DRAFT FINAL

REVIEWER COMMENTS

External Peer Review Meeting on the *Toxicological Review of 1,4-Dioxane* (CASRN 123-91-1)

Prepared for:

Eva D. McLanahan, Ph.D.
National Center for Environmental Assessment
U.S. Environmental Protection Agency
109 T.W. Alexander Drive
Research Triangle Park, NC 27711

Prepared by:

Versar, Inc.
Contract No. EP-C-07-025
Task Order 51

Peer Reviewers:

George V. Alexeeff, Ph.D., D.A.B.T.
Bruce C. Allen, M.S.
James V. Bruckner, Ph.D.
Harvey J. Clewell III, Ph.D., D.A.B.T.
Lena Ernstgård, Ph.D.
Frederick J. Kaskel, M.D., Ph.D.
Kannan Krishnan, Ph.D., D.A.B.T.
Raghubir P. Sharma, DVM, Ph.D.

September 9, 2009

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 within EPA's National Center for Environmental Assessment (NCEA) developed a Toxicological Review of 1,4-dioxane. 1,4-Dioxane was nominated for IRIS assessment by the United States Air Force, ARCADIS, and the Celanese Corporation. The draft document slated for the external peer review contains a chronic oral reference dose and an oral cancer slope factor.

Peer Reviewers:

George V. Alexeeff, Ph.D., D.A.B.T.
Office of Environmental Health Hazard Assessment (OEHHA)
California EPA
Oakland, CA 94612

Bruce C. Allen, M.S.
Bruce Allen Consulting
Chapel Hill, NC 27514

James V. Bruckner, Ph.D. ***(Chair)***
Department of Pharmaceutical and Biomedical Sciences
College of Pharmacy
University of Georgia
Athens, GA 30602

Harvey J. Clewell III, Ph.D., D.A.B.T.
Center for Human Health Assessment
The Hamner Institutes for Health Sciences
Research Triangle Park, NC 27703

Lena Ernstgård, Ph.D.
Institute of Environmental Medicine
Karolinska Institutet
Stockholm, Sweden

Frederick J. Kaskel, M.D., Ph.D.
Children's Hospital at Montefiore
Albert Einstein College of Medicine of Yeshiva University
Bronx, New York 10467

Kannan Krishnan, Ph.D., D.A.B.T.
Inter-University Toxicology Research Center (CIRTOX)
Université de Montréal
Montréal, Canada

Raghubir P. Sharma, DVM, Ph.D.
Department of Physiology and Pharmacology
College of Veterinary Medicine *(Retired)*
The University of Georgia
Athens, GA 30602

II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of 1,4-dioxane 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). There is a current assessment on the IRIS database for the health effects associated with 1,4-dioxane exposure which was first available in 1988.

The draft health assessment includes a chronic Reference Dose (RfD) and a carcinogenicity assessment. An inhalation Reference Concentration (RfC) and inhalation unit risk (IUR) were not derived in this assessment. EPA will evaluate the recently published 1,4-dioxane inhalation data for the potential to derive an RfC and IUR in a separate document to follow this assessment. Below are a set of charge questions that address scientific issues in the current assessment of 1,4-dioxane. Please provide detailed explanations for responses to the charge questions.

(A) General Charge Questions:

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

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of 1,4-dioxane.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of 1,4-dioxane.

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

Chemical-Specific Charge Questions:

(B) Oral reference dose (RfD) for 1,4-dioxane

1. A chronic RfD for 1,4-dioxane has been derived from a 2-year drinking water study (Kociba et al., 1974) in rats and mice. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has the selection of this study been transparently and objectively described in the document? Are the criteria and rationale for this selection transparently and objectively described in the document?

Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Degenerative liver and kidney effects were selected as the critical effect. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed explanation. Please comment on whether EPA's rationale regarding adversity of the critical effect for the RfD has been adequately and transparently described and is scientifically supported by the available data. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. Kociba et al. (1974) derived a NOAEL based upon the observation of degenerative liver and kidney effects and these data were utilized to derive the point of departure (POD) for the RfD. Please provide comments with regard to whether the NOAEL approach is the best approach for determining the POD. Has the approach been appropriately conducted and objectively and transparently described? Please identify and provide rationales for any alternative approaches for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

4. EPA evaluated the PBPK and empirical models available to describe kinetics following inhalation of 1,4-dioxane (Reitz et al., 1990; Young et al., 1978, 1977). EPA concluded that the use of existing, revised, and recalibrated PBPK models for 1,4-dioxane were not superior to default approaches for the dose-extrapolation between species. Please comment on whether EPA's rationale regarding the decision to not utilize existing or revised PBPK models has been adequately and transparently described and is supported by the available data. Please identify and provide the rationale for any alternative approaches that should be considered or preferred to the approach presented in the toxicological review.

5. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factors:

- An interspecies uncertainty factor of 10 was used to account for uncertainties in extrapolating from laboratory animals to humans because a PBPK model to support interspecies extrapolation was not suitable.

- An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the RfD because the available information on the variability in human response to 1,4-dioxane is considered insufficient to move away from the default uncertainty factor of 10.

- A database uncertainty factor of 3 was used to account for lack of adequate reproductive toxicity data for 1,4-dioxane, and in particular absence of a multigeneration

reproductive toxicity study. Has the rationale for the selection of these uncertainty factors been transparently and objectively described in the document? Please comment on whether the application of these uncertainty factors has been scientifically justified.

(C) Carcinogenicity of 1,4-dioxane

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that 1,4-dioxane is *likely to be carcinogenic to humans*. Please comment on the cancer weight of evidence characterization. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described? Do the available data for both liver tumors in rats and mice and nasal, mammary, and peritoneal tumors in rats support the conclusion that 1,4-dioxane is a likely human carcinogen?

2. Evidence indicating the mode of action of carcinogenicity of 1,4-dioxane was considered. Several hypothesized MOAs were evaluated within the Toxicological Review and EPA reached the conclusion that a MOA(s) could not be supported for any tumor types observed in animal models. Please comment on whether the weight of the scientific evidence supports this conclusion. Please comment on whether the rationale for this conclusion has been transparently and objectively described. Please comment on data available for 1,4-dioxane that may provide significant biological support for a MOA beyond what has been described in the Toxicological Review. Considerations should include the scientific support regarding the plausibility for the hypothesized MOA(s), and the characterization of uncertainty regarding the MOA(s).

3. A two-year drinking water cancer bioassay (JBRC, 1998a) was selected as the principal study for the development of an oral slope factor (OSF). Please comment on the appropriateness of the selection of the principal study. Has the rationale for this choice been transparently and objectively described?

4. Combined liver tumors (adenomas and carcinomas) in female Cjr:BDF_1 mice from the JBRC (1998a) study were chosen as the most sensitive species and gender for the derivation of the final OSF. Please comment on the appropriateness of the selections of species and gender. Please comment on whether the rationale for these selections is scientifically justified. Has the rationale for these choices been transparently and objectively described?

5. Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described? Regarding liver cancer, a linear low-dose extrapolation approach was utilized to derive the OSF. Please provide detailed comments on whether this approach to dose-response assessment is scientifically sound, appropriately conducted, and objectively and transparently described in the document. Please identify and provide the rationale for any alternative approaches for the determination of the OSF and discuss whether such approaches are preferred to EPA's approach.

III. GENERAL IMPRESSIONS

George V. Alexeeff

My overall impression is that the document is sound, well reasoned and concise. As indicated in the document, exposure to 1, 4-dioxane has resulted in multiple tumor types in multiple species, including liver adenomas and carcinomas, nasal carcinomas, mammary adenomas and fibroadenomas, and mesothiolomos of the peritoneal cavity. This document lays out the new evidence reported since the previous EPA assessment which shows that the carcinogenic potency is much greater than previously thought, that nasal tumors as well as liver tumors have been reproduced in several studies, and that additional tumor sites of the mammary gland and peritoneal cavity must be considered for 1,4-dioxane. The available mechanistic data were laid out well. The document has conducted one of the more thorough and comprehensive evaluations of mode of action for a compound. The extensive evaluation of the mode of action accurately and transparently establishes that "the weight of evidence is inadequate to establish a MOA by which 1, 4-dioxane induces peritoneal, mammary, or nasal tumors in rats and liver tumors in rats and mice." The analysis of the 1, 4-dioxane in this report follows the EPA 2005 Guidelines for Carcinogen Risk Assessment on all major scientific and policy issues. The document considers and weighs all of the available science. The use of Hill criteria in evaluating carcinogenic endpoints is strength in the analysis. The carcinogenic assessment is based on the appropriate statistical model of liver tumors in female mice and the linear extrapolation approach is clearly supported based on sound science, public health protection and the EPA 2005 Guidelines for Carcinogen Risk Assessment. The use of ¾ power of body weight scaling was appropriate since the use of pharmacokinetic models at this time do not adequately fit the data.

