngelo et al., 2008) was selected as the basis for quantification of the oral cancer slope factor. Please comment on whether the selection of this study is scientifically justified.
5. The oral cancer slope factor was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for liver tumors). Has the modeling approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the slope factor and discuss whether such approaches are preferred to EPA's approach.
6. An inhalation unit risk (IUR) for cancer was not derived for trichloroacetic acid. Is the determination that the available data for trichloroacetic acid do not support derivation of an IUR scientifically justified?

### III. GENERAL IMPRESSIONS

#### *Penelope A. Fenner-Crisp*

##### 1) Accuracy of information presented.

It is difficult to reach a firm judgment on this aspect of the document, in light of the fact that I (and, presumably, the other reviewers) did not receive hard copies/pdf files of every paper referenced in this document when provided with the draft document to review and comment upon. Therefore, I could not confirm accuracy to the 100% level by reading each paper myself, as I did not have access to every published paper cited in the references. Nonetheless, on the basis of my reading a substantial subset of the papers cited and many additional PubMed abstracts, it appears that EPA did a credible job in summarizing data/results accurately.

##### 2) Clarity of presentation

As a frequent reader of EPA documents constructed in the format employed for the TCA draft, I found most of it to be clear in presentation (except for the MOA section), with the appropriate information to be found in the expected places and accurately replicated when, by necessity, the same information required recapitulation.

##### 3) Soundness of conclusions

I agree with some and disagree with others. Specifics can be found in comments on subsequent questions.

[Note: I did not have time to systematically compare the summarization/description of all TCA-specific information included in the draft Trichloroethylene document that has recently been issued in anticipation of external peer review. It is imperative that the Agency makes sure that all relevant studies be included in both documents, and that they are presented accurately and similarly in both. ]

#### *David W. Gaylor*

The Document is comprehensive and clearly written. The Document generally meets the high standards for the IRIS. The conclusions appear to be correct for the RfD, but are not adequately supported by the analysis of the information presented (see response to charge Question II(A)2).

It appears that future studies might possibly show that the precursors for tumors exhibit non-linear dose response relationships at low doses which may suggest the use of non-linear procedures for conducting risk assessments for potential carcinogenicity of TCA at low doses.

***Ronald L. Melnick***

In general, I found this draft document to be a comprehensive review and assessment of the health effects of trichloroacetic acid. Several issues that need to be addressed in order to improve the accuracy and clarity of the presented information are addressed below under Response to Charge Questions. Some of these issues include the need for 1) a table that lays out consistencies/inconsistencies and data gaps regarding the PPAR $\alpha$  MOA for peroxisome proliferators in general and specifically for TCA; 2) the inclusion of all of the tumor data from the multiple studies by DeAngelo et al. (2008) for the estimation of the oral cancer slope factor; and 3) discussion on the impact of incomplete histopathologic evaluations from the critical studies that were selected as the basis for derivation of the RfD and the oral cancer slope factor.

***Martha M. Moore***

The authors have done a very nice job of summarizing all of the information related to TCA. There is a good degree of detail included for each of the research studies summarized. This provides the reader with an appropriate amount of information and allows the reader to understand the overall conclusions that were drawn. In particular, they have provided an integration of a number of studies related to each reviewed aspect of TCA including toxicokinetics and the available hazard identification information. When they found conflicting information from the literature reviewed, they were generally successful in providing a perspective as to why there might be reasons for the differences. That is, they provided an analysis of the individual studies. If they could not find an explanation for the differences and conflicts contained in the available literature, they did a good job of indicating this.

I think it is appropriate that they included information about DCA as well as TCA, even though they did present a compelling argument that DCA is not a significant metabolite from TCA. These two chemicals do have some differences in their toxicological properties and it is important to make a determination as to whether the overall assessment of TCA should include the toxicology for DCA.

I think that the format used for the tables provides both useful detail and a good summary of the data. It would be helpful if the results column include a summary of which doses/concentrations were positive. I think that it is important to factor the dose levels into the overall conclusions. That is, for some chemicals, positive results are seen at only irrelevantly high doses. The response and the mode of action for responses at environmentally relevant levels may be totally different than those observed at high dose levels.

***Michael A. Pereira***

The document is an excellent review of the literature pertaining to trichloroacetic acid (TCA). The clarity of the presentation is also excellent and very easy to follow. However, there is concern about the soundness of the conclusions of the document. The

major concern is the conclusion that TCA is "likely to be carcinogenic to humans" is not justified. In fact, the literature indicates the opposite, that TCA is not likely to be carcinogenic to humans. This classification is based on the evidence that the carcinogenic response to TCA in mouse liver is in a strain with a very high incidence of spontaneous liver tumors and is due to peroxisome proliferation, as well as the facts that TCA was not carcinogenic in rats, and that the exposure of humans to TCA would never be sufficient to cause cancer by this mechanism. The reason for this classification of TCA is discussed further in my review.

The use of liver necrosis in mice is not the best endpoint for calculation of the RfD, since it is related to peroxisome proliferation. Since humans are much less sensitive to peroxisome proliferators than mice, the UF for mouse-to-human extrapolation should be 0.1 and not 10. The use of testicular tubular degeneration would be a better choice and result in a higher RfD. Even though testicular tubular degeneration had a higher NOAEL of 68 mg/kg-day (LOAEL = 602 mg/kg-day). The higher UF of 10 would result in a higher RfD than the NOAEL of 8 mg/kg-day for liver necrosis with a UF of 1.

***Ivan Rusyn***

Overall, the report and its summary seem objective and the authors should be commended for their efforts. Trichloroacetic acid (TCA) is a chemical of concern due to a number of potential direct and indirect exposure scenarios. It is a major long-lived metabolite of several chlorinated solvents, such as tri- and tetra-chloroethylene which are major environmental contaminants of concern. Several key animal studies with TCA have become available only recently and the toxicological review is well timed. It is also notable that the evaluation of TCA coincides with that of tetrachloroethylene and a recent National Research Council's review of health risks from exposures to trichloroethylene. The debate over the human relevance of the peroxisome proliferator mode of action continues and the current document rightfully pays considerable attention to this issue. Most of the conclusions and recommendations seem adequately supported by evidence and argument. Nonetheless, I suggest addressing several points of concern as detailed in the "Specific Observations" section of this document.

***Andrew G. Salmon***

EPA has done an excellent job in identifying and summarizing the available literature on TCA toxicity, and provides a clear analysis of the likely modes of action. The Toxicological Review deals with a considerable range of available data relating not only to TCA itself but to other related chlorinated compounds at to widely researched mechanistic issues such as peroxisome proliferation. Study descriptions are appropriately detailed, providing key data for the reader to form an independent opinion as well as reporting the conclusions both of the study authors and of EPA's analysts. As would be expected there are limits to its coverage, particularly in regard to some earlier publications, but in general the key studies and discussions in the literature have been identified and are clearly reported.

The risk assessment and derivations of health protective values (RfD, slope factor) are logically developed and clearly laid out, and closely follow standard EPA policy. The conclusions presented are careful and defensible, and the description appropriately covers the chain of logic supporting these conclusions and the uncertainties inevitably remaining. Although there are some specific choices made with which I disagree (as explained in my responses to the detailed charge questions below), the overall impression is that this is a thoroughly and carefully prepared document and that the conclusions reached are basically sound. If anything, the EPA analysts have been overly cautious in a few cases such as the decision not to attempt route-to-route extrapolations for an RfC or inhalation slope factor. On the other hand, the cancer hazard identification and risk assessment for the oral route, which involves some highly complex analyses and difficult choices, is well handled with the appropriate balance of open-minded presentation and skeptical evaluation of competing mechanistic theories.

***Anthony R. Scialli***

The review is very well written and the presentation is clear. The conclusions with respect to cancer appear unsound, but the other conclusions are adequately supported. The reproductive and developmental studies, to which I gave most of my attention, are generally not of adequate quality for risk assessment, and I believe some of these studies are given too much credence. There is a particularly uncritical acceptance of studies using outrageous exposure levels. I recommend that EPA reviewers take a stand on these kinds of studies. In addition to these studies constituting a waste of animal resources, they also risk calling attention to “hazard” that does not represent conceivable real world exposure conditions. This concern is especially important in *in vitro* studies, which are treated far too kindly in this draft. I recommend consistent attention to exposure levels, even in *in vitro* studies. I understand that concentrations used *in vitro* are not the same as plasma concentrations achieved *in vivo*, but some context can be given. In some cases, the concentrations use *in vitro* are orders of magnitude beyond concentrations that are achievable *in vivo* under any realistic exposure scenarios.

***Alan H. Stern***

The toxicology of trichloroacetic acid (TCA) is fascinating in its complexity and the uncertainties relating to the relevance of the available animal toxicity data to human exposure. This is particularly the case for the carcinogenic potential of TCA. The document does an admirable job of synthesizing the data on the potential MOAs for TCA carcinogenicity including the possible role and relevance of PPAR $\alpha$  activation/peroxisome proliferation. The document also does an excellent job of attempting to balance the seemingly contradictory lines of evidence regarding the potential applicability of TCA-mediated mouse liver tumors to humans. However, partly because of the complexity of the possible carcinogenic mechanisms, and partly because of the structure of the document, the discussions regarding the potentially relevant carcinogenicity data and their relevance to human cancer risk are repeated with various levels of interpretation several times (sections 4.2, 4.5, 4.7, 5.4 and section 6). As the arguments are developed and then compared in these various sections, it becomes

difficult to keep all the information in view and in perspective. I found myself going back numerous times to acquaint myself with the basis for various hypotheses and explanations. While I understand that the structure used in this document is consistent with that of all other EPA Toxicological Reviews, in this case, perhaps a more concise and direct structure would be helpful. Also, while the argument for hypomethylation of DNA resulting from TCA exposure is developed at some length in the middle of the document, it disappears in section 5.4 (Cancer Assessment). Also, while the document discusses DEHP to at some length, dieldrin is not mentioned. Dieldrin has some chemical similarity to TCA, is a PPAR $\alpha$  agonist and also appears to have the potential to cause mammary gland tumors with gestational exposure. As discussed below, one major area of weakness in the document appears to be the use of the DeAngelo et al. (2008) 104 week study for derivation of the cancer potency. There does not appear to be sufficient data available from that study to justify the derivation of a cancer potency.

#### IV. RESPONSE TO CHARGE QUESTIONS

##### General Charge Questions

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

***Penelope A. Fenner-Crisp***

It's generally ok, until you get to the MOA discussion. It's hard to follow and non-compliant with the Agency's own Framework described in the 2005 cancer guidelines.

***David W. Gaylor***

The Toxicological Review is logical, clear and concise. The EPA has clearly synthesized the scientific evidence for a non-cancer hazard of TCA above 8 mg/kg-day in rodents (DeAngelo *et al.*, 2008) and a cancer hazard at human equivalent doses of TCA above 2.1 mg/kg-day (see Table 5-10).

***Ronald L. Melnick***

While the Toxicological Review is clear and comprehensive, it is not obvious why a particular dose response model was selected for the determination of the benchmark dose when multiple models provide adequate fits to the data. Also, the selection of 10% extra risk for the benchmark response for noncancer effects was not adequately justified. More discussion is needed to support the selection of a specific dose-response model and the benchmark response.

The MOA associated with PPAR $\alpha$  activation is a critical component of this review. Although the issues related to this MOA are adequately described in the document, a table on consistencies/inconsistencies and data gaps regarding the PPAR $\alpha$  activation MOA would provide greater clarity for the current review on TCA as well as for future assessments of other peroxisome proliferators (see comments below).

It is not clear why human equivalent doses were estimated for the cancer assessment but not for the derivation of the oral RfD. This issue needs to be addressed in the EPA review.

***Martha M. Moore***

Yes, I think that the authors have done a good job of providing both a summary of the available information, as well as a clearly synthesized summary for both noncancer and cancer.

***Michael A. Pereira***

The Toxicological Review is a complete and excellent review of the literature pertaining to TCA and is logical, clear and concise in its presentation of the literature review. However, the conclusions derived from the literature review are too speculative, especially with respect to modes of action other than peroxisome proliferation for which there is no evidence.

***Ivan Rusyn***

The document is a product of thorough and comprehensive review of the data available to date. It does a good job of synthesizing the information and presenting it in a rather concise fashion. There is room for improving the clarity, completeness and rationale as described in the detailed comments at the end of this document.

***Andrew G. Salmon***

The Review is logically and clearly written. To describe a document which exceeds 200 pages with its appendices as “concise” might be considered to be stretching the usual meaning of the word. However, the length and detail of the descriptive and analytical sections are appropriate and dictated by necessity rather than loquacity. The evidence for health hazards associated with exposure to trichloroacetic acid is thoroughly described and evaluated. The summary describing the major conclusions of the Review (Section 6, page 143) is clear and accurately reflects the more detailed evaluations presented earlier.

***Anthony R. Scialli***

Yes. Overall, the report is clear and well written. I have some specific requests for more explanation, which will appear below. By and large, reproducing the conclusions of individual study authors has some value but results in some highly questionable statements appearing in the document. I would favor some commentary when a study author’s conclusions are dubious. For example, “The study authors concluded XYZ, but support for this conclusion is not evident.”

***Alan H. Stern***

The document is logical and mostly clear. However, it would benefit from less use of acronyms. I found myself constantly going back to the list of abbreviations. The document is not, however, concise. As above, this is largely a function of the complexity of the subject and the standard structure of the document. Nonetheless, this makes for a overly long document where much of the length is due to repetition.

## General Charge Questions

***2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of trichloroacetic acid.***

***Penelope A. Fenner-Crisp***

See below.

***David W. Gaylor***

Not aware of any additional studies.

***Ronald L. Melnick***

Other than updating recent literature on PPAR $\alpha$  activation, no additional studies were found that would significantly impact the overall assessment.

The carcinogenicity data from Study 2 of DeAngelo et al. (2008) should be included in this review. This study is listed in Table 4-2b, but the data were not included in the cancer assessment.

***Martha M. Moore***

I have no suggestions.

***Michael A. Pereira***

A: The following additional studies of TCA in humans should be included:

1) Allen and Fisher, Risk Anal. 13: 71-86, 1993 should be discussed along with the Fisher et al., 1998 reference included in the document.

2) The following references on the elimination half-life of TCA should be included: Breimer et al., J. Chromatography 88: 55-63, 1974, Muller et al., Arch. Toxikol. 29: 335-340, 1972; Arch. Toxikol. 32: 283-295, 1972.

B: The document needs to include the literature pertaining to the histopathology and molecular biology of the tumors induced by other peroxisome proliferators and then discuss the similarity between these tumors and those found in TCA-treated mice. The comparison of the mouse liver tumors induced by TCA to those induced by other PPAR $\alpha$  agonists would demonstrate that the tumors found in TCA-treated mice are identical to those found in mice administered other peroxisome proliferators. Hence, a critical review of the similarity of these tumors would demonstrate that peroxisome proliferation could account for mode of action of the tumors induced by TCA. This

would also indicate that only an extremely small percentage, if any, of the tumors induced by TCA were the result of a different mode of action.

The following reviews of TCA need to be added to the document:

The document needs to compare the pathology, histopathology, biology and molecular biology of the mouse liver tumors found in TCA-treated mice to those tumors found in the liver of mice treated with other PPAR- $\alpha$  agonists. This is critical since the EPA is proposing that TCA has MOAs other than those of PPAR- $\alpha$  agonists. The following four articles, as well as many others in the literature, came to a conclusion different from the EPA. These references concluded that the TCA MOA is that of a PPAR- $\alpha$  agonist and that the liver tumors found in TCA-treated mice do not indicate that TCA, or for that matter, the other PPAR- $\alpha$  agonists are likely to be human carcinogens.