The synthesis of major noncancer effects was well done. The uncertainty factors used were appropriate and justified. Conclusions drawn and not drawn from the data were well described and clearly stated. The diagrams accompanying the document were helpful. The uncertainty analysis was straightforward. Finally, the appendices for more specific information were helpful.

Bruce C. Allen

The Toxicological Review of 1,4-dioxane has provided a nice summary of the data available for deriving an oral reference dose (RfD) and an oral cancer slope factor (CSF). There are, however several issues associated with the derivation of the numerical values of the RfD and the CSF that need to be addressed and corrected. Primary among them are:

- the use of the Kociba (1974) study to obtain a point of departure (POD) for the RfD;
- a flawed model selection procedure for the CSF derivation.

Moreover, several issues related to mode of action, pharmacokinetic data/modeling results, and, particularly, uncertainty analysis/presentation need to be addressed.

James V. Bruckner

This draft of the *Toxicological Review of 1,4-Dioxane* (1,4-D) is the best of its type that I have reviewed. The format and organization of the document make it easy to read and find desired information. The accounts of the major studies' designs and findings are quite comprehensive and appear to be accurate and complete. The text is very well written. Paragraphs usually begin with introductory sentences that tell what is to be discussed. I normally make quite a lot of editorial changes. In this case I have not made any, although I was tempted to add commas in a number of places where they were needed. The tables are simple, easy to understand and have clear footnotes.

Harvey J. Clewell III

My overall impression of this document is very positive. It is well written, clear and transparent. It appears to be a very accurate, open-minded and balanced analysis of the literature evidence regarding dioxane's dosimetry, toxicity, and mode of action. I particularly like the excellent use of the MOA schematics (Figures 4-1 and 4-2), the key event temporal sequence/dose-response table (Table 4-18), and the POD plots (Figures 5-1 to 5-4). They really helped to pull the assessment together and make it easier to follow the decision logic. In general, I found the conclusions presented in the document to be sound and reasonable. However, there were a few places (sections 3.2, 3.3, and 4.5.2.2) where I could not evaluate the authors' conclusions because no information was provided on the concentrations/doses used in the studies being discussed.

Sadly, I have to agree with the conclusion that the existing PBPK models are inadequate to perform route-to-route or cross-species dosimetry for either the cancer or noncancer dose-response assessments. However, I felt that the discussion in the appendix (sections B4 and B5) on the evaluation of the PBPK model was hard to follow. Nevertheless, I do not think that further attempts to improve the PBPK model are warranted unless they include an experimental component (as in the work of Sweeney et al. 2008). I think the main document should include a more complete description of the model refinement effort performed by Sweeney et al. 2008, but having looked over that publication, it does not give me reason to believe that a PBPK model for dioxane can be used in current risk assessments without performing additional laboratory experiments to improve the understanding of dioxane kinetics in the human. In particular, there is a need for additional kinetic data from exposures of human subjects to dioxane to confirm the data reported by Young et al. (1977).

I agree with the conclusions regarding the inability to use Benchmark dose analysis for the noncancer endpoints. On the other hand, I don't understand why an animal to human uncertainty factor of 10 was used instead of using BW^3/4 scaling for kinetics and a factor of three for dynamics. I thought that BW^3/4 scaling had recently been adopted by EPA for noncancer cross-species dosimetry in the derivation of RfDs.

I agree with the conclusion that the MOA for dioxane carcinogenicity is likely to be nonlinear, but that there is inadequate evidence to support a specific MOA hypothesis with any confidence, so that a default linear low-dose extrapolation approach is

(unfortunately) necessary. However, I was surprised to see the apparent acceptance of a mode of action involving direct effects of dioxane rather than a metabolite. I don't think the available evidence is sufficient to rule out a toxic metabolite that is produced disproportionately at higher concentrations where high affinity, low capacity cyps like 2e1 are saturated, but other low affinity, high capacity cyps like 3a4 are not. My bet would be on the dialdehyde. Obviously, there is a need for experimental support of this suggestion, but it seems plausible and mentioning it in the assessment might drive additional research that could benefit future assessments. Until the question of the appropriate dose metric for liver toxicity and carcinogenicity (concentration of dioxane in the liver, concentration of a metabolite in the liver, or production of a reactive metabolite in the liver) can be resolved by additional experimental studies it will not be possible to depart from default approaches for cross-species dosimetry and it will not be possible to use a PBPK model to conduct route-to-route extrapolation in the rat.

I agree that there is currently inadequate data to support an inhalation risk assessment. I am glad to hear that the EPA plans to evaluate the recently reported inhalation studies by Kasai and colleagues. Hopefully, these new data might serve as the basis for an RfC and inhalation potency factor. Importantly, plasma dioxane concentrations were also reported in the 90-day study, providing an opportunity to evaluate the relationship between the plasma concentrations and the observed preneoplastic lesions and toxicity in the liver.