The difference in the conclusions of these articles is important since one is written by an EPA employee, another by an NCI employee and the third by an IARC Panel of experts that included EPA and NCI employees.

The Four References to be added and discussed:

1) J. Christopher Corton, (National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA) Evaluation of the Role of Peroxisome Proliferator-Activated Receptor  $\alpha$  (PPAR $\alpha$ ) in Mouse Liver Tumor Induction by Trichloroethylene and Metabolites. *Critical Reviews in Toxicology*, 38:857–875, 2008. Summary: "In summary, the WOE indicates that TCA-induced liver tumors arise by a PPAR $\alpha$ -dependent MOA. Although the TCE MOA is likely dominated by a PPAR $\alpha$ -dependent contribution from TCA, the contribution of a PPAR $\alpha$ -independent MOA from DCA cannot be ruled out."

2) Christoph Köhle; Michael Schwarz; Karl Walter Bock. Promotion of hepatocarcinogenesis in humans and animal models. *Arch Toxicol* (2008) 82:623-631.

3) Frank J. Gonzalez; Yatrik M. Shah. Laboratory of Metabolism, Center for Cancer Research, National Cancer Research, National Institutes of Health, Bethesda, MD 20892, United States. PPAR: Mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology* 246 (2008) 2-8.

4) International Agency for Research on Cancer (IARC). Some Drinking-water Disinfectants and Contaminants, including Arsenic. Vol 84, pp 403-440, 2004. Summary:

**5.5 Evaluation**

“There is *inadequate evidence* in humans for the carcinogenicity of trichloroacetic acid.”

“There is *limited evidence* in experimental animals for the carcinogenicity of trichloroacetic acid.”

**Overall evaluation**

“Trichloroacetic acid is *not classifiable as to its carcinogenicity to humans (Group 3)*.”

The document should state the maximum possible exposure of TCA to humans. This is of interest and important since should the maximum possible exposure to humans be below the calculated cancer risk then TCA should be classified as not being carcinogenic to humans from environmental exposure.

***Ivan Rusyn***

None are essential.

***Andrew G. Salmon***

The descriptions of studies given in the section describing toxic effects of TCA include many of the key studies, but there are a few cases where it would be helpful to give details additional studies where these are use elsewhere to support points raised in the discussion. For instance, the report by Elcombe (1985) is cited twice in the narrative in relation to species differences in peroxisome proliferation response between rats and mice: this is an important topic given that there is a crucial divergence in the carcinogenic response also between these two species. It would therefore be helpful if a brief description of this early study of TCA-induced peroxisome proliferation, and presentation of its actual results, were included along with the more recent reports which were described in some detail.

Obviously when preparing a report of this type for review there is an inevitable cut-off point in the literature surveyed which is likely to be some months at least prior to appearance of the review draft. The literature surveyed in the Toxicological Review includes citation of a number of fairly recent papers in the discussion sections. However, in a rapidly evolving field such as the analysis of peroxisome proliferation, there will inevitably be further developments occurring all the time, which may affect the interpretations presented. This is certainly true of the report which has just appeared as an electronic publication by Ren et al. (2009)<sup>1</sup>. This important study of the effects of PPAR $\alpha$  agonists in wild-type and PPAR $\alpha$ -null mice shows how much more complicated the real situation is than has been assumed in relatively simplistic shorter-term experiments with PPAR $\alpha$ -null mice purporting to show that certain responses are exclusively dependent on PPAR $\alpha$  activation. It also helps to extend and clarify the initially puzzling findings by Ito et al. (2007) which are noted in the Review. Finally, and of particular relevance in the present context, Ren et al. (2009) demonstrate that not all PPAR $\alpha$  agonists are alike in their impact on other receptors: for instance, xenobiotic peroxisome proliferators such as DEHP (and perhaps by implication TCA) may affect a different and broader spectrum of receptors than some hypolipidemic drugs or drug candidates such as clofibrate or WY14643. It would be helpful if the Review compared these different PPAR $\alpha$  agonists and evaluated evidence as to where TCA stands in this spectrum of properties. The

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<sup>1</sup> Ren H, Aleksunes LM, Wood C, Vallant B, George MH, Klaassen CD, Corton JC (2009). Characterization of Peroxisome Proliferator-Activated Receptor  $\alpha$  (PPAR $\alpha$ ) – Independent effects of PPAR $\alpha$  activators in the rodent liver: Di-(2-ethylhexyl) phthalate also activates the Constitutive Activated Receptor. *Toxicological Sciences*, advance access e-published 10/22/09.

authors of the Review should consider updating the final version to reflect these latest developments.

***Anthony R. Scialli***

I don't know of any.

***Alan H. Stern***

As above, it appears that dieldrin has much in common toxicologically with TCA in that it is a PPAR $\alpha$  agonist, a peroxisome proliferator, and causes liver tumors in mice but not rats. Dieldrin also causes mammary gland tumors with gestational exposure (Cameron HL, Foster WG. Developmental and lactational exposure to dieldrin alters mammary tumorigenesis in Her2/neu transgenic mice. PLoS One. 2009;4(1):e4303. Epub 2009 Jan 28). Also, it appears that dieldrin can cause transformation of breast cells by a PPAR $\alpha$ -independent mechanism (Cameron HL, Foster WG. Dieldrin promotes resistance to anoikis in breast cancer cells in vitro. *Reprod Toxicol.* 2008 Feb;25(2):256-62.)

## Chemical Specific Charge Questions

### (A) Oral Reference Dose (RfD) for Trichloroacetic Acid

***1. A 60-week drinking water study in mice (DeAngelo et al., 2008) was selected as the basis for derivation of the RfD for trichloroacetic acid. Please comment on whether the selection of DeAngelo et al. (2008) as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.***

#### ***Penelope A. Fenner-Crisp***

To meet the definition of a chronic, repeated-dose study, according to EPA's and OECD's test guidelines, the exposure duration must be at least 12 months' long. The 60-week exposure duration study described in DeAngelo, et al. (2008) fits that definition, as does the 104 week in F344 rats (DeAngelo, et al, 1997). The two DeAngelo, et al. studies (1997, 2008) and the developmental toxicity study in Long Evans rats (Smith et al., 1989) are the only studies in the existing TCA database which present sufficient information suitable for the derivation of a traditional (i.e. lifetime) Reference Dose.

While modeling of the developmental toxicity data was an interesting exercise for comparison's sake, it probably was not really necessary, given that there was no no-effect level observed and the lowest effect level was about 5-fold higher than the lowest effect level observed in the mouse drinking water study. However, better safe than sorry, given that the endpoints measured were substantially different from those in the drinking water study and are among those of potentially significant concern to human health.

In the final analysis, the study chosen for RfD derivation was the appropriate one.

#### ***David W. Gaylor***

Since the study of DeAngelo *et al.* (2008) produced the most sensitive endpoints for exposures to TCA, it is the proper study to use for the derivation of the RfD.

#### ***Ronald L. Melnick***

The selection of DeAngelo et al. (2008) as the principal study for the derivation of the RfD for TCA is justified because this study included multiple dose groups, biweekly/monthly measurements of water consumption and body weights (to allow accurate estimations of mean daily dose), interim sacrifices at multiple time points up to 60 weeks of exposure, complete necropsies, and microscopic evaluations of gross lesions and several potential target organs. In addition, mice are more sensitive than rats to TCA-induced effects. A deficiency of this study is that other than gross lesions, liver, kidney, spleen and testis, complete histopathologic examinations were reportedly performed on only 5 mice from the high-dose and control groups. EPA should clarify with DeAngelo et al. on the extent of histopathologic examinations that were performed at the interim

sacrifices and at the termination of the 60-week study. A second major deficiency is that this study was limited to male mice. Thus, effects at sites other than those examined microscopically or in female mice might have been missed. These limitations need further discussion in the hazard identification chapter as well as in the section on the selection of uncertainty factors.

The developmental study of TCA in rats by Smith et al. (1989), which was the critical study for the derivation of the RfD in 1994, should be given equal consideration as the principal study for derivation of the RfD. Data from this study are amenable to dose-response modeling or to a NOAEL/UF approach for derivation of the RfD. Particularly noteworthy is the estimation of similar RfDs from the experimental data in these two studies (Smith et al., 1989, and DeAngelo et al., 2008). The Smith et al. (1989) study included multiple doses, but all of these showed evidence of maternal toxicity and developmental effects. Several other studies have demonstrated fetal effects of TCA, but none of these used doses low enough to reach a developmental NOAEL.

***Martha M. Moore***

I have no suggestions.

***Michael A. Pereira***

The rationale for choosing this study is sound and very well justified in the document. It does have the lowest NOAEL level based on hepatocellular necrosis.

***Ivan Rusyn***

This reviewer agrees with the study selection.

***Andrew G. Salmon***

This report describes a large study (50 mice per group) conducted using current methodology including thorough clinical and pathology evaluations. Reporting is thorough, including detailed analysis of dose rates (which can be a problem for drinking water studies if this calculation is not addressed specifically by the study authors). Mice appear to be a sensitive species for the liver effects of concern: the NOAEL and LOAEL reported in the parallel study in rats (De Angelo et al., 1997) are about five times higher than those reported for the mouse study. It is therefore an excellent choice for the basis of the RfD derivation. Some other studies might be considered for derivation of a principal or supporting RfD value, but this particular report is the most suitable. Results from this study are consistent with other studies in mice.

***Anthony R. Scialli***

Selection of this study is appropriate and well justified. The Smith et al. developmental study could also be used, but I would recommend using only the fetal growth endpoints and not the malformation endpoints.

***Alan H. Stern***

The choice of the DeAngelo et al. (2008) study for the derivation of the RfD was appropriate for several reasons: 1. The NOAEL and LOAEL were the lowest among the available studies; 2. The study was based on mice, which were more sensitive than rats; and 3. The inclusions of microscopic examination of tissues other than the liver provided a reasonable basis for identifying hepatic necrosis as the critical effect. Ideally, however, the study forming the basis of the RfD should have been a lifetime duration study rather than a 1-year study. Nonetheless, this study is still a chronic duration study and thus appropriate in terms of duration.

**(A) Oral Reference Dose (RfD) for Trichloroacetic Acid**

**2. Liver toxicity (hepatocellular necrosis) was selected as the critical effect for the determination of the point of departure (POD). Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.**

*Penelope A. Fenner-Crisp*

In principle, hepatocellular necrosis is an appropriate endpoint to use as a critical effect, on the assumption that the mode of action by which it occurs is relevant to humans. If the necrosis seen in mice is a consequence/key event in the PPARalpha agonism MOA leading to tumors, then it may not be. In that case, one or more of the endpoints identified in the long-term rat study or the testicular effects in mice or the developmental effects in rats would be more appropriate.

*David W. Gaylor*

In Table 5-1, an increase in liver weight and an increase in liver peroxisome proliferation were indicated for the study by DeAngelo *et al.* (2008). However, these two endpoints were totally ignored in the determination of the Point-of-departure (POD) for the derivation of the RfD. Presumably the impact of liver peroxisome proliferation would be reflected in the risk assessment for cancer based on liver tumors. The EPA Document does not investigate whether or not the increase in liver weight has a POD (BMDL<sub>10</sub>) lower than the value of 18 mg/kg-day calculated for hepatocellular necrosis (Table 5-3). Liver weight is a continuous data measurement. Continuous data often are more sensitive than quantal data. For continuous data, measurements in the unexposed control animals can be used to identify animals with abnormally (not necessarily adverse) low or high measurements, e.g., below the 1<sup>st</sup> percentile or above the 99<sup>th</sup> percentile. If the measurements are approximately normally distributed, the dose that produces a change in the mean response equivalent to 1.1 times the standard deviation, i.e., a BMR = 1.1 x (standard deviation), produces an estimated excess risk of 10% (BMD<sub>10</sub>) of animals with values in the abnormal range. The associated BMDL<sub>10</sub> can then be calculated for the POD and subsequent RfD. For more discussion on the calculation of benchmark doses for continuous data see: Crump, KS. Calculation of benchmark doses from continuous data. *Risk Analysis* 15: 79-89 (1995); Gaylor, DW and Slikker, W. Risk assessment for neurotoxic effects. *NeuroToxicology* 11: 211-218 (1990).

Liver weights as a function of dose are displayed in Table 3 (DeAngelo *et al.*, 2008). This reviewer fit these results to the continuous data models provided by the EPA Benchmark Dose Software, Version 1.4.1, using a Benchmark Response (BMR) = 1.1x(standard deviation). Among the models with adequate goodness-of-fit, the polynomial model had the lowest (best) Akaike Information Criteria (AIC) resulting in a POD (BMDL<sub>10</sub>) of 63 mg/kg-day of TCA. This value is well above the BMDL<sub>10</sub> of

18 mg/kg-day for hepatocellular necrosis. In the data presented by DeAngelo *et al.* (2008), for the dose of 602 mg/kg-day the average liver weight was 3.2 g with a standard deviation of 5.0 mg. Since standard deviations for liver weight tend to range from about 20-30% of the mean, as was observed here for the controls and lower doses, the standard deviation of 5.0 at the high dose is extremely excessive. It appears that the standard deviation of 5.0 is a typographical error, miscalculation, or the result of an outlier value in the high dose group. Dropping the high dose group resulted in a BMDL<sub>10</sub> = 58 mg/kg-day, which is still above the BMDL<sub>10</sub> = 18 mg/kg-day for hepatocellular necrosis chosen for the POD.

***Ronald L. Melnick***

Hepatocellular necrosis is an appropriate critical effect for the determination of the POD. This is an adverse effect that showed a clear monotonic dose-response relationship. The selection of this effect is validated by the correlated dose-response for increased serum LDH activity in the same study (DeAngelo *et al.*, 2008, Fig. 2). In addition, studies of TCA in rats also provided evidence of liver toxicity (e.g., Celik, 2007; Acharya *et al.*, 1997; DeAngelo *et al.*, 1997). Because similar RfDs were derived for liver toxicity and developmental toxicity, both endpoints should be emphasized throughout this review. The derivation of similar RfDs for two different effects strengthens the level of confidence in the estimated value.

***Martha M. Moore***

I have no suggestions.

***Michael A. Pereira***

Hepatocellular necrosis is not the best choice for the critical effect since it was very mild and similar mild central lobular necrosis has been reported for some PPAR- $\alpha$  agonists. The relationship to other PPAR- $\alpha$  agonists indicates that the UF for mouse to human extrapolation should be 3 not 10. Also, the database UF should be 3 not 10 since histopathologic evaluation of the liver in both mice and rats was reported in the study selected as the basis for derivation of the RfD. Testicular tubular degeneration had a higher POD of 127.4 mg/kg-day (page 123). Using a UF of 10 for each of the three uncertainty factors for testicular tubular degeneration would result in a slightly lower RfD of 0.127 mg/kg-day than using the POD of 18 mg/kg-day for liver necrosis with the three UFs totaling 100 (3 X 3 X 10); RfD = 0.18 mg/kg-day. Since the RfD for testicular degeneration and liver necrosis are very similar using my recommended uncertainty factors, I would recommend that the text of the document and Figure 5-2 include the RfD for both effects. This is especially so since Figure 5-2 also contains the comparison to the mouse liver toxicity to rat liver and rat developmental.

I would also recommend that when the BMDL<sub>10</sub>/POD of two effects are within 30, if not 100, of each other that both be used to derive the RfD. This is because the use of different uncertainty factors could result in the POD with the higher level actually resulting in a

lower RfD. Hence, it was premature on page 123 to pick the POD to be used in deriving the RfD. This should be done after the application of the uncertainty factors to both PODs on page 131.