Lena Ernstgård

The draft of the "Toxicological Review of 1,4-Dioxane"document is well written and has a good organization. The available data about dioxane is presented clearly. The table of contents, list of tables, list of figures, and list of abbreviations and acronyms are well done and help the reader. The selection of a key study, as well as the quantitative approaches is clearly is presented. The methods used for deriving the reference dose and cancer slope factor are described clearly and with transparency. The uncertainty in data is extensively discussed. Several uncertainty factors are used to derive an RfD due to limited data. The use of uncertainty factors is described transparently and discussed. The major conclusions in the characterizations of hazard and dose response are well described. The tables and the figures are appropriate, well formed, well referred to in the text and a good supplement for the reader. The appendices for more specific information are helpful, although it was a little hard to follow. The evaluation of the existing PBPK models showed that the use of the models, in their present form, are not useable to perform route-to-route or cross-species extrapolations for either the cancer or non-cancer dose-response assessments. More experimental data are needed.

Frederick J. Kaskel

This Integrated Risk Information System (IRIS) document is an extensive current review of the hazard and dose-response assessment of 1,4-dioxane including oral reference dose (RfD) and inhalation reference concentration (RfD) values for chronic and subchronic exposure durations, and a carcinogenicity assessment. As such, it provides quantitative information for use in risk assessments for health effects produced through a nonlinear mode of action. The RfD is an estimate of a daily exposure in humans that is

considerable not a significant risk over a lifetime. The inhalation RFC is analogous to the oral RfD but provides an continuous inhalation exposure estimate involving both respiratory and extrarespiratory exposures. Reference values are derived for chronic (lifetime), acute (<24 hrs), short-term (>24 hrs through 30 days), and subchronic (>30 days through 10% of a lifetime) exposures.

The carcinogenicity assessment provides quantitative estimates on the carcinogenic hazard risk of exposures from oral and inhalation routes. A weight-of-evidence judgment for the agent is provided in terms of risk as a carcinogen using a low-dose extrapolation procedure that estimates an upper bound on the estimates of risk per mg/kg-day of oral and ug/m^3 air breathed, respectively.

Kannan Krishnan

This document summarizes the current state of knowledge regarding the toxicokinetics, toxicity, carcinogenicity and mode of action of 1,4-dioxane as it relates to the dose-response assessment of cancer and non-cancer endpoints. The information developed in this process is presented along with the sources of uncertainty. The selection of a critical (key) study, as well as the quantitative approaches (including the application of uncertainty factors), are clearly presented. The methods used for deriving the reference dose and cancer slope factor are described in a systematic and transparent manner. Overall, the document is clear, concise and comprehensible.

However, this reviewer feels that the level of supporting information provided in the document, regarding the following aspects, is inadequate:

- Is the mouse strain Cjr:BDF1 previously used for assessing human risks?

- Why was the high dose to low dose extrapolation (re: cancer) not performed on the basis of pharmacokinetic data, given the existing information/concern on the non-linearity?

- Was it feasible at all to conduct BMD analyses (and high dose to low dose extrapolation) on the basis of internal dose, for non-cancer effects?

Clarification to these questions would help determine the level of scientific soundness of the assessment as presented.

Raghubir P. Sharma

The document reviewed is a well-prepared draft of the Toxicological Review of 1,4-dioxane. The review draft is clear, complete and concise, and follows the general format for *Toxicological Reviews* released by the EPA. The introduction is a general template for such reviews followed by adequate chemical and physical information on this compound. This section also includes production volumes, general uses or sources of exposure, and environmental movement of 1,4-dioxane. The next chapter on

toxicokinetics is brief but adequate and consists of all of the available information on this topic. The following chapter is titled as "Hazard Identification," however, it includes all toxicological information. This chapter summarizes relevant studies on acute, subchronic and chronic information on 1,4-dioxane, including both non-cancer and cancer effects of this chemical. The text includes both the description of available studies as well as an analytical critique of these as to their adequacy for deriving the necessary toxicity parameters for this document. The next section is on dose-response assessment and derives the oral RfD and slope factor for the chemical. The uncertainty factors are fairly well described and so are the reasons as to why inhalation values were not derived. The rationale for selected studies, the derivation and uncertainties for RfD and BMD are adequate. The HED are derived in a rational manner. Estimates for oral RfD are adequate and the rationale for its derivation is well described; however the estimates for cancer slope factor may be reexamined (as indicated later in this review). The final chapter is a summary of all the information presented in previous sections. References are listed following this concluding section and are followed by appendices. The appendices are clear with graphic or tabular presentation wherever possible. The presentation of the information is clear, accurate, and the conclusions are sound.

IV. RESPONSE TO CHARGE QUESTIONS

(A) General Charge Questions:

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

George V. Alexeeff

In general, the Toxicological Review was logical, clear and concise. The Introduction provides a useful overview of the IRIS program and identifies the key guidelines that are followed in preparation of the document. The chemical and physical information was concise and contained most of the needed relevant information. The toxicokinetics section was laid out nicely with the key information. The cancer and noncancer hazards have been objectively discussed and synthesized.

The document transparently describes that 1, 4-dioxane produced tumors at multiple sites in multiple animal species, and in multiple animals strains. The analysis clearly describes the evaluation of potential modes of action (MOA) for carcinogenicity and why an MOA could not be identified for any of the tumor sites. The weight of evidence clearly supports this conclusion. Adverse effects, primarily of liver and kidney toxicity are presented clearly. NOAELs and LOAELs are clearly identified for key studies. The discussion of uncertainty synthesized the available evidence and the uncertainty factors used in the noncancer assessment are appropriate and well-justified.

Bruce C. Allen

The review is logical and, for the most part, clear with respect to the presentation of the background data. It is difficult to be concise given the format/structure of such reviews – there is a great deal of repetition when the same data sets are considered for different purposes in different sections of the document.

It appears that the summaries are accurate reflections of the observations from the source studies. The results of those studies appear to be objectively presented; the synthesis of the results across studies appears to be fair and balanced, although the lack of a stronger conclusion or statement about MOA may down-play the evidence that hyperplasia (with or without cytotoxicity) is responsible for the occurrence of tumors after 1,4-dioxane exposure.

James V. Bruckner

As noted elsewhere, the document is clear, presented in a logical sequence, and as concise as the required EPA format (with its redundancy) allows.

Harvey J. Clewell III

My overall impression of this document is very positive. It is well written, clear and transparent. It appears to be a very accurate, open-minded and balanced analysis of the literature evidence regarding dioxane's dosimetry, toxicity, and mode of action. I particularly like the excellent use of the MOA schematics (Figures 4-1 and 4-2), the key event temporal sequence/dose-response table (Table 4-18), and the POD plots (Figures 5-1 to 5-4). They really helped to pull the assessment together and make it easier to follow the decision logic. In general, I found the conclusions presented in the document to be sound and reasonable. However, there were a few places (sections 3.2, 3.3, and 4.5.2.2) where I could not evaluate the authors' conclusions because no information was provided on the concentrations/doses used in the studies being discussed.

Lena Ernstgård

The toxicological review is logical, clear and concise. The predominant noncancer effect of chronic exposure to 1,4-dioxane found is degenerative effects in the liver and kidney. The scientific evidence for these effects is clearly and objectively presented. The effects in liver and kidney are well supported by several studies. I also believe that the cancer hazard is evaluated sufficiently. The scientific evidence is clearly and objectively presented.

Frederick J. Kaskel

Yes; the review is presented in a concise yet extensive presentation that is clear, accurate and objective. The scientific evidence reviewing the potential noncancer and cancer hazards of the 1,4-dioxane are well-reviewed and focused.

Kannan Krishnan

Yes. The document is clear and concise. The Agency has clearly represented and synthesized the scientific evidence for cancer and non-cancer effects. However, the objectivity and transparency might be improved regarding the (i) description of mode of action and how that feeds into the choice of the extrapolation model for cancer endpoint, as well as (ii) the presentation of the outcome based on the consideration of internal dose (high dose to low dose extrapolation in rodents) in cancer and non-cancer assessments.

Raghubir P. Sharma

Yes, the review is clear, concise, accurate and objectively presented. Both noncancer and cancer hazards are discussed appropriately. The scientific evidence has been properly synthesized. None of the calculations were verified as they have been derived using specialized software.

(A) General Charge Questions:

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of 1,4-dioxane.

George V. Alexeeff

The following studies are suggested for consideration in the assessment:

California Department of Health Services (1989) Risk Specific Intake Levels for the Proposition 65 Carcinogen 1, 4-dioxane. Reproductive and Cancer Hazard Assessment Section. Office of Environmental Health Hazard Assessment.

In this report the Hoch-Ligeti et al. study is fully analyzed taking into account that the study clearly states that five groups of 30 rats were used in the analysis. This results in an incidence rate of 0/30, 1/30, 1/30, 2/30, and 2/30. A Mantel-Haenszell trend test conducted for significance resulted in a highly significant result of $p<0.001$.