***Ivan Rusyn***

The necrosis phenotype in this study has been assessed by histopathological evaluation and graded from 0 to 4. While there is no doubt in the quality of the pathological assessment, the subjective nature of this evaluation and a somewhat narrow range and qualitative nature of the biomarker may raise challenges to the strength of this endpoint as a quantitative critical effect. A more appropriate endpoint should be one with a more dynamic range and one that is relevant to the MOA, such as one of the markers of peroxisome proliferation (e.g., Cyanide-insensitive palmitoyl coenzyme A oxidase activity) assessed in this study. The latter endpoint is amenable to statistical interrogation and shows consistent, dose- and time-responsive changes.

***Andrew G. Salmon***

This endpoint is consistently observed in rodents and has been thoroughly examined from the biochemical and histological standpoint, as well as being the subject of a number of mechanistic investigations. This histological definition of the effect is a clearly adverse response. In principle it would be desirable to consider more upstream effects in the mechanistic chain of responses. These tend to be both sensitive indicators of effect, and also susceptible to more precise and statistically powerful measurements (e.g. continuous biochemical or physiological parameters) compared to the strictly quantal definition of histological damage responses. In the case of TCA there are a number of candidate parameters, such as peroxisome proliferation biomarkers for use in this approach. It would be interesting to see how these compare to the results obtained for hepatic necrosis, even if it were eventually concluded that the mechanism(s) of action are insufficiently well defined to justify the use of these measures as the primary basis of an RfD.

Based on the data presented it appears that selection of hepatic necrosis at 30 to 45 weeks as the critical effect is appropriate. Other endpoints that might be considered (e.g. other hepatocellular responses, testicular effects, developmental toxicity) are observed only at higher doses. However, the authors of the toxicological review are to be commended for including thorough analyses of these alternative endpoints for comparison with the chosen analysis based on hepatic necrosis.

***Anthony R. Scialli***

This endpoint was appropriately selected and justified; however, consideration should be given to using liver weight instead of necrosis. Liver weight offers the advantage of being continuous, which permits a more appealing benchmark dose calculation. In addition, liver weight is less subjective than pathologist ratings of subtle histologic observations.

***Alan H. Stern***

Hepatic necrosis is clearly an appropriate endpoint in general for derivation of an RfD. However, it does not appear that necrosis was identified in other studies, including studies of equal or longer duration at higher doses. Also, most, if not all, of the other toxic effects of TCA appear to occur through relative subtle and indirect mechanisms (e.g., PPAR $\alpha$  agonism, interaction with DNA methylation). Necrosis would generally be expected to result from direct chemical action or from severe oxidative stress, neither of which appear to be significant mechanisms in other studies. Thus, while the DeAngelo et al. (2008) study appears to be a valid and well conducted study, the observation of hepatic necrosis in this study raises some questions for me about the generalizability of this endpoint. Nonetheless, the observation cannot be dismissed and is appropriate for the derivation of the RfD.

**(A) Oral Reference Dose (RfD) for Trichloroacetic Acid**

***3. Benchmark dose (BMD) modeling was conducted on the liver and testicular effects in male mice exposed to trichloroacetic acid in the drinking water study by DeAngelo et al. (2008) in order to determine the POD. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., 10% extra risk of hepatocellular necrosis) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.***

***Penelope A. Fenner-Crisp***

I agree that the BMD modeling procedure has been conducted appropriately. However, modeling should also be done on one or more of the endpoints that identified the NOAEL in the long-term rat drinking water study.

***David W. Gaylor***

The BMD modeling was appropriate for the quantal data endpoints. An extra risk of 10% is appropriate. As discussed in the response to the preceding charge question, the BMDL<sub>10</sub> for the continuous data endpoint of liver weight should be added to the Document in order to fully justify the selection of liver necrosis for the POD and resulting RfD.

For a quantal endpoint, it is noted that some dose response models often produce similar or even identical values for the chi-square goodness-of-fit test, Akaike Information Criterion (AIC), BMD<sub>10</sub>, and BMDL<sub>10</sub>. This is not unexpected when only a few dose levels are used. The gamma, multistage, and Weibull are mathematically similar exponential models with dose raised to powers greater than one. With a small number of dose levels, it often is not possible to distinguish between these models. This is the case for hepatocellular necrosis. The comments from the OMB seemed to imply that the BMDL<sub>10</sub> from these three models should be given three times as much weight. This would not be appropriate as the mathematical model fit to these data was identical for the gamma, multistage, and Weibull. Hence, these three results are not independent.

***Ronald L. Melnick***

BMD modeling is the preferred approach to derive the POD because it uses all of the dose response data and is less impacted by the group size. BMD modeling of the developmental toxicity data from Smith et al. (1989) was also conducted for comparison with the POD for liver effects from the DeAngelo et al. (2008) study. Because the developmental toxicity study did not include low enough doses to achieve a NOAEL, an alternative approach to consider is the application of an additional uncertainty factor of 10X to the LOAEL for fetal body weight from Smith et al. (1989).

For liver or testicular toxicity, several models provided adequate fits to the data, with somewhat different estimates of BMDL<sub>10</sub>. Based on this outcome, it is not obvious why a particular dose response model was selected for the determination of the POD. If it is EPA's policy to select the model that yielded the lowest AIC value, then that rationale should be explicitly noted. Otherwise, model selection seems a bit arbitrary. EPA needs to justify the selection of 10% extra risk for the benchmark response. Simply stating "a BMR of 10% is generally used in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparisons across assessments" is inadequate.

In addition, EPA needs to explain why human equivalent doses were not estimated for the derivation of the oral RfD.

***Martha M. Moore***

While I am not technically qualified to fully address this question, in general, I think that the BMD approach is a good approach to data evaluation. I think that the comparisons of RfDs across target organs shown in figure 5-2 (page 133) is a good approach and provides support for the RfD that was determined.

***Michael A. Pereira***

The POD of 18 mg/kg-day derived for hepatocellular inflammation is less than the 127.4 mg/kg-day derived for testicular tubular degeneration. However, since the hepatocellular effect results from peroxisome proliferation, it is not related to a toxic response in humans so that the UF for mouse-to-human extrapolation should be 1 not 10. If this is the case than testicular tubular degeneration with a UF of 10 would result in males of a lower RfD of 0.127 mg/kg-day than the 0.18 mg/kg-day for liver inflammation using a UF of 1.

***Ivan Rusyn***

This reviewer deems the BMD modeling to be satisfactory, rigorous and scientifically sound. The EPA should, however, consider performing modeling on the rat liver (DeAngelo et al., 1997) data as well as on some additional, more continuous and with a wider range, endpoints from the principal study. Finally, the EPA shall make an explicit reference to any documents that are used as guidance for selecting the models (e.g., US EPA (2000b) document) in all cases when a model selection step was performed. A brief explanation of what the policy is will be desirable and will improve the transparency of the document.

***Andrew G. Salmon***

The application of the benchmark dose modeling approach in determining the POD follows standard U.S. EPA practice and the guidance offered by the authors of the BMDS software. Presentation of detailed program output and rationale for model choice is helpful in demonstrating that the analysis has been performed appropriately. Of the

various models tried, some were properly rejected as providing markedly worse fit than the better fitting models. Although differences in fit quality measures and BMDL estimates among these methods were not large, benchmark dose methodology guidance recommends the selection of a single best-fitting model. Of those models providing a reasonable fit, the log-logistic model is preferred for the hepatocellular necrosis data. This is justified by the lower AIC and/or higher p-value for the Chi-square goodness of fit. It should be noted that some models produce essentially identical results when they all optimize to a common reduced model, *e.g.* several exponential models may converge to an exponent of 1 (linear). Although an updated version of the software has been released since this analysis was performed, there have not been any substantive changes in the specific models of interest in this analysis, and the results for these models do not differ between versions.

The quantal analysis presented is the standard way of undertaking a benchmark analysis of this kind of data. However, the fact that DeAngelo et al. (2008) report severity as well as incidence data for their various histological endpoints does open the possibility of treating the overall incidence and severity data as a pseudo-continuous variable with greater statistical power than the dichotomously truncated data input to the quantal model (examples: see some recent Cal/EPA Reference Exposure Level determinations).

I have a specific concern with the choice of the ED<sub>10</sub> as the benchmark. The usual approach in using the benchmark approach is to attempt to define a benchmark dose at a response level which would be considered equivalent to a NOAEL. This allows similar application of uncertainty factors as have been conventionally used with NOAEL determinations, as was done here (see below). A 10% response rate above background in a well-designed study with reasonable statistical power would seldom be seen as a NOAEL, especially if the control rate is zero and the response is clearly adverse, as is the case for the hepatocellular necrosis and inflammation endpoints in the study by DeAngelo et al. (2008). It is somewhat unfortunate that the dataset chosen as the basis for the analysis is from the small interim sacrifices (ten animals per group), and lacks the statistical power of the larger datasets from these studies. If a study with 50 animals per group (such as the 104 week Study 2 [DeAngelo et al., 2008]) had been used as an indicator, a 10 % response rate would have been clearly identified as an effect level:

One-sided Fisher exact test for  $r2/n2 > r1/n1$

Enter r1 n1 r2 n2 --> 0 50 5 50

One-sided Fisher exact p-value is 2.8142E-0002

Fortunately, the benchmark dose methodology is not limited to simplistic pairwise comparisons but uses the entire dataset (like a trend test, but not limited to a linear model), so in fact even small studies can be analyzed with some confidence. However, the basic point is not so much about the statistical properties of an individual dataset, as the question of what response level should generally be considered a “minimally biologically significant change.” Extensive experience with this type of analysis in regulatory agencies (such as Cal/EPA, in addition to a number of specific examples presented by U.S. EPA) suggests that the 5% response rate yields a more appropriate

benchmark for quantal data in animal studies, if the aim is to select a benchmark which is similar to a NOAEL for extrapolation purposes. In this particular case, the ED<sub>05</sub> is within the range of the doses for which observations are reported.

There is a slight anomaly in the data for Study 1 reported by DeAngelo et al. (2008) in that at the 60 week time point the low dose group (0.05 g/L TCA) showed a considerably higher incidence and greater severity of hepatocellular cytoplasmic alteration than the mid-dose group (0.5 g/L), but incidence and severity of hepatocellular inflammation and testicular tubular degeneration were reported as uniformly zero, in contrast to even the control group where the incidence of these effects at mild severity was 7% and 10% respectively. Obviously these things happen from time to time without an obvious cause and may be produced by simple statistical fluctuations, but it does raise the question of whether there was something anomalous about the treatment or analysis of the low-dose group at 60 weeks. Therefore, by extension, the validity of the low-dose 0/10 response for hepatocellular necrosis at 30-45 weeks may be questionable: if this observation were an underestimate of the “proper” result at this dose that could affect the calculated BMDL significantly. I did not see any comment on this point either in the study description of the report of the dose response analysis.

***Anthony R. Scialli***

The benchmark dose modeling has been appropriately conducted. Inasmuch as the benchmark response of 10% is arbitrary, some justification would be helpful. At present, the text says, “A BMR of 10% is generally used in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparison across assessments.” In other words, it’s arbitrary, but we want to do it the same way each time. It might be preferable to indicate what studies have shown a BMDL<sub>10</sub> means with respect to LOAEL-NOAEL determinations. This kind of information is available, at least for developmental endpoints. Consideration can be given to using liver weight as an endpoint, which would permit use of a BMR related to the control response (e.g., a standard deviation based BMR).

***Alan H. Stern***

I have checked the benchmark dose calculations for the DeAngelo et al. (2008) hepatocellular necrosis using the EPA BMD software (ver. 2.0) and obtained the exact values given in Table 5-3 for chi-sq p-value, AIC and BMDL. However, the BMD modeling in this document should reflect the latest version of the software.

The document (pg. 123, par. 1) justifies the choice of a BMR of 10% on the basis that “A BMR of 10% is generally used in the absence of information regarding what level of change is considered biologically significant and also to facilitate a consistent basis of comparison across assessments.” I believe that this is both factually incorrect and inappropriate. Lack of clarity as to the extent of change that is biologically significant implies that the parameter under consideration is a continuous variable (e.g., body weight). However, the variables under consideration (hepatocellular inflammation,

hepatocellular necrosis, and testicular tubular degeneration) are all defined in the document as dichotomous variables. These determinations may, in fact, require an implicit judgment on the part of the pathologist as to what level of observed change constitutes a “positive” finding. Nonetheless, as compiled in the original papers and as presented in the document, these are dichotomous endpoints. Thus, the rationale presented for the selection of a BMR of 10% for continuous data is not valid. Furthermore, even if these were continuous, rather than dichotomous endpoints, there is still no *a priori* basis for selecting a 10% BMR. Depending on the particular data, BMR for continuous data can also be set at 5% or on the basis of a z-value. For dichotomous data, the appropriate basis for selecting a BMR should be the distribution of the data. The BMR should be close to the lower end of the observed data. In addition, the notion that a BMR of 10% should be used “...to facilitate a consistent basis of comparison across assessments” is contrary to the one of the original ideas underlying the use of the BMD approach – that the BMDL should be an estimate of the study-independent NOAEL. There is no reason why the BMR underlying such a value would or should be consistent across studies. In the DeAngelo et al. (2008) study, it appears that for hepatocellular inflammation, a BMR of 5% could reasonably be justified on the basis of the distribution of the data. If, however, there are other reasons for selecting a value of 10% (e.g., minimal nature of the effect), this should be explicitly stated.

The difference in Table 5-2 between the logistic model with a chi-sq p-value = 0.24 and AIC = 74.19 (considered the best fitting model) and the probit model with a chi-sq p-value of 0.24 and AIC = 74.20 (not considered a best-fitting model) is not meaningful. This also applies to Table 5-4 where the difference between the logistic model (chi-sq p-value = 0.19, AIC = 76.08) and the gamma, multistage 1<sup>o</sup>, Weibul models (chi-sq p-value = 0.19, AIC = 76.16) is also not meaningful. Minor differences in the fit of non-biological, purely mathematical models should not be overinterpreted since none of these fits necessarily reflect true differences in the underlying dose-response and perfect fit is not the goal of the curve fitting exercise. If it were, the appropriate degree polynomial giving an exact fit could be used.

The quantal linear model (available in the BMDS ver. 2.0 software) gives an identical fit to the gamma, multistage 1<sup>o</sup> and Wiebul models and should be added to that list.

**(A) Oral Reference Dose (RfD) for Trichloroacetic Acid**

***4. Please comment on the rationale for the selection of the uncertainty factors applied to the POD for the derivation of the RfD. If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s).***

***Penelope A. Fenner-Crisp***

I agree with the composite 1000X uncertainty factor, although I think some further analysis of the ADME data might allow for some modest refinement of the animal-to-human factor.

In the section presenting the justification for the 10X for interspecies extrapolation, I think that the last sentence needs to be revised to read “Insufficient information is currently available to assess ~~rat~~ **MOUSE**-to-human differences in TCA toxicokinetics or toxicodynamics,” since the RfD is being based upon data from the mouse, not the rat.

***David W. Gaylor***

The selections of the uncertainty factors were appropriate.