National Research Council (2008) Science and Decisions: Advancing Risk Assessment Committee on Improving Risk Analysis Approaches Used by the U.S. EPA. Washington, D.C., National Academy Press.

Bruce C. Allen

I know of no other studies.

James V. Bruckner

ATSDR (2007) Toxicological Profile for 1,4-dioxane. Agency for Toxic Substances and Disease Registry. Atlanta, GA.

Kasai T; Saito H; Senoh Y; et al. (2008) Thirteen-week inhalation toxicity of 1,4-dioxane in rats. Inhal Toxicol 20: 961-971.

Kasai T; Kano Y; Umeda T; et al. (2009) Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal Toxicol 20: in press.

Stickney JA; Sager SL; Clarkson JR; et al. (2003) An updated evaluation of the carcinogenic potential of 1,4-dioxane. Regul Toxicol Pharmacol 38: 183-195.

Yamamoto S; Ohsawa M; Nishizawa T; et al. (2000) Long-term toxicology study of 1,4-dioxane in R344 rats by multiple-route exposure (drinking water and inhalation). J Toxicol Sci 25: 347.

Harvey J. Clewell III

None that I'm aware of, other than the recently published inhalation studies of Kasai et al. Hopefully, these might serve as the basis for an RfC and inhalation potency estimate. Importantly, plasma dioxane concentrations were also reported in the 90-day study, providing an opportunity to evaluate the relationship between the plasma concentrations and the observed preneoplastic lesions and toxicity in the liver.

Lena Ernstgård

There were only a few inhalation studies available for the review; so an additional study, Kasai et al. (2008), could be included. It is a subchronic (13 week) inhalation study.. (Ref: Kasai T, Saito M, Senoh H, Umeda Y, Aiso S, Ohbayashi H, Nishizawa T, Nagano K, Fukushima S. Thirteen-week inhalation toxicity of 1,4-dioxane in rats. Inhal Toxicol. 2008 Aug;20(10):961-71).

Frederick J. Kaskel

Noncancer effects:

- Further investigations are needed in order to characterize the mechanisms responsible for the acute and chronic nephrotoxicity.
- Is the acute kidney injury (AKI) multifactorial?

- Are there both tubular and glomerular/vascular toxicities that result in cortical tubule degeneration and evidence for glomerulonephrities?

- What are the functional correlates of the histologic changes in terms of assessment of renal function?

- Exposure in utero and risk to the fetus and newborn. Only one 1985 experimental study examined effect on fetal body weight and decrease in ossification; effect on kidney development and nephrogenesis has not been examined.

- Concentrations in breast milk following exposure.

- Risk for use of drinking water to constitute infant formula.

- Exposures during early development.

- Application of newer biomarkers for risk of renal injury; i.e., NGAL and KIM.

- Pharmacokinetics and metabolism of 1,4-dioxane in development.

- Risk with decreased renal function or chronic renal disease.

- Risk of storage in fat cells and obesity.

- Gender differences and effects of aging on susceptibility to injury.

- Studies on renal metabolism free radical generation, gene expression, AKI to supplement data on CYP450 induction.

Kannan Krishnan

I am not aware of any other oral bioassays for this chemical. The newer inhalation study (Kasai et al.) might provide qualitative support to the overall toxicological database on dioxane, but not provide a unique data source for deriving RfD.

Raghubir P. Sharma

No relevant additional studies after oral administration were identified from an on-line literature search during 2008-2009 that would be appropriate for this review. The search was not performed for the prior period, as the document indicates (Page 2, line 16) that it had been conducted. The studies presented have been carefully selected, and objectively and analytically discussed. An additional reference on inhalation exposure of this chemical was located as recently published. The copy of this article by Kasai et. al., 2009, regarding a 13-week inhalation study, is now available and was provided by EPA to the reviewers.

(A) General Charge Questions:

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of 1,4-dioxane.

George V. Alexeeff

The following are suggestions for future research:

- Research that would likely increase the confidence in the database regarding carcinogenicity would be an evaluation of potential epigenetic mechanisms of carcinogenicity.

- Increased information on sources of exposure and biological concentrations would be helpful. Currently, concerns are being raised for drinking water contaminated with 1,4-dioxane, however, it appears that there are significant exposures from consumer products. More information along these lines would be helpful to better understand the level of concern of the compound and background exposures.