***Ronald L. Melnick***

The selection of uncertainty factors of 10X for human variation, 10X for animal-to-human variation, and 10X for database insufficiencies are reasonable and consistent with EPA policy. However, it is not possible to know if 10X adequately accounts for the extent of human variability. Additional discussion is needed on database insufficiencies. One explanation “there are no TCA-specific systemic toxicity data in humans” is already captured in the UF for animal-to-human extrapolation. The developmental effects of TCA in rats raise serious concerns. While the lack of a multigeneration reproductive toxicity study is certainly a major data gap, other data gaps related to developmental effects should be noted, e.g., none of the studies included doses that would allow identification of a NOAEL, none of the studies included evaluations beyond gestational exposure, and no data are available on potential neurodevelopmental toxicity or reproductive toxicity. In addition, the study that was used to derive the RfD (DeAngelo et al., 2008) did not include female mice, and it is not clear to what extent complete histopathological evaluations were performed in that study. These limitations and critical data gaps support the 10X uncertainty factor for database insufficiencies. An additional UF of 3-10X should be applied to an RfD based on a BMR of 10% extra risk.

***Martha M. Moore***

Again, not my area of expertise--but I think that the factors used are appropriate given that there does not appear to be any information that would suggest using something other than the default values.

***Michael A. Pereira***

See the above comment about the UF for mouse-to-human extrapolation that should be 3 not 10.

Furthermore, the use of a UF of 10 for database insufficiencies does not appear to be justified, because the DeAngelo studies were conducted in two species (mice and rats) and there are developmental data. Hence, this UF should be 3, if not 1, because there does not appear to be any significant database insufficiencies, especially any insufficiency that could lower the calculated RfD. The database insufficiencies are those that would increase the RfD such as further determination that the liver toxicity is not relevant to humans since it involves PPAR- $\alpha$ .

Also, the use of 10 as the UF for human variation needs to be better justified, since a UF of 3 would appear to be more justified. This is because: a) TCA is metabolized to a limited extent; b) the toxicokinetics of TCA in mice and rats; and c) the mode of action i.e., peroxisome proliferation does not suggest that humans would vary significantly in their susceptibility to TCA.

***Ivan Rusyn***

The EPA provides excellent rationale for each uncertainty factor applied in the document. The EPA will be well advised to review the recent work of the NTP's Host Susceptibility Branch which has begun examining the inter-strain differences in ADME and toxicity of chemicals in toxicity studies in mice. A recent published report on the effects of acetaminophen administered to a panel of inbred mouse strains (Harrill et al., 2009) examined a dose-response for liver necrosis in several strains. This work suggests that about a 10-fold difference exists in the EC50 for acetaminophen-induced elevation of serum ALT. The principal study used for RfD derivation (DeAngelo et al., 2008) has used B6C3F1 mice which are genetically homogeneous animals and there is little data to determine whether the toxicity in this strain appropriately represents the range of responses that may exist in the mouse population. Thus, omitting an uncertainty factor for within-mouse (or rat) variability from the overall application of the uncertainty factors may be a limitation that needs a broader consideration in risk assessment process.

***Andrew G. Salmon***

The selection of uncertainty factors applied to the POD for the RfD derivation follows standard U.S. EPA practice from PODs determined from NOAELs or BMDLs in animal toxicity studies. As such, they are acceptable provided that an appropriate POD has been identified. As noted above, the recommended POD in this case (and any similar cases) is the BMDL<sub>05</sub> rather than the BMDL<sub>10</sub>. Provided this change is accepted, the uncertainty factors are appropriate: if the BMDL<sub>10</sub> is retained a LOAEL-to-NOAEL uncertainty factor is required.

There have been a number of publications and guidance documents suggesting that the default value of 10 for human variability is insufficient, particularly when the range of human metabolic capabilities is considered and the need to protect children, among other sensitive subpopulations, is recognized. For instance, Cal/EPA's Air Toxics Hot Spots program currently recommends a value of 30 for metabolized systemic toxicants. Using an alternative approach, some U.S. EPA programs recommend a separate tenfold uncertainty factor to be used (in appropriate cases) to protect children. In this case, however, the extent of metabolism is minor and (as far as we can determine) not a major determinant of either the toxicity or clearance of TCA. This significantly reduces the extent to which genetic polymorphisms in xenobiotic metabolizing enzymes or physiological variations can affect the impact of TCA, so in this case a tenfold UF for human diversity is probably sufficient. However, uncritical use of this value, such as here where it is simply described (page 132) as the default in the absence of other compound specific information, should be reconsidered, especially where children are among the potential target population.

Inclusion of a database uncertainty factor in this case is appropriately described and justified. Although there are some reproductive toxicity data, these are relatively weak: the key study observed no NOAEL and reported a relatively high NOAEL. The lack of a multigeneration study emphasizes the potential concern for effects in children, and inclusion of this uncertainty factor helps to address any residual uncertainty which may remain about the adequacy of the value used for the uncertainty factor for human variation.

***Anthony R. Scialli***

The uncertainty factors are appropriate. Another database insufficiency to consider is that the developmental studies are of limited utility. The Smith paper is the only developmental study worth considering, and the selected doses were so high that BMD modeling ended up way below the observed range.

***Alan H. Stern***

I think that the UFs that were selected are reasonable. Since the DeAngelo studies did conduct extensive histopathological examination in two species (mice and rats), and there are developmental data, I don't find significant insufficiencies for the chronic endpoint given that full histopathological examination appears to have been limited to the DeAngelo studies.

In general, lack of human-specific data is addressed by the animal to human UF. Most RfDs are based on animal data without useful human data. I don't, however, recall that being used as a contributing value to a full UF for database insufficiencies. However, the lack of long-term follow-up developmental studies, as well as the lack of two-generational reproduction studies, warrants a UF of 10 for database insufficiencies.

**(B) Inhalation Reference Concentration (RfC) for Trichloroacetic Acid**

***1. An RfC was not derived for trichloroacetic acid. Has the scientific justification for not deriving an RfC been clearly described in the document? Please identify and provide the rationale for any studies that should be selected as the principal study.***

***Penelope A. Fenner-Crisp***

The justification for not deriving an RfC is summarized in only three sentences on Page 133. This is inadequate. The Agency has issued a methods document which provides a decision tree which helps to guide the risk assessor in determining if, when and how one might conduct a route-to-route extrapolation (EPA, 1994. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA /600/8-90/066F). The document includes examples of when it would be inappropriate to carry out this calculation. The Agency has an obligation to provide, in greater detail, the reason(s) it has not drafted an RfC for TCA. If it is because, for instance, a decision has been made to never again do a route-to-route extrapolation under any circumstances or in the absence of a PBPK model, say so. Whatever the reason, it needs an explanation beyond “The available information was inadequate for a route-to-route extrapolation from the oral pathway to the inhalation pathway.”

The justification for not doing an inhalation unit risk is a bit more illuminating, but still unsatisfying.

***David W. Gaylor***

Not deriving an inhalation RfC for TCA has been justified.

***Ronald L. Melnick***

The lack of inhalation toxicity data on TCA, which is likely due to the low vapor pressure of this chemical, make it difficult to derive an RfC. However, because TCA undergoes minimal metabolism and it is likely that a major percentage of inhaled TCA will be absorbed, an inhalation RfC could be obtained by assuming 100% absorption. Thus, EPA should pursue the derivation of an RfC with explicit description of all assumptions.

***Martha M. Moore***

The justification for not deriving an RfC is that there are no inhalation studies available. That appears to be a clear statement and justification.

***Michael A. Pereira***

The document is justified in not deriving an RfC for inhalation exposure to TCA. This is because there are no studies available in which to calculate an RfC.

***Ivan Rusyn***

This reviewer agrees with Drs. Fenner-Crisp and Salmon and would like to suggest that the EPA considers route-to-route extrapolation or state explicitly why such an exercise has not been considered.

***Andrew G. Salmon***

The lack of any appropriate data on inhalation toxicity presents a barrier to satisfactory risk assessment for inhalation exposures to TCA. It is most likely an acute hazard by inhalation simply as a result of its acidity, but it is not entirely clear how this would reflect on chronic exposures by inhalation, especially to aerosols of neutral pH. Whether such exposures actually occur to any substantial extent in the general environment is unclear, but it is well-known that indoor aerosol exposures to drinking water contaminants do result from other domestic uses of the supplied water, such as showering and use of toilets. Swimming in pools filled from the piped supply may also result in additional exposure to water contaminants by various routes. The Agency's decision not to derive a RfC is defended simply by the lack of data. It is acknowledged that there is no sophisticated PBPK model for TCA by inhalation or any other route, but in view of the relatively minor importance of metabolism for this compound, and its prompt excretion primarily in the urine as unchanged material, it is not obvious that a model of any great sophistication is necessary to undertake a route-to route extrapolation. It appears to me that a simple set of assumptions such as 100% absorption by the inhalation route, followed by systemic distribution via the bloodstream, could be justified by consideration of the limited metabolic and pharmacokinetic data which are available via the oral route and the simple water-soluble nature of the chemical of concern. This would allow derivation of an RfC which would be protective of those systemic effects observed by the oral route, for which there is no reason to suppose they would not appear if uptake were instead by inhalation. The assumption of 100% uptake is admittedly arbitrary, but not unreasonable for a water soluble material, and it is unlikely to be in error by a factor of more than about two, which is less than the other uncertainties inherent in an RfC or RfD derivation.

***Anthony R. Scialli***

No studies, no RfC. Makes sense to me; however, I agree with the sentiment that justification should be given for not doing a route-to-route extrapolation.

***Alan H. Stern***

In general, it is best to derive an RfC from inhalation-specific data. Nonetheless, it is still possible to derive an RfC from ingestion-specific data. EPA's guidance for inhalation dosimetry can provide a reasonable estimation of the extent of absorption by the inhalation route in the absence of inhalation-specific data. The larger uncertainty, however, generally comes into play when considering extrapolating inhalation-based toxicity from ingestion-based toxicity data. In the case of TCA, given the lack of

significant metabolism when ingested, it seems unlikely that there would be respiratory-specific toxicity and it might, therefore, be reasonable to derive an RfC from the ingestion specific data. However, since inhalation of TCA is not likely to be a major factor due to volatilization under normal environmental conditions, the major inhalation exposure is likely to occur due to showering. This is particularly the case since TCA is a widespread water contaminant due to its occurrence as a chlorination by-product. Nonetheless, given the possibility of respiratory-specific toxicity, and the lack of data that can address respiratory-specific toxicity, it may be more appropriate to simply provide the exposure relationship that would allow an estimate of cumulative (i.e., ingestion plus inhalation) exposure under standard exposure assumptions.

(C) **Carcinogenicity of Trichloroacetic Acid**

***1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgr-d.htm), the Agency concluded that trichloroacetic acid is likely to be carcinogenic to humans by all routes of exposure. Please comment on the cancer weight of evidence characterization. Is the weight of evidence characterization scientifically justified?***

***Penelope A. Fenner-Crisp***

1) At best, one might characterize TCA as exhibiting “suggestive evidence of carcinogenic potential.” However, it more likely should be placed into the “Not likely to be carcinogenic to humans” category.

2) I disagree with EPA's conclusion that TCA is likely to be carcinogenic **by all routes of exposure** (emphasis added). The 2005 cancer guidelines state: “For a route-to-route exposure extrapolation, *the default option is that an agent that causes internal tumors by one route of exposure will be carcinogenic by another route if it is absorbed by the second route to give an internal dose.* TCA data are available showing that it is absorbed dermally in humans. On the other hand, the document notes that there are no studies which determine whether or not there is TCA absorption following inhalation exposure. In addition, no scientific argument is offered to support the conclusion that uptake into the blood of intact parent TCA by the inhalation route could/would occur.

Therefore, one can conclude ONLY “..... **by the oral and dermal** routes of exposure.”

***David W. Gaylor***

The weight of evidence characterization appears to be appropriate for high doses.

***Ronald L. Melnick***

The only identified carcinogenic effect of TCA is the induction of hepatocellular adenomas and carcinomas in mice. The carcinogenicity of TCA in male and female mice has been demonstrated in multiple studies, even after only 52 weeks of exposure. According to EPA's 2005 Guidelines for cancer risk assessment, data supporting the descriptor “likely to be carcinogenic to humans” may include positive findings in animal experiments in more than one sex OR early age at the onset of response, with or without evidence of carcinogenicity in humans. Thus, the carcinogenicity data on TCA are consistent with the descriptor “likely to be carcinogenic to humans.”

Based on the fact that a large percentage of orally administered TCA is excreted in the urine of rats and mice, it is likely that absorbed TCA from any route of administration will be systemically distributed. Based on its high water solubility, it is likely that a major portion of inhaled TCA would be absorbed. The finding of increased urinary excretion of TCA by human subjects following 30-minute sessions in chlorinated swimming pool

water indicates that TCA can be absorbed through the skin. Because TCA undergoes minimal metabolism, it is likely that the carcinogenic effects observed with oral exposure would also occur with dermal or inhalation exposure.

The evidence on TCA supporting the descriptor “likely to be carcinogenic to humans” is one of the weakest among chemicals listed in IRIS with this descriptor. EPA should provide some perspective on the extent of evidence on TCA carcinogenicity relative to other chemicals that share this descriptor.

***Martha M. Moore***

The summary of the overall weight of the evidence concerning carcinogenicity is relatively brief. The conclusion that TCA is “likely to be carcinogenic to humans” by all routes, is largely based on the lack of evidence to the contrary.

***Michael A. Pereira***

The conclusion that TCA is "likely to be carcinogenic to humans" is not justified. In fact, the literature indicates the opposite, that is, TCA is likely not carcinogenic to humans. TCA is unlikely to be carcinogenic to humans because:

- 1) In a well-performed carcinogenesis bioassay in rats, it did not exhibit any indication of a carcinogenic response;
- 2) In mice, it has only been reported to induce liver tumors in a strain with a very high incidence of spontaneous tumors. Furthermore, no other tumors in any other site were report at necropsy;
- 3) TCA is not genotoxic and induces liver tumors by a non-genotoxic mechanism related to peroxisome proliferation. Humans are much less sensitive than mice to peroxisome proliferators, so that this mode of action is not important or in humans. Hence, the mouse liver tumors found in TCA-treated mice are not relevant to humans; and
- 4) The presence of an increase yield of liver tumors in a strain of mice with a high background incidence of liver tumors is not sufficient to suggest that a chemical is a human carcinogen. Suggestive evidence that a chemical is a human carcinogen would require at least an increased yield of tumors in another organ or in another species with a low yield of background liver tumors.

The four references cited above under General Charge Question 2 need to be added and discussed since they came to a conclusion similar to mine that TCA is not likely to be a human carcinogen. Their conclusion is based on TCA mouse liver tumors having the identical pathology, histopathology, biology and molecular biology as those tumors found in liver of mice treated with other PPAR- $\alpha$  agonists. This is critical since the EPA is proposing that TCA has MOAs other than those of PPAR- $\alpha$  agonists. These four articles, as well as many other reviews of the literature, came to conclusion that the TCA MOA is that of a PPAR- $\alpha$  agonist and that the liver tumors found in TCA-treated mice do not indicate that TCA is likely to be a human carcinogen.

Taking all this into consideration, it is very unlikely that TCA will induce tumors in another organ of mice. Hence, it is very unlikely to be carcinogenic to humans. It cannot be stated at this time that TCA is not carcinogenic to humans until a two-year carcinogenesis bioassay is performed in mice that evaluates all the organs. Furthermore, the exposure of humans to TCA would never be sufficient to cause cancer by the non-genotoxic mechanism whether or not it is related to PPAR- $\alpha$ .

***Ivan Rusyn***

The authors of the document did a good job in presenting scientific justification for the characterization of the weight of evidence.

***Andrew G. Salmon***

The Review provides a thorough consideration of both the direct evidence of carcinogenicity, and the mechanistic investigations which, although not identifying a single unequivocal mechanism of action, provide important insights into the likely significance of the carcinogenicity observations for human cancer risk.

I have some reservations about the way the genetic toxicity information has been characterized. It is probably correct to conclude that TCA is at best a weak mutagen, and that any such activity does not derive from direct reaction of TCA or metabolites of this compound with DNA. However, although the case of mutagenesis related to oxidative stress, generation of reactive oxidants either via peroxisome activation or other mechanisms (*e.g.* macrophage/Kupffer cell interactions, whether associated with PPAR $\alpha$  activation or otherwise) is discussed in the section on mechanisms, it has not been sufficiently recognized that the available genetic toxicity assay results since for the most part the tests employed, particularly those *in vitro*, either lacked the ability to detect oxidative effects or, even if they had such sensitivity, the likely metabolic or cellular mechanisms for generating activated oxygen species were absent from the test systems.

Overall, however, the characterization presented is carefully balanced and scientifically justified. The data meet the criteria for identification of TCA as “likely to be carcinogenic to humans.” It is worth pointing out that current guidelines do not limit the characterization to this simple categorization, but also require provision of a narrative statement of the overall context of the finding, including comparison of the strength of the evidence and the degree of “likeliness” or “possibility” of an identified carcinogenic risk to humans. The Report provides this background information, although the authors may want to extend or clarify this if other comments are seen to require it.

***Anthony R. Scialli***

This characterization is not scientifically justified. You have multiple studies but all in a single species (mouse) with lack of concordance in a closely related species (rat). So how does this make trichloroacetic acid likely to be carcinogenic in humans? Maybe I am missing something, but if the EPA 2005 Guidelines require this kind of disregard of

common sense, then the Guidelines should be changed or ignored. Even after consideration of the criteria in the Guidelines, I remain unmoved. Considerable weight appears to have been given to both sexes being affected, but I fail to see the biological significance of both sexes being affected by liver tumors after administration of a PPAR $\alpha$  agonist as a signal of the likelihood of human carcinogenicity.

***Alan H. Stern***

Under EPA's 2005 Guidelines, the examples for criteria consistent with the description "likely to be carcinogenic to humans" are:

- an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence...OR
- an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route...OR
- a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset; OR
- a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case.

Within the narrow form of these criteria, it is clear that TCA is an agent that has tested positive in animal experiment in more than one sex and that, by definition, it meets the definition of "likely to be carcinogenic to humans." Therefore, within the narrow requirements of the 2005 Guidelines, I agree that this characterization is justified. However, in the broader scientific context, it seems to me that the appropriate descriptor for TCA should be "suggestive evidence of carcinogenic potential." Even though TCA was positive for cancer in mouse liver, with respect to its implication for human cancer risk it is clear that some positive animal carcinogenesis studies produce more plausible associations with potential human cancer than others. The document expresses the argument for TCA as "There are data gaps that preclude a determination that TCA *is not carcinogenic to humans* [my emphasis]." The document makes a reasonable case for such a statement. However, such a statement is not the same as saying that the data from animal studies *are likely* to be relevant to human exposure. It is a considerably weaker statement than a similar statement made in a positive form (e.g., the data suggest that carcinogenicity observed in animal studies is likely to be relevant to humans). However, the document apparently does not feel that the evidence supports such a statement and I agree.

With respect to the other criteria from the EPA Guidelines, TCA has tested positive in only one strain of one species (B63CF1 mouse), albeit in both sexes. Thus, the evidence for carcinogenicity across strains and species is not compelling. Liver tumors are common in rodents and the tumor response while statistically significant does not appear

to be extraordinary. Thus the data do not appear to raise “additional biological concerns beyond that of a statistically significant result.” Finally supporting evidence for events associated with tumor formation such as DNA reactivity or effects on cell growth exists, but the evidence is somewhat speculative rather than direct and clear cut – i.e., that TCA produces certain effects (e.g., hypomethylation, effects of Kupffer cells) that may be part of a causal chain of events leading to carcinogenesis.

**(C) Carcinogenicity of Trichloroacetic Acid**

***2. Have the studies supporting the discussion of the mode(s) of carcinogenic action been clearly described?***

*Penelope A. Fenner-Crisp*

In my view, the whole discussion on MOA needs to be restructured in order to achieve adequate clarity.

Those studies that are included in the discussion of MOA generally are clearly described. However, they aren't all of the ones that should be included in this section.

This section represents a very weak example of the application of EPA's Framework for evaluating the hypothesized mode(s) of action. To me, the discussion is muddled and does not flow well in a manner consistent with the Framework. The Framework was created for a good reason - to provide a useful structure for presenting a complex story in a clear and transparent way. Information on the proposed PPARalpha-agonism MOA is intermingled with data on other MOAs or alternative explanations. Data which do or do not support TCA's involvement in each key event posited for the PPARalpha-agonism MOA are not systematically presented.

It is not made clear in the document where and how the information on gene expression (pages 97-98, 102-103), DNA hypomethylation (pages 98-100, 102-104) and altered cell proliferation (pages 112-114) fit into the MOA discussion. Is the Agency trying to suggest that these are key events not previously identified as being a part of PPARalpha agonism? Are they separate stand-alone MOAs? Or, are they simply examples of effects representing already identified key events in the PPAR MOA, as it appears to this reviewer?

A second possible MOA (direct damage to DNA) for which a robust data base exists for TCA and about which one can reach a conclusion as to whether or not it applies to TCA is not subjected to a "formal" MOA analysis, but is buried in the Summary section. Preston and Williams (2005. DNA-reactive carcinogens: Mode of action and human cancer hazard. *Crit. Revs. Toxicol.* 33:673-683) have described the key events for tumor development for DNA-reactive carcinogens. The results of the genotoxicity studies for TCA should be measured against the elements of these Key Events in a separate MOA analysis.

There also is no separate discussion/section of other possible MOAs which currently don't have sufficient information to be subjected to a formal MOA analysis. However, such a section should be included.

Topics to be covered in this new section include a) role of NPCs, e.g. Kupffer cells; b) role of other nuclear receptors such as CAR; and 3) GJIC-intercellular communication [move from Summary section].

Useful additions to the existing discussion of NPCs include:

- 1) Roberts, et al. (2007). Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. *Toxicol. Sci.* 96(1): 2-15).
- 2) Woods, et al. (2007a). Sustained formation of POBN radical adducts in mouse liver by peroxisome proliferators is dependent upon PPAR $\alpha$ , but not NADPH oxidase. *Free Radic. Biol. Med.* 42(3): 335-342.
- 3) Woods, et al (2007b). Time-course investigation of PPAR $\alpha$ - and Kupffer cell-dependent effects of Wy-14,643 in mouse liver using microarray gene expression. *Toxicol. APpl. Pharmacol.* 225(3):267-277.

Also, this new section is where the Ito et al. (2007) paper on tumorigenesis of DEHP in PPAR $\alpha$  null mice can be described. They are not the only investigators who have speculated that peroxisome proliferators may also induce tumors by a PPAR $\alpha$  independent pathway. Important questions to be answered include whether or not PPAR $\alpha$ -independent pathway(s) is/are functional in the intact mouse, or only in the absence of the predominant PPAR $\alpha$  pathway in the intact animal (as, perhaps, illustrated by Takashima, et al. 2008).

Some additional papers should be included here. One is a study which postulates a role for constitutive androstane receptor (CAR) (Ren, et al. 2009. Characterization of peroxisome-proliferator-activated receptor{ $\alpha$ } (PPAR{ $\alpha$ } activators in the rodent liver: Di-(2-ethylhexyl) phthalate A activates the constitutive androstane receptor. *Toxicol. Sci.* October 22 Advance access). The results of another study (Guo, et al (2007) Induction of nuclear translocation of constitutive androstane receptor by peroxisome proliferator-activated receptor  $\alpha$  synthetic ligands in mouse liver. *J. Biol. Chem.* 282(50):36766-36776) would suggest that PPAR $\alpha$  ligands not only serve as PPAR $\alpha$  agonists, but possibly act as CAR antagonists.” This would suggest that in removing the PPAR $\alpha$  from the wild-type mouse to create a null mouse, a normally dormant or unused pathway would be unmasked and unleashed.

Nonetheless, since the document concludes on page 101 that “...TCA-induced hepatocyte hypertrophy is PPAR $\alpha$  dependent,” based upon results of the Laughter et al (2004) study, perhaps TCA exposure in a null mouse would not mimic the DEHP/PPAR $\alpha$  null mouse results. Long-term administration of Wy 14,643 doesn't.

Another useful addition to the paper would be Zhen et al. (2007. Metabolomic and genetic analysis of biomarkers for peroxisome proliferator-activated receptor  $\alpha$  expression and activation. *Mol. Epidemiol.* 21(9): 2136-2151) which described different metabolic sets observed when comparing groups of wild type and null mice, untreated or treated with Wy-14,643.

Other papers that also would be helpful:

The series of papers that NHEERL researchers and others have been publishing on PPAR $\alpha$  agonism related effects of the perfluoroalkyl acids (i.e., PFOA, etc.)

- 1) Wolf, et al (2008). Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. *Toxicol. Sci.* 106(1): 162-171.
- 2) Foreman, et al. (2009). Differential hepatic effects of perfluorobutyrate mediated by mouse and human PPAR-alpha. *Toxicol. Sci.* 110(1): 204-211.
- 3) Rosen, et al. (2008). Gene profiling in the livers of wild-type and PPARalpha-null mice exposed to perfluorooctanoic acid. *Toxicol. Pathol.* 36(4):592-607.
- 4) Wolf, et al (2009). Comparative hepatic effects of perfluorooctanoic acid and WY 14,643 in PPAR-alpha knockout and wild-type mice. *Toxicol. Pathol.* 36(4):632-639.
- 5) Cheng and Klaassen (2009b) Critical role of PPAR $\alpha$  in perfluorooctanoic acid- and perfluorodecanoic acid-induced downregulation of Oatp uptake transporters in mouse livers *Toxicol. Sci.* 106(1):37-45.
- 6) Bjork and Wallace (2009) Structure-activity relationships and human relevance for perfluoroalkyl acid-induced transcriptional activation of peroxisome proliferation in liver cell cultures. *Toxicol. Sci.* 111(1):89-99.

TABLES! TABLES! TABLES! This is the mantra that has been presented over the past decade on Mode of Action/Human Relevance analyses. Tables are wonderful tools to assist the risk assessor in clearly presenting proposed key events, along with the data that show/don't show if, and how, the chemical of interest is involved in that key event and whether or not, in the final analysis, it is all relevant to human health.

The authors of the TCA document refer to Klaunig (2003) several times in the PPAR MOA discussion, but solely with regard to the discussion on the basic generic building blocks of the MOA (Figure 4 and Table 12 in Klaunig et al.). I would recommend that the authors also study Table 17 and the tables embedded in the case studies in Klaunig et al. (e.g., Tables 19 and 20 for DEHP; Tables 23 and 24 for clofibrate and Table 25 for oxadiazon), in the hope that they can appreciate their value as visual displays of the available data.

There are also a number of other exemplary case studies that have been published in the peer reviewed literature since then as well, the structure of which makes them easy to comprehend. Summarizing data in tables and/or lists makes it so much easier then to craft the presentation patterned after the modified Hill criteria.

Good case examples published in the peer-reviewed literature:

1) Metofluthrin

Yamada, et al (2009). Case study: An evaluation of the human relevance of the synthetic pyrethroid metofluthrin-induced liver tumors in rats based upon mode of action. *Toxicol. Sci.* 108(1): 59-68.

Deguchi, et al., (2009). Mode of action analysis for the synthetic pyrethroid metofluthrin-induced rat liver tumors: Evidence for hepatic CYP2B induction and hepatocyte proliferation. *Toxicol. Sci.* 108(1): 69-80.

2) Thiamethoxam

Pastoor, et al. (2005). Case study: Weight of evidence of the human health relevance of thiamethoxam-related mouse liver tumors. *Toxicol. Sci.* 86(1):56-60

Green, et al (2005a) Thiamethoxam-induced mouse liver tumors and their relevance to humans. Part 1: Mode of action studies in the mouse. *Toxicol.Sci.* 86(1):36-47.

Green, et al (2005) Thiamethoxam-induced mouse liver tumors and their relevance to humans. Part 2: Species difference in response. *Toxicol.Sci.* 86(1):48-55.

3) Pyrethrins

Osimitz, T and B. Lake. (2009). Mode-of-action analysis for induction of rat liver tumors by pyrethrins: relevance to human cancer risk. *Crit. Rev. Toxicol.* 39(6):501-511.

Price, et al. (2007). A mode of action for induction of liver tumors by pyrethrins in the rat. *Toxicol. Appl. Pharmacol.* 218(2):186-195.

4) Carbon tetrachloride

Manibusan, et al. (2007). Postulated carbon tetrachloride mode of action: A review. *J. Environ. Sci. Health Part C* 25: 185-209

5) Formaldehyde and glutaraldehyde

McGregor, et al (2006). Formaldehyde and glutaraldehyde and nasal cytotoxicity: Case study within the context of the 2006 IPCS human framework for the analysis of a cancer mode of action in humans. *Crit. Revs. Toxicol.* 36:821-835.

***David W. Gaylor***

Although the studies are rather complex, they have been described adequately.

**Ronald L. Melnick**

The document provides an extensive and comprehensive review on the mode of action for TCA carcinogenicity, particularly on the literature related to PPAR $\alpha$  activation in the liver. The discussion notes “there are a number of inconsistencies and data gaps that reduce the confidence in the conclusion that TCA induced hepatocarcinogenesis through a PPAR $\alpha$  MOA.” Several inconsistencies are then noted. Because of the importance of this issue in characterizing the potential carcinogenic risk of TCA, this document as well as future assessments on peroxisome proliferators would benefit from a table that identifies consistencies/inconsistencies and data gaps for a few well studied peroxisome proliferators (e.g., DEHP, Wy-14,643, etc.) and for TCA.

Experimental Observation	DEHP	Wy-14,643	TCA
Carcinogenicity in PPAR $\alpha$ -null mice	+ (22 month exposure)	- (11 month exposure)	Not tested
Hepatocarcinogenicity in rats	+	+	-
Hepatocarcinogenicity in mice	+	+	+
Cell proliferation, in vivo	+, transient	+, sustained	+
Cell proliferation, in vitro +/- Kupffer cells			
Inhibition of apoptosis			
Let-7C miRNA inhibition			
DNA hypomethylation, PPAR $\alpha$ -dependent			
Increased 8-OHdG			
Other phenotypic characteristics			
Kupffer cell activation			
Hepatocellular necrosis			
Dose-response relationships: e.g. peroxisome proliferation and liver tumor incidence			

**Martha M. Moore**

Yes, I think that the authors have done a very thorough job of outlining and summarizing a relatively large number of studies related to the MOA for cancer.

**Michael A. Pereira**

The discussion of the mode(s) of action for carcinogenic action of TCA in mouse liver is not complete or clearly described. This is because the carcinogenic modes of action, other than PPAR $\alpha$  agonist-induced peroxisome proliferation are purely speculative without support. This needs to be clearly stated in the document, or these other proposed modes of action should not be discussed. Also, as stated above, the histopathology, biology and molecular biology of the liver tumors in TCA-treated mice are completely consistent with

those found in mice treated with other inducers of peroxisome proliferation and are not consistent with tumors found in mice treated with agents that act via the other speculated modes of action proposed in the document.

The document needs to address why there have never been found any tumors with the biology or histopathology similar to those that act via mechanisms other than that of a PPAR- $\alpha$  agonist. This is the most critical criticism of the document. It is also a major concern of the four references listed above in General Charge Question 2. Chemicals that are not PPAR- $\alpha$  agonists and produce mouse liver tumors by the other proposed mechanisms consistently result in eosinophilic tumors with many pretumorous altered foci both of which are GST- $\pi$  positive. However, these foci and tumors have never been found in mouse liver tumors produced by TCA. Thus, TCA could not have produced any tumors by a MOA similar to a non-PPAR- $\alpha$  agonist. Hence, the document has to clearly and completely describe how the liver tumors found in TCA-treated mice are identical in histopathology, biology, and molecular biology to liver tumors found in mice treated with other peroxisome proliferators.