- Only very limited information is available on human toxicokinetics parameters; additional studies should be undertaken with a varied population of subjects to identify the range and variation in the response parameters. The current database reflects four individuals exposed by inhalation without any external breath being analyzed.

- A study using metabolic inhibitors could be used to examine if increases in toxicity are due to the parent compound.

Bruce C. Allen

Given EPA's inability to make a stronger conclusion about the MOA of 1,4-dioxane, it appears that research directed at that determination would increase confidence in future assessments. This, of course, depends on the extent to which resolving the issue of whether hyperplasia is or is not associated with cytotoxicity would have any impact on the quantitative approaches ultimately used for the RfD or cancer slope factor derivations.

In fact, until and unless EPA provides researchers with fixed decision criteria for making MOA determinations, there may be little benefit from further research. That is the case because no one (perhaps including EPA personnel) knows what pieces of evidence will change any of the decisions that have been made in this assessment. I am not saying that EPA needs to come up with a generic set of decision criteria, but a useful function of the detailed toxicological review of 1,4-dioxane should be to identify not only data gaps but the specific results for 1,4-dioxane that would change the decisions that have been made for that specific compound. As an example, the Toxicological Review says repeatedly that inconsistencies between results from JBRC (1998) and Kociba et al. (1974) affect

decisions regarding MOA as it relates to cytotoxicity. Given the entire set of observations and analyses that have been reviewed at this point, EPA should be able to tell researchers exactly what result would allow them to make a conclusion. For example, what would it take for EPA to conclude that one of those studies is just wrong or its observations are immaterial (or out-weighed, or outliers, or whatever other term is appropriate) to the MOA determination. Then, experiments can be designed (perhaps in consultation with EPA to ensure that proposed procedures are consistent with the expressed data need) to produce a result that, depending on its outcome, will tip the balance one way on the other.

James V. Bruckner

There are several serious deficiencies in the 1,4-D database. There is a paucity of information on the toxicokinetics (TK) and metabolism of the chemical in common test animals and humans. The only absorption, disposition and elimination data appear to be for ^{14}C following administration of the radiolabeled compound. ^{14}C levels, of course, do not distinguish among the parent compound, its (major) metabolites, CO_2 and carbon incorporated into macromolecules. It would be worthwhile to administer a series of doses of 1,4-D to male and female mice and rats. Time-course of the parent compound in blood and target tissues in each sex and species should be obtained for comparison with one another and with any human data. The dose-dependency of urinary excretion of β-hydroxyethoxy acetic acid (HEAA) should also be established. It would be important to also monitor the time-course of putative cytotoxic metabolites in target tissues, and, if possible, to correlate their concentration(s) with manifestations of adverse effects *in situ*. Such an *in vivo* approach may provide information about the identity of toxic moieties, be they parent compound or metabolite(s). *In vitro* experiments should also be conducted, in which different concentrations of the compounds are incubated with representative cell types. Such *in vitro* and *in vivo* experiments should provide much-needed information on the mode of action (MOA). Detailed *in vivo* experiments could be carried out in which the onset, progression and regression of inflammation, necrosis or apoptosis, regeneration, etc. are carefully monitored in the liver, kidney, nasal tissue, etc. to verify whether 1,4-D acts via a proliferative regeneration mechanism. A "stop" experiment, in which 1,4-D is stopped after cell death and hyperplasia are fully manifest, could provide information in support of a cytotoxicity MOA, if no tumors developed. Molecular studies could focus on potential effects of 1,4-D/metabolites on protooncogenes, tumor suppressor genes, and overall gene expression.

Harvey J. Clewell III

I don't think the available evidence is sufficient to rule out a toxic metabolite that is produced disproportionately at higher concentrations where high affinity, low capacity cyps like 2e1 are saturated but other low affinity, high capacity cyps like 3a4 are not. My bet would be on the dialdehyde. Obviously, there is a need for experimental support of this suggestion, but it seems plausible and mentioning it in the assessment might drive additional research that could benefit future assessments.

Studies to improve the human PBPK model are needed that include an experimental component (as in the work of Sweeney et al. 2008). In particular, there is a need for additional kinetic data from exposures of human subjects to dioxane to confirm the data reported by Young et al. (1977).

Lena Ernstgård

For future assessments of 1,4-dioxane, more research in uptake and disposition in humans is desired. There is only one study (Young et al. 1977) designed for this purpose, and it has limitations. The uptake could not be calculated since no measurements in exhaled air were performed. The study is performed in only four subjects. Further, a new toxicokinetic study will probably improve a PBPK model for dioxane A multigeneration reproductive toxicity study could also be appropriate.