***Ivan Rusyn***

While some of the parts of the MOA are discussed quite extensively (e.g., the discussion on the PPAR $\alpha$ -mediated events and their possible role in species differences with regard to liver carcinogenic potency of TCA), other important components of the MOA have been described with little detail or receive little attention. Most of all, the document suffers from inconsistencies in listing the components of the MOA in different chapters (see specific comments below) which detracts from driving the message home that the MOA is complex. Re-focusing such discussions would further strengthen EPA's MOA analysis and subsequent conclusions. Furthermore, the EPA may strengthen the quantitative assessment of the relative potency of PPAR $\alpha$  activation by TCA in comparison to other chlorinated solvents; as well as compare the potency indicators for mouse hepatocarcinogenicity of various peroxisome proliferators, including chloroacetic acids, with common short-term markers of PPAR $\alpha$  activation and in vitro transactivation of PPAR $\alpha$ . An excellent analysis that may serve as an example is presented in a recent article by Guyton et al. (2009) published in *Environmental Health Perspectives*.

***Andrew G. Salmon***

In general, yes. Most of the recent key studies have been described in sufficient detail to understand what was done and the significance of the observations in the discussion of the modes of action. Once or two cases where more explanation would be helpful: the case of the early report by Elcombe (1985) has already been noted. If, as I suggest, the Agency is able to provide further analysis of the important studies with PPAR $\alpha$ -null mice based on very recently published new information, it would be desirable to include a fuller description of some of these studies, in particular the pivotal report by Ito et al. (2007) and the new paper by Ren et al. (2009).

***Anthony R. Scialli***

The discussion of PPAR $\alpha$  is nicely done. The short section on decreased cell-cell communication seems speculative. The sections on altered cell proliferation and genotoxicity are good, although it might be helpful to put the conclusion at the beginning of the section as well as at the end.

***Alan H. Stern***

Yes. While the structure of the document could benefit from a more direct presentation, the document does an excellent job of laying out the quite complex considerations and sometimes conflicting evidence regarding the possible models of TCA carcinogenicity. In particular, the summary presented in the introduction to section 5.4 is an excellent overview.

(C) **Carcinogenicity of Trichloroacetic Acid**

***3. EPA has concluded that the available data do not support any specific mode of action. In addition, EPA has determined that the data are not supportive of PPARalpha agonist-induced peroxisome proliferation as the sole mode of action leading to tumor formation. Please comment on whether these determinations are scientifically justified.***

*Penelope A. Fenner-Crisp*

I disagree with the conclusion that there are insufficient data to establish PPARalpha agonism as a mode of action, in the fact the preponderant MOA. The Agency hasn't done a very good job of "lining up" the TCA-specific data that support (or not) its involvement in the key events and it fails to acknowledge, and include discussion of, relevant data on chemicals other than TCA itself in order to develop a credible MOA analysis.

An additional reference useful in documenting key events associated with PPAR $\alpha$ -agonism MOA includes:

Xiao, et al. (2006). Activation of peroxisome proliferator-activated receptor alpha enhances apoptosis in the mouse liver. *Toxicol.Sci.* 92(2): 368-377.

More than one conclusion must come out of the discussion on MOA. One is with regard to whether or not sufficient information exists to show if TCA exerts its carcinogenic effect by an MOA that has been characterized well enough generically. In this instance, there are two: PPARalpha-agonism and direct DNA-reactivity. The second conclusion would be with regard to the feasibility/likelihood that one or more additional MOAs may be in play as well. We must remember that it is possible that more than one MOA may be involved in a specific response, and if so, we are obligated to determine under what the circumstances/conditions this could occur, e.g., stand-alone or interrelated with the PPARalpha agonism MOA.

As for the question as to whether or not other modes of action may be at play, EPA cites the long-term study with DEHP in which PPARalpha null mice still showed a positive tumor response. There is a fairly simple study that could be done-repeat the study with DEHP and/or other known agonists in transgenic mice in which the mouse PPARalpha has been replaced with the human PPARalpha. Shorter term studies generally show that the hPPAR mouse responds differently from the intact mouse, and from the null mouse as well.

The discussion regarding the human relevance of the PPARalpha agonist MOA is woefully inadequate - two sentences near the bottom of page 108. There is a growing body of evidence which reveals the differences between the rodent PPARalpha and the human PPARalpha cascade and explains why the human PPARalpha cascade does not/cannot include a tumorigenic response. It appears not to be simply a quantitative difference, but a qualitative one. This body of data needs to be summarized in this document.

***David W. Gaylor***

The current conclusions regarding the carcinogenic MOA for TCA are appropriate. It appears that future studies might be able to justify the use of a non-linear MOA at low doses for a carcinogenic risk assessment of TCA.

***Ronald L. Melnick***

Peroxisome proliferation is not a MOA leading to cell proliferation (counter to the statement on page 91, 2<sup>nd</sup> paragraph, line 8) or to tumor induction; peroxisome proliferation in laboratory animals is an endpoint dependent on PPAR $\alpha$  activation. In fact, the document cites Klaunig et al. (2003) who noted that peroxisome proliferation is “an associative rather than a causal event in development of liver tumors” (page 95, 1<sup>st</sup> paragraph). The issue of concern for TCA is whether or not tumor formation is the result of events dependent SOLELY on PPAR $\alpha$  activation. The completion of the table suggested above might help clarify which inconsistencies in the PPAR $\alpha$  activation MOA, data gaps for TCA, and additional effects induced by TCA justify the EPA determination that available data are not supportive of PPAR $\alpha$  activation as the sole MOA leading to tumor formation. Available data do not support any MOA exclusively; multiple processes are likely involved. As noted in the EPA document, “dose-response concordance between proposed key events and tumor response is lacking.” The lack of correlation between peroxisome proliferation and liver cancer potency has been demonstrated (Marsman et al., 1988). Further studies and analyses are needed on age- and duration-dependent dose-response relationships for proposed key PPAR $\alpha$ -dependent events and liver tumor incidence for several peroxisome proliferators, including TCA, in rats and mice.

While studies using PPAR $\alpha$ -null mice can provide important insights on biological activities that are dependent on this transcription factor, the relevance of such findings to humans is not always obvious because humans produce a functional PPAR $\alpha$ . Furthermore, the distribution of PPAR $\alpha$  expression levels in humans is not known. The lack of induction of certain cell cycle regulated genes in PPAR $\alpha$ -humanized mice may be due to differences in binding of activated hPPAR $\alpha$  to mouse co-activators or in binding to mouse peroxisome proliferator response elements. These points as well as discussion on the reliability of studies using primary human hepatocyte cultures should be included in the discussion on human relevance.

***Martha M. Moore***

Yes, I agree that their conclusion is scientifically justified based on all of the available information.

***Michael A. Pereira***

As stated above, the EPA is not justified in concluding that PPARalpha agonist-induced peroxisome proliferation is not the sole mode of action leading to tumor formation in mice exposed to TCA. Of course, it is almost impossible to prove that one mode of action is the sole mode, like proving a negative. However, all the data indicate that TCA acts solely as a PPAR-alpha agonist so that this is the sole MOA for TCA to produce mouse liver tumors. If another mode of action is active then it can only be responsible for an extremely small and insignificant incidence of liver tumors. Hence, another mode of action is pure speculative without support.

***Ivan Rusyn***

The first conclusion here may be rephrased to state that the data available to date suggests that there are multiple modes of action that may not be mutually exclusive. The second statement is also technically sound but may be changed to reflect the fact that while PPARalpha-related events represent some of the major components of the overall mechanism of toxicity and carcinogenicity, it is premature to conclude at this time, especially with regards to TCA, that this is the only mode of action. In addition, since new scientific evidence that challenges the hypothesis that PPARalpha is absolutely required for hepatocarcinogenesis of peroxisome proliferators in mice has been presented recently, the strengths of this linkage becomes more uncertain.

***Andrew G. Salmon***

This conclusion is justified. There is a clear role of PPAR $\alpha$  agonism as a contributor to the observed effects of TCA in rodents, but it is not at all demonstrated that the PPAR-associated peroxisome proliferation response is part of the direct chain of events leading to carcinogenesis. Similarly, there appear to be several other TCA-related responses not linked to PPAR $\alpha$ , or at least not exclusively tied to this pathway, which may contribute to the cancer response.

***Anthony R. Scialli***

Not my area of expertise, but the case for PPAR $\alpha$  agonist mode of action looks pretty convincing to me. Even after reading this document, I am not sure why the EPA authors have determined that the data don't support this mechanism as the mode of action.

***Alan H. Stern***

I agree that the data do not support a clear MOA (although it seems likely the peroxisome proliferation is *a* mode of action). The data most strongly suggest MOAs that proceed through PPAR $\alpha$  agonism. These include peroxisome proliferation as well as cell proliferation associated with PPAR $\alpha$ . However, the data do not make a strong case for any particular PPAR $\alpha$  associated MOA and it would not be implausible that peroxisome proliferation is both necessary and sufficient to produce liver tumors in mice. Other

possible MOAs (e.g., DNA hypomethylation, Kupffer cell-mediated effects, inhibition of gap junction communication) appear to be mostly, if not entirely, speculative. Based on my reading of the data, I believe that it is too strong a statement to say that the data are not supportive of PPAR $\alpha$  induced peroxisome proliferation as the sole MOA. Rather, I would say that the data do not identify any specific MOA (including peroxisome proliferation) as exclusive, but also do not rule out any specific MOA (including peroxisome proliferation) as a sole MOA.

(C) **Carcinogenicity of Trichloroacetic Acid**

***4. A 104-week drinking water study in mice (DeAngelo et al., 2008) was selected as the basis for quantification of the oral cancer slope factor. Please comment on whether the selection of this study is scientifically justified.***

***Penelope A. Fenner-Crisp***

Section 5.4 describes the proposed elements of the quantification of estimated human carcinogenic potential. Tables 5-8 through 5-12 briefly summarize the relevant liver tumor incidences observed in five mouse studies, the data from which might be suitable to serve as the basis for a quantitative estimate. Exposure duration scaling factors were applied to the four studies conducted for 52 (2), 60 (1) or 82 (1) weeks, but not to the one 104 week study.

I agree that it was not necessary to make the duration adjustment to the 104 week study. However, I would disagree with its application to the 82 week study data. While NTP has traditionally incorporated a 104-week exposure in its chemical bioassay design for both mice and rats, regulatory testing authorities, in the U.S. (including EPA) and internationally (OECD), have determined that 18 months of exposure is sufficient for a carcinogenicity study in mice<sup>2</sup>. I would suggest that the 82-week data be re-adjusted by excluding the exposure duration factor. The result should then be re-compared to the calculations based upon the 104 week study, before reaching a final decision as to which of the two studies provides the best data set for quantitative assessment, if one must be done.

The results, and oral slope factors calculated there from, of the 52 and 60 week studies should be excluded from consideration in deriving the estimates. Incorporating an exposure duration factor introduces an unnecessary uncertainty into the extrapolation exercise.

***David W. Gaylor***

The selection of this study for the calculation of the oral cancer slope factor is justified by the lowest LED<sub>10</sub> (most sensitive result) as displayed in Table 5-13.

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<sup>2</sup> US EPA 1998. Health Effects Test Guidelines OPPTS 870.4200 Carcinogenicity. p.5 “(i) **Oral studies.** If the test substance is administered by gavage, the animals are dosed with the test substance on a 7-day per week basis for a period of **at least 18 months for mice** (emphasis added) and hamsters and 24 months for rats. However, based primarily on practical considerations, dosing by gavage on a 5-day per week basis is acceptable. If the test substance is administered in the drinking water or mixed in the diet, then exposure should be on a 7-day per week basis.”

**Ronald L. Melnick**

Numerous studies clearly establish TCA as a hepatocarcinogen in male and female mice. The selection of the 2-year drinking water study of TCA in male mice by DeAngelo et al. (2008) for quantification of the oral cancer slope factor is justified because it is the only adequate study of sufficient duration with a positive neoplastic response. Also, pH-adjusted dosing solutions were analyzed throughout the study, measurements of water consumption and body weights were made regularly throughout the study to allow accurate estimations of mean daily dose, and there were sufficient numbers of animals surviving until study termination. Although complete necropsies and microscopic evaluations of gross lesions and several potential target organs were performed, a deficiency of this study is that other than gross lesions, liver, kidney, spleen and testis, complete histopathologic examinations were performed on only 5 mice from the high-dose and control groups. A deficiency in EPA's cancer assessment is that not all of the liver tumor data from DeAngelo et al. (2008) were used for the quantification of the oral cancer slope factor and there are issues with the data shown in Table 4-6 that need to be resolved. The use of both terms "prevalence" and "incidence" for the liver tumor data (Table 4-6) is confusing. Also, based on the number of animals in each group at final necropsy, the number of animals examined microscopically, and the number of unscheduled deaths, it appears that a large number of unscheduled death animals in each group was not examined for hepatocellular neoplasia. EPA needs to obtain from DeAngelo et al. an explanation on why these animals were not examined. An explanation is also needed on the impact of this missing data on the cancer potency estimate. Some explanation is needed on why there is such a large difference in the liver tumor incidence in the control group from study #2 (12%) versus the control group from study #3 (64%). It is not likely that neutralized acetic acid (1.5g/L) provided to the study #2 control group would have such an impact.

Because the focus of most cancer bioassays was on effects in the liver, the evaluation of the carcinogenic potential of TCA at other sites in laboratory animals is incomplete.

***Martha M. Moore***

I think it is justified.

***Michael A. Pereira***

This bioassay, although performed correctly, was not designed to evaluate whether TCA induced tumors in organs other than the liver. As stated above, the liver tumors induced by TCA are not sufficient to classify TCA as a potential human carcinogen. Hence, without supporting evidence that TCA induces tumors in another organ, the derivation of an oral cancer slope factor is not justified. In fact, the derivation of an oral cancer slope factor should not be performed since it gives the erroneous impression that there is concern about the carcinogenicity of TCA. This could lead to changes in drinking water disinfection and engineering to remove TCA that are likely to be more harmful to humans and the environment than exposure to TCA.

***Ivan Rusyn***

It is scientifically justified and the rationale is clearly established in the document.

***Andrew G. Salmon***

The choice of this study as the basis for the cancer dose response assessment is justified. It is a study using the relevant route of exposure for derivation of an oral slope factor: it is of sufficient size and appropriate design, and thoroughly reported. Other similar studies suitable for quantitative analysis are of lower power (in terms of group sizes and number of dose groups) and were reported in less detail in some areas, but their results are generally supportive of the results of DeAngelo et al. (2008).

***Anthony R. Scialli***

I disagree with calculation of an oral cancer slope factor because I do not find evidence for considering TCA a likely human carcinogen.

***Alan H. Stern***

The DeAngelo et al. (2008) 104 week duration study had a very high incidence of combined adenomas and carcinomas in the control group (64%). This is much higher than in Ball et al. (2002) (0%); Ball et al. (1990) (0%); the DeAngelo et al. (2008) 60 week duration study (13%); or Pereira et al. (1996) (4%), all of which used the same strain of mouse and all (except Pereira et al.) used male mice. This raises serious questions about the background rate of liver tumors in the DeAngelo et al. (2008) 104 week study. The B6C3F1 is, apparently, a sensitive strain for liver tumors and the 104 week study was the longest duration study among the cited studies. Thus, it may be the case that the high rate of tumors in the controls in that study is not unexpected. Nonetheless, this does not appear to be discussed in the document. This is a serious omission in the document. Furthermore, since there are only two doses in that study (in addition to the controls) and the incidence of combined adenomas and carcinomas at the lower of the two doses is less than the incidence in the control mice, the derivation of the POD is based essentially on the observation from a single dose. Furthermore, the background incidence of liver tumors is not only high but highly variable. Thus while the 104 week study is the longest duration study, it does not provide much useable data. Thus, the 104 week DeAngelo et al. (2008) study does not appear to be a good choice for modeling of the cancer slope factor. However, the comparison of the cancer potency slopes among the several other candidate studies is based on the exposure duration scaling applied to those studies.

**(C) Carcinogenicity of Trichloroacetic Acid**

***5. The oral cancer slope factor was calculated by linear extrapolation from the POD (lower 95% confidence limit on the dose associated with 10% extra risk for liver tumors). Has the modeling approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the slope factor and discuss whether such approaches are preferred to EPA's approach.***

***Penelope A. Fenner-Crisp***

Quantitative risk assessment using a margin-of-exposure approach would be more appropriate for a “suggestive” WOE characterization. No quantitative assessment is necessary for an “Unlikely” characterization.

***David W. Gaylor***

The benchmark dose modeling approach was appropriately conducted. The calculation of human equivalent doses described in Section 5.4.3 is wholeheartedly supported.

***Ronald L. Melnick***

Based on data gaps and lack of understanding of the processes involved in TCA induced liver tumors in mice, a linear extrapolation from the POD is consistent with EPA's 2005 Guidelines for Carcinogen Risk Assessment. For exposure duration scaling, EPA assumes that 104-week exposure duration represents a lifetime exposure in mice. This assumption underestimates the true lifetime of B6C3F1 mice. The mouse lifetime should be documented or longer lifetimes should be used for exposure duration scaling.

Why was the multistage model the only one fit to the liver tumor data? Other models, e.g., Weibull, should also have been fit to these data and evaluated for goodness-of-fit. The cancer potency estimates based on the male mouse tumor data from DeAngelo et al. (2008) differ by about 3-fold. Is this difference due to different control rates, experimental variability, exposure duration scaling, or some other reason? An alternative approach would be to combine the liver tumor data from male mice exposed for 60 weeks with the data from the 104-week exposure for the determination of the LED<sub>10</sub> and the oral cancer slope factor.

***Martha M. Moore***

I am not really technically qualified to address this question.

***Michael A. Pereira***

See the above comment.

***Ivan Rusyn***

No comments.

***Andrew G. Salmon***

Modeling of the cancer dose response and derivation of the oral cancer slope factor was appropriately undertaken using the standard approach recommended by U.S. EPA cancer risk assessment guidelines. In view of the established uncertainties about the various possible mechanisms of action for TCA carcinogenesis, alternative approaches such as explicit biologically based response models would not be appropriate.

***Anthony R. Scialli***

I can't comment.

***Alan H. Stern***

In general, the approach of deriving the POD using benchmark dose modeling and calculating the potency slope from a line dropped from the POD to the origin is a reasonable approach. However, it needs to be appreciated that the advantage of benchmark dose modeling over the LOAEL/NOAEL approach to defining the POD is that benchmark dose modeling uses all of the available data to derive a metric that is independent of the specific doses in the study under consideration. It is, therefore, independent of the specific NOAEL or LOAEL derived from the study-specific doses. This means that benchmark dose modeling requires sufficient data to define the dose-response curve and thus, for the distinction between a BMDL and a NOAEL to be meaningful. Often, with two dose-response observations (in addition to the control) and certainly with one observation, benchmark dose modeling becomes a data independent exercise. As discussed above, the DeAngelo et al. (2008) 104-week study had only two dose levels and it appears that tumors were observed at an incidence significantly above the inordinately high control value at only one of those doses. Thus, with two values that are both essentially control values and one positive value for tumor incidence, it does not make sense to me to employ benchmark dose modeling. For such a data set, the only reasonable approach would be to define the dose-response value with the positive response as the POD and proceed from that value. However, this solution begs the question of whether given these considerations, it is *a priori* appropriate to use this study at all for deriving the cancer potency.

**(C) Carcinogenicity of Trichloroacetic Acid**

***6. An inhalation unit risk (IUR) for cancer was not derived for trichloroacetic acid. Is the determination that the available data for trichloroacetic acid do not support derivation of an IUR scientifically justified?***

***Penelope A. Fenner-Crisp***

See Comment B1.

***David W. Gaylor***

Data are not available to derive an inhalation unit risk for cancer for TCA.

***Ronald L. Melnick***

Yes, the lack of inhalation carcinogenicity data and the lack of information on the disposition of TCA after inhalation exposure justify not deriving an IUR for this chemical. The lack of inhalation studies on TCA is likely related to the low vapor pressure of this chemical.

***Martha M. Moore***

As with the RfC, the justification is based on lack of available data.

***Michael A. Pereira***

Since there are no inhalation data pertaining to TCA being a carcinogen by this route, it is completely justifiable not to derive an IUR for cancer. The document needs to justify its conclusion that TCA is carcinogenic by all routes of exposure which is assumed to include inhalation. If there are no data to indicate that inhalation of TCA is carcinogenic in laboratory animals and human, how can the document support this conclusion.

***Ivan Rusyn***

Appears to be justified.

***Andrew G. Salmon***

I will re-state, with some specific changes relevant to the inhalation unit risk, my concerns expressed earlier about the decision not to calculate an RfC for trichloroacetic acid:

The Agency's decision not to derive an IUR is defended simply by the lack of data. It is acknowledged that there is no sophisticated PBPK model for TCA by inhalation or any other route, but in view of the relatively minor importance of metabolism for this

compound, and its prompt excretion primarily in the urine as unchanged material, it is not obvious that a model of any great sophistication is necessary to undertake a route-to route extrapolation. It appears to me that a simple set of assumptions such as 100% absorption by the inhalation route, followed by systemic distribution via the bloodstream, could be justified by consideration of the limited metabolic and pharmacokinetic data which are available via the oral route and the simple water-soluble nature of the chemical of concern. This would allow derivation of an IUR. The Review already addresses the question of whether the carcinogenic effect is likely to be independent of the route of exposure, and concludes that there is no reason to suppose they would not appear if uptake were by inhalation or other routes instead of the oral route used in the key studies. The assumption of 100% uptake is admittedly arbitrary, but not unreasonable for a non-volatile water soluble material, and it is unlikely to be in error by a factor of more than about two, which is less than the other uncertainties inherent in a cancer slope factor derivation.

***Anthony R. Scialli***

No comment.

***Alan H. Stern***

Given that TCA is not particularly volatile and especially given that there appear to be no data on inhalation toxicity, I agree that the decision not to derive an IUR is justified.

## V. SPECIFIC OBSERVATIONS

### *Penelope A. Fenner-Crisp*

[The reviewer did not submit specific observations.]

### *David W. Gaylor*

Page 120, Table 5-1, Comments Column for the Developmental Study by Smith et al. (1989):

- (a) 10% extra risk should read 5% extra risk.
- (b) Change Tables 5-3 and 5-4 to Tables 5-6 and 5-7.

Page 147, Section 6.2.3, 2<sup>nd</sup> paragraph, line 3:

- (a) Change (mg/kg)<sup>3/4</sup> to mg/kg<sup>3/4</sup>.

### *Ronald L. Melnick*

Pages 7-11. The relevance of TCA binding to plasma proteins is not clear, especially since the elimination rate constant in rat plasma is about 2-4 times faster than in liver (page 7, 1<sup>st</sup> paragraph). Is protein binding specific or non-specific? If binding is specific, why was the liver:blood partition coefficient greater than one (page 11, 2<sup>nd</sup> paragraph, PC: 1.18)? What is the basis for species differences in binding capacity/binding sites to albumin (Page 10, 2<sup>nd</sup> paragraph)? The dissociation constants listed on page 10 (2<sup>nd</sup> paragraph) should be included in Table 3-1. While peak levels of free TCA in plasma might be lower in species with greater plasma protein binding, the AUCs for free TCA might not be very different due to plasma protein binding causing a reduction in the rate of elimination in the urine. Species differences in the elimination kinetics of TCA should be noted in the dose-response assessment.

Pages 12-17. According to the proposed metabolic scheme (Figure 3-1), metabolism of TCA to CO<sub>2</sub> occurs via the formation of DCA. If DCA or subsequent metabolites are important in the toxicity or carcinogenicity of TCA, then effects at low doses may be relatively more potent than those at high doses (CO<sub>2</sub> exhalation decreased from 12% to 8% as iv doses increased from 1 to 50 mg/kg) (Page 12, 1<sup>st</sup> paragraph). The dose-dependent decrease in CO<sub>2</sub> exhalation may be due to the greater rate and extent of urinary excretion of TCA at higher doses (Page 17, 3<sup>rd</sup> paragraph). These points should be noted in the dose-response assessment.

Page 97, 2<sup>nd</sup> paragraph. The discussion of the paper by Woods et al. (2007) fails to note the author's conclusion that suppression of apoptosis was dependent on both Kupffer cell NADPH oxidase activity and PPAR $\alpha$ .

Page 109-110. The lack of induction of certain cell cycle regulated genes in PPAR $\alpha$ -humanized mice may be due to differences in binding of activated hPPAR $\alpha$  to mouse co-activators or to certain mouse PPREs.

Page 111, line 8. There are not data demonstrating “less susceptibility of humans than mice to TCA-induced liver tumors.”

Page 133, Figure 5-2. Show derived RfDs for all of the liver and developmental toxicity endpoints for which PODs were obtained.

***Martha M. Moore***

There is substantial available information on TCA. Unfortunately, as is generally the case, the information comes from a wide assortment of studies that were not coordinated in much, if any, way. I think it is important that “we” start to move from describing the array of studies, and try to array all the data into some type of framework that provides information (perhaps by route of exposure and species/strain) as to what doses were used, how long a treatment was used and what effects were observed at what doses and at what time. Obviously, it would be ideal if researchers started to design their studies based on trying to understand all possible modes of action and if both a temporal and dose response framework were used. This is obviously best done in a large well coordinated study. In the absence of this information, it would be helpful if the available information is arrayed in a way that at least attempts to address temporality and dose response concordance for all the various biological effects.

I have one correction for the genotox section. Page 84. The mouse lymphoma study of Harrington-Brock et al. actually found TCA positive without S9 in a single culture in one experiment. This culture had a relative total growth (the measure of cytotoxicity for this assay) of 11%. The authors state that this response was at less than or equal to 11%. In a repeat experiment cultures giving the same level of cytotoxicity were not positive—therefore the overall call is equivocal. I also note, that the criteria for calling responses positive in the mouse lymphoma assay has changed. No longer is a two fold response considered to be positive. Rather there is a requirement that the response exceed a global evaluation factor of  $90 \times 10^{-6}$ . That is, the induced mutant frequency (the response above the background mutant frequency) should exceed  $90 \times 10^{-6}$ . In this particular case, the application of the new criteria does not change the overall call for TCA.

Reference for the new criteria: Moore, M.M., M. Honma, J. Clements, G. Bolcsfoldi, B. Burlinson, M. Cifone, J. Clarke, R. Delongchamp, R. Durward, M. Fellows, B. Gollapudi, S. Hou, P. Jenkinson, M. Lloyd, J. Majeska, B. Myhr, M. O’Donovan, T. Omori, C. Riach, R. San, L.F. Stankowski, Jr., A. Thakur, F. Van Goethem, S. Wakuri and I. Yoshimura. (2006) Mouse Lymphoma Thymidine Kinase Gene Mutation Assay: Follow-up Meeting of the International Workshop on Genotoxicity Tests—Aberdeen, Scotland, 2003-- Assay acceptance criteria, positive controls, and data evaluation. *Environ. Mol. Mutagen.* 47, 1-5.

Table 4-9 lists sperm-head abnormalities as a genotoxicity endpoint. This is not a correct classification.

Given that information for DCA is included in some of the sections and there is a genotoxicity data base for DCA, I think it would be good to include that in the document.

***Michael A. Pereira***

None.

***Ivan Rusyn***

Page 5, section 3.1:

Considerations of absorption of TCA lack important details on the type of vehicle and the route of administration used in each study. It is well known that vehicle effects are considerable with regards to bioavailability of many toxicants, including the class of chlorinated solvents.

Page 19, section 3.5:

While the authors are correct in pointing out that no PBPK model for TCA has been reported yet, there is some recent literature on the pharmacokinetic modeling of TCA after exposure to TCE (e.g., Kim et al., 2009). In absence of direct modeling of TCA, the authors may wish to consider the kinetic profile of TCA as a metabolite of chlorinated solvents.

Pages 25-27, table 4-1:

Exposure route column should contain information on the type of a vehicle used in each study.

Pages 42-42, table 4-2b:

Cancer bioassays and tumor promotion studies are viewed by many in the field as studies that are difficult to compare directly as the pathophysiology of the two types of models may vary considerably. To avoid potential confusion and undue criticism, the authors may wish to present these separately.

Page 43, table 4-2b:

The "Species" column on this page needs to provide the information on the sex of animals used for Bull et al. (1990). Likewise, for studies that included more than one sex (i.e., "Results" column), complete explanation of the findings in each sex should be included.

Page 49, table 4-6:

The "Prevalence" rows seem to be presenting the data in an erroneous format. Note "b" indicates that the numbers should represent a fraction (i.e., range btw 0 and 1) while the data seem to be expressed as percent.

Page 58, table 4-7:

Descriptions of most of the studies listed should state the vehicle used.

Section 4.5:

The phenomenon of peroxisome proliferation is an important consideration for the mode of action discussion on TCA. There are a number of studies that have evaluated this endpoint and it is curious that it is not included in this section.

Page 71:

The authors should explicitly state the magnitude and significance (if any) of the observed changes in cell proliferation for the two studies detailed on this page. This also applies to other parts of the document where this critical quantitative information is omitted. A careful evaluation of the accuracy and completeness of the statements should be conducted.

Page 75:

The reference to Tao et al (2000) should be corrected as there are two references for this first author/year combination.

Page 92, section 4.7.2:

This section may benefit from inclusion of the references to the tables included in the previous sections.

Pages 93-94, section 4.7.3:

The authors need to make strong statement regarding the fact that only one long-term study was conducted in rats to evaluate the carcinogenicity of TCA.

The authors make vague statements regarding the MOA. The arguments would be strengthened if it is stated that the MOA for TCA is complex, a position well articulated in section 4.5 and throughout the document. It is not only “possible” that there is “more than one MOA,” but this document provides considerable rationale and lists compelling experimental evidence to reach such a conclusion.

Figure 4-1 needs to be reconciled with the arguments in section 4.5. For example, “activation of non-parenchymal cells” is shown in the figure yet there is no mention of the data that would support this.

Page 95, section 4.7.3.1.1:

The review by Klaunig et al. (2003) is cited 8 times on this page. The authors are advised to strengthen the arguments by referencing the original articles.

Pages 112-115, section 4.7.3.1.1.5:

This section should be consistent with the rest of the document where it discusses potential components of MOA. As written, this particular section omits mention of studies investigating the potential role for epigenetic events. Inconsistencies in MOA discussion create confusion and distraction.

Page 129:

The BMDS version used for the analysis here appears to be 1.3.1 while section 5.4.1 (page 137) mentions version 1.4.1. The authors are advised to make sure the versions are listed correctly.

Page 135, section 5.4:

This reviewer disagrees that the MOA for TCA can be characterized as “unknown.” Considerable experimental evidence, as stated elsewhere in this document, suggests that the MOA is complex and no single event can establish unequivocally the mechanistic basis for TCA carcinogenicity in mice.

Page 138, tables 5-8 through 5-10:

The statements “see text for...” in table notes should include a reference to a specific location (e.g., chapter number) in this document.

There are ~2x differences in estimated daily intake between three studies listed. This needs to be carefully explained in the text.

***Andrew G. Salmon***

In view of the acknowledged possibility of oxidative damage playing a role in TCA responses, it is unjustified to make blanket statements such as “The data do not support a mutagenic mechanism” (page 115, paragraph 3, line 3) without further qualification, even if some published authors have ventured such assertions. The authors of the Review have elsewhere been more cautious and accurate in their statements, such as “data from recent TCA studies that have investigated the MOA for hepatocarcinogenesis do not support a direct mutagenic mechanism” (page 135, 3<sup>rd</sup> paragraph, lines 1-2). The Agency’s attempts to define a “mutagenic mode of action” and deduce risk assessment choices therefrom have failed to establish a scientifically robust definition of what this phrase means, and have generally resulted in more derision than enlightenment. Therefore the reviewers here would do well to steer clear of this nebulous concept and to be careful to define exactly what they mean in discussing the role of mutations in the mechanism of cancer causation.

Figure 4-1 (page 94) presents a series of possible key events in TCA carcinogenesis, but appears to focus more on the PPAR $\alpha$ -related effects than the others. Several interesting investigations, such as DNA hypomethylation, are omitted (or subsumed under general headings). There is no indication of the possibility of oxidative stress resulting from macrophage (Kupffer cell) activation, rather than peroxisome proliferation in hepatocytes, as described in Section 4.5.1.5 (page 78). This could occur either as a result of or independent of PPAR $\alpha$  activation. While admittedly there is a limit as to how much can be crammed into such a figure without creating an impenetrable rat’s-nest effect, some thought should be given to expanding the figure and/or acknowledging its limitations.

**Anthony R. Scialli**

1. It would help a great deal if you used line numbering in drafts sent for comment.
2. Page 11, lines 22-26: The gestational age of the mice used in this experiment should be inserted.
3. Page 20, second paragraph: I recommend against citing data such as Klotz and Pyrch in which haloacetic acids or other disinfection byproducts were studied together. Only data specifically on TCA would be relevant to this review. In fact, EPA would provide a service by explicitly stating that data on mixtures cannot be applied to the individual components of the mixture.
4. Page 20, line 22: If you keep the discussion of Klotz and Pyrch, please do not characterize the findings as an excess risk. The point estimate is low and the confidence interval overlaps unity by a mile. There is no excess risk here.
5. Page 24: Table 4-1: I can't discern an order for these studies within species. I recommend chronological order within species order.
6. Page 57, section 4.3.1: I disagree that an in vitro study can suggest that TCA might decrease fertilization. Within this paragraph, I recommend that all units be given as molar in addition to the units reported in the original papers. It should be clear that failure to fertilize at a high concentration of any chemical (even salt or glucose) would not be predictive of much of anything in the real world.
7. Page 58, Table 4-7: Kudos to the authors for pointing out the inadequacies of Johnson et al., 1998.
8. Page 59, Table 4-7: For the papers by Singh and Warren, it would be important to indicate parental toxicity if it occurred. Maternal toxicity is indicated for one of the Singh studies (2006) but not the other one. Failure to evaluate maternal or parental toxicity is an important limitation of a study.
9. Pages 60-61: The Smith study is well summarized; however, the heart defects noted by the authors were almost all levocardia. Take a look in the Discussion section of the Smith paper. The authors indicate that levocardia is ill-defined and "of trivial appearance." I suspect that it represents sectioning artifact. This endpoint should be downplayed, particularly in light of the lack of confirmation by Fisher et al. I recommend adding maternal weights to Table 4-8.
10. Pages 61-62: The deficiencies of the Johnson et al. study are described, but the study is not dismissed from further consideration. I recommend a conclusion sentence that the study is unreliable and cannot be used for risk assessment.
11. Page 63, last paragraph, continuing to page 64: The Singh rat poisoning exercise

- used dose levels that were too high and did not indicate parental toxicity. It is unclear if data were analyzed per litter. The fetal brain weight changes should be expressed as relative as well as absolute brain weight change. These studies do not offer a reliable assessment of TCA developmental toxicity.
12. Page 64, last paragraph: I recommend spelling out retinoic acid.
  13. Page 65, first full paragraph: Maternal weight is characterized as reduced, but it was not reduced. The same applies to the lens and globe areas and mean medial canthus and interocular distances. Statistical significance is important, even more so in the face of multiple comparisons.
  14. Page 65, discussion of Collier et al.: Please estimate dose in mg/kg bw/day from the drinking water concentrations. I would suggest, however, that the gene expression study is irrelevant. Alterations in gene expression may be adaptive, and this sort of experiment can be nothing more than a hypothesis-generating exercise. The study has to be mentioned, but recounting the results is a waste of ink.
  15. Pages 65-67: I agree with summarizing the in vitro studies, but I would downplay their utility. All concentrations should be given in molar terms, so please convert the FETAX concentrations. It would also be helpful for context to indicate what plasma concentrations are achievable in humans or in rats under specific exposure conditions so the reader can understand how unreasonable some of the in vitro conditions are.
  16. Page 66, line 26: saying that the Hydra assay “is considered to be useful” is a stretch. Perhaps you meant to say that the assay is considered to be useful by the author of this paper (and 2 or 3 other people on the planet), but I am not sure EPA wants to sound like it is endorsing this test.
  17. Page 89, section 4.6.1.3: To say that TCA is a developmental toxicant makes no sense independent of specifying the exposure conditions. Although it was at one time fashionable to do hazard identification independent of dose-response, the 1991 Risk Assessment Guidelines emphasized the need to interpret hazard in the context of dose. Thus, TCA cannot and should not be characterized as a developmental toxicant without specification of the conditions under which developmental toxicity occurs.
  18. Same paragraph: I object to citing the effects reported by Johnson et al. This study is unreliable. Smith et al. is the only reliable study, and the developmental effects noted in the Smith study occurred only in the presence of excessive maternal toxicity.

19. Page 89, section 4.6.1.3, second paragraph: I recommend dropping the paragraph or reducing the paragraph to a condemnation of Singh for using excessive doses and not reporting parental toxicity.
20. Page 89, section 4.6.1.3, third paragraph: In vitro studies are given too much emphasis. Whole embryo culture is not useful to “assess the potential for developmental toxicity.” The characterization of TCA as inducing a variety of morphologic changes in cultured rodent embryos or in frogs ignores the unrealistic concentrations of TCA used in these experiments, which would probably not be survivable in an intact mammal.
21. Page 120, Table 5-1: For the Smith et al. study, I would include in the comments that the heart effects were not confirmed by Fisher and that the heart effects consisted of levocardia, which is by the study authors’ own admission an ill-defined malformation that was probably a function of the way the Wilson sectioning was performed. Levocardia should not be used for benchmark dose analysis.
22. Page 120, Table 5-1: Delete Johnson et al. from this table. It is not reliable and should not be a candidate study.
23. Page 126, first paragraph: I recommend analyzing the fetal body weight and crown rump lengths using a BMR of 1 control standard deviation. I do not recommend converting these data to quantal form. The levocardia should not be used for benchmark dose analysis.
24. Bottom of page 131: The use of a BMDL<sub>05</sub> for developmental endpoints is not well justified. The greater sensitivity of the studies would argue against using a lower POD. I know it has become conventional to use a BMDL<sub>05</sub> for reproductive-developmental toxicity and a BMDL<sub>10</sub> for other endpoints, but it doesn’t make scientific sense. It would be better to base the decision on empirical data such as have been outlined by some authors in this field or to use a BMR based on a control standard deviation if possible.
25. Page 144, line 16: Please remove from the list of observed effects cardiac malformations, decreased fetal testis weight, decreased fetal ovary weight, apoptosis of gonocytes, and decreased fetal brain weight. The cardiac malformations were not reliable (levocardia, not even reliable according to the study authors) and the other abnormalities were identified in poorly reported studies using inappropriate high exposure levels and no information on parental toxicity.

***Alan H. Stern***

Pg. 7, par. 1 - The rate constants should have units (e.g., hr<sup>-1</sup>).

Pg. 10, par. 3 - The human plasma binding data should be included in this context.

Pg. 13, fig. - In the step for the formation of glyoxilic acid only one of the two oxygens is accounted for.

Pg. 32, par. 4 and ff - This is confusing. The relationship between the Austin (1996) study and the “earlier” Larson and Ball (1992) study is unclear. Furthermore, in apparent contradiction to the text, Larson and Ball administered a range of doses, not a “high single dose,” although this apparently means a one-time administration. Also, it is not clear why the induction of TBARS and 8OHdG are not considered a “standard measure of liver toxicity.”

Pg. 34, par. 3 - Is it possible that TBARS levels were reduced following TCA pre-treatment because pre-treatment induced an anti-oxidant response?

Pg. 38, par. 2 - *“The results of this study indicate that TCA induces liver effects through activation of PPAR $\alpha$ .”* It seems me that this conclusion regarding “liver effects” in general is too broad given the parameters of the Laughter et al. (2004) study. It is not clear from those findings that liver effects such as necrosis and DNA-thymidine uptake are PPAR $\alpha$  mediated.

Pg. 44, par. 2 - The abbreviation “GGT” has not previously been presented and its significance has not been explained.

*“Thus, TCA does not appear to be an initiator based on the results of this assay.”* - The interpretation of this is not clear since the assay has not been explained and is not intuitive.

Pg. 45, par. 4 - As was done with study 2 and 3, the corresponding mg/L dose should be given.

Pg. 51, par. 2 - The meaning of the terminology “increased hepatocyte labeling” is not clear.

Pg. 52, par. 3 - As presented, the data from this study are very difficult to follow. This paragraph would benefit greatly from a table instead of the narrative.

Pg. 53, par. 4 - This is confusing. On the one hand, the yield of tumors remained stable. On the other hand, the yield of hepatocellular carcinomas was increased following the recovery period. Does this imply that the yield of adenomas was decreased?

*“These findings indicate that...”* - Given the above lack of clarity, this statement is difficult to evaluate.

Pg. 55, par. 1 - Since TCA is metabolized to some extent to DCA, the tumorigenic mechanism of DCA is arguably related to that for TCA.

Pg. 60, par. 2 - "...cardiovascular malformations, particularly levocardia..." - My understanding is that levocardia is not a cardiovascular malformation, but an overall visceral malformation that essentially involves all of the viscera except the heart.

Pg. 75, par. 3 and ff. - Presumably, the significance of the mRNA increase in TCA-treated tissue and, in general, in TCA mediated tumors is that the decrease in methylation leads to increased IGFII expression. However, this is not made explicit here.

Pg. 80, par. 3 - On the fourth line, I believe that "TCA" should be "DCA."

Pg. 88, par. 2 - Why isn't DeAngelo et al. (2008) listed here given that liver toxicity (necrosis) in that study is the critical effect for the RfD?

Pg. 93, par. 4 - "Global DNA methylation" should more properly be "hypomethylation."

Pg. 123, sec. 5.1.2 - Given the importance of the interpretation of the benchmark dose modeling, benchmark dose modeling figures (at least those yielding the POD) should be presented in the body of the text rather than the appendix.

Par. 1 - Although this document in its present form may represent the culmination of much earlier drafts, the most current version of the EPA BMD software should be used. I believe that the current version is at least 2.1 as opposed to the version 1.4.1 used in calculations in the document. As stated elsewhere, I have recalculated the BMDLs for the critical effect (hepatic necrosis) using version 2.0 and obtained the same results.

"A BMR of 10% is generally used in the absence of information regarding what level of change is considered biologically significant..." The appropriate basis for selecting a BNMR should be the distribution of the data. The BMR should be close to the lower end of the observed data. In the DeAngelo et al. (2008) study, it appears that a BMR of 5% could reasonably be justified. If there are other reasons for selecting a value of 10% (e.g., minimal nature of the effect), this should be explicitly stated.

Par. 3 - BMDL values should be compared to NOAEL and LOAEL values.

Pg. 124 - The difference in Table 5-2 between the log probit and logistic models' chi-sq of 0.24, AIC of 47.19 (ultimately considered to be the best-fitting models) and the probit model's chi-sq of 0.24, AIC of 74.20 (not considered a best-fitting model) is not meaningful. This also applies to Table 5-4 where the difference between the log logistic model (chi-sq = 0.19, AIC = 76.08) and the gamma/multistate 1<sup>o</sup>/Weibul models (chi-sq = 0.19, AIC = 76.16) is also not meaningful. Minor differences between the fit of non-biologically-based, purely mathematical models should not be overinterpreted since none of these fits necessarily reflect true differences in the underlying dose-response, and perfect fit is not the goal. If it were, the appropriate-degree polynomial could be used to give an exact fit to any data.

Pg. 125, Table 5-3 - The quantal linear model should be added to the first group (gamma, etc.). The values I obtained from the BMD (ver. 2.0) software for that model are identical to those for this group.

Pg. 126, par. 1 - “*Nested developmental toxicity models were employed in order to account for interindividual correlation of toxicity endpoints within litters.*” This needs more explanation, but on its face, it raises the question as to why interindividual differences within litters was even an issue since the general procedure is that the relevant metric is litters rather than fetuses.

Pg. 127 - “*This conversion method assumes that the control group has a 5% background response rate...*” Where does this value come from? The actual background response rate for visceral malformation is 3% and for levocardia is 0%.

Pg. 128, par. 3 - Why is body weight decrease treated as a quantal response? It should be a continuous response as stated on pg. 126.

pg. 135, sec. 5.4 - The Cancer Assessment summary and particularly the text beginning on the third paragraph is an excellent summary of an difficult and complex subject.

pg. 135, par. 4 - Hypomethylation should be mentioned here.

Pg. 139, exposure duration scaling factors - Neither this scaling approach nor any similar approach is referenced in the 2005 Cancer Guidelines. Nor have I encountered it previously. This needs more discussion and justification than a 30 year-old citation.

Pg. 140, par. 2 - There is no clear rationale presented for limiting the BMD modeling to the multistage model. At very least, the rationale should be presented.

Pg. 140, par. 3 - Apparently the slope factors derived from the  $LED_{10}$  and  $ED_{10}$  refer to the  $BMD_{10}$  and the  $BMDL_{10}$ . This shift in terminology is confusing. In addition, however, it is not clear why the former slope is calculated at all since it is not the basis for the calculation of the slope factor.

Pg. 141 - “*...the tumorigenic response of TCA exhibited a linear relationship with increasing doses.*” The shape of the dose-response curve within the observable range (i.e., linear) is not a strong argument for the appropriate extrapolation model since the observable data accounts for approximately 1 order of magnitude while the extrapolation generally accounts for 5 orders of magnitude (assuming that the dose corresponding to  $1 \times 10^{-6}$  risk is the general goal). The observation of linearity does not necessarily imply linearity at much lower doses.

Pg. 145, par. 3 - The third argument presented in support of the characterization of “likely to be carcinogenic to humans” is essentially a negative argument – i.e., that the data do not support *excluding* TCA as a human carcinogen. Logically, this is an argument for a “possible” rather than a “likely” human carcinogen because it says that at most, we

cannot say that TCA is *not* a human carcinogen – i.e., that it is possible that it is a carcinogen.

Pg. 147, par. 4 - As pointed out previously, the shape of the dose-response curve in the observed range provides little indication of the shape of the extrapolated curve 5 orders of magnitude below the observed range.