Frederick J. Kaskel

Additional investigations examining some of the suggestions listed above [in response to Question A.2].

Kannan Krishnan

Cancer risk assessment:

- Focused mode of action (e.g., cell proliferation) studies are needed with relevance to the doses used in bioassays to inform on the type of dose-response model to be used.

Non-cancer risk assessment:

- A multigen study is needed to address or reduce the database uncertainty factor.

Additionally, conducting the following analyses with the existing datasets may also enhance the confidence in the assessment:

- Combined analysis of the multiple datasets and outcomes for cancer and non-cancer endpoints, where appropriate;

- Evaluation of dose metrics relevant to the mode of action, to improve confidence in the extrapolation approach and uncertainty factors used;

- Bayesian analysis of human PK data and estimation of the human variability in the key determinants (metabolism rates, tissue partition coefficients).

Raghubir P. Sharma

There are a limited number of studies that involve 1,4-dioxane for subchronic or chronic exposures. Some of these studies are fairly dated (e.g., Fairley et al., 1934); however, the

conclusions drawn from these are appropriate. The study by Kociba et al. (1974) has been chosen for the derivation of the oral RfD; the findings of this study have been strengthened by later reports such as NCI, 1978, and JBRC, 1998. It is unlikely that further studies would be any more helpful in suggesting an adequate NOAEL for rodents. However, very limited studies following inhalation exposure to this compound have been conducted (hence no RfC calculated). Inhalation is perhaps the most appropriate mode of exposure in humans; therefore additional inhalation studies would be helpful. Similarly there is little information on reproductive/developmental effects of 1,4-dioxane (limited to one report only). The possible exposure of newborn via milk is not understood. Indeed exposure levels in individuals possibly exposed to this chemical, including body burden, have not been characterized. Identification of susceptible populations including factors that may make an individual more susceptible will be helpful in estimating the safe exposure levels to 1,4-dioxane. Additional studies on mode of action, pharmacokinetics in humans, and characterization of human exposure, will be helpful in establishing risk levels for this compound.

(A) General Charge Questions:

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

George V. Alexeeff

With regards to the oral reference dose, Section 5 reviews the range of animal toxicology data. Unfortunately, an adequate inhalation study is lacking and the key oral studies were conducted in the 1970's utilizing relatively insensitive indicators and in some cases incomplete reporting. Choice of the Kociba et al. (1974) study NOAEL as the POD appears to be a strong estimate of the true NOAEL for the study. The ratio of the LOAEL to NOAEL is close to 10 which is what would be expected for the severe endpoint of hepatic and renal degeneration and necrosis. Utilization of at least a 10-fold interspecies uncertainty factor is necessary since methods to reduce uncertainty, such as PBPK modeling, are not supported or internally consistent. The use of a full intraspecies uncertainty factor is also necessary since a full identification, understanding and consideration of sensitive subpopulations is not available. Finally, the absence of key studies, such as adequate chronic inhalation studies and a multigeneration reproductive toxicity study, much less specialized developmental toxicity studies, represents another important source of uncertainty.

Regarding the discussion of cancer risk assessment uncertainty, the document thoroughly discusses the uncertainty in the model selection, scaling, and dose metric. The document also describes the range in responses based on species or gender selection. Finally the assessment also brings to light the uncertainty in human metabolism and human relevance.

In general, these have been adequately discussed. It would appear to be important to identify the data gaps regarding protecting infants and children. There is an absence of a complete developmental and reproductive toxicity information to understand the pre- and post-natal effects. There is also a lack of exposure information via consumer products and breast milk. Finally, the absence of a clear mode of action raises additional uncertainty regarding the increased susceptibility of infants and children to the carcinogenic effects of dioxane. However, the choices and assumptions made in the discussion of uncertainty have been described transparently and objectively. The description of the impact of the uncertainty on the assessment is transparent and objective. Finally these uncertainty issues are summarized in the Section 6, the conclusion, in a succinct manner.

Bruce C. Allen

As discussed below, I believe there are several serious potential problems with using the Kociba et al. (1974) study as the basis of the RfD derivation. Those problems could, and should, have been mentioned in the uncertainty section for the RfD (Section 5.3), but they were not.

There is a great deal of uncertainty associated with choosing an older study (Kociba et al. 1974) that did not meet some very minimal reporting requirements (no incidence data were reported) and, apparently, for which those data could not be retrieved. The designation of NOAEL and LOAEL status to the dose groups from that study is therefore based entirely on the evaluations of the authors, with no recourse to confirmation. Furthermore, the many known problems with the NOAEL/LOAEL approach to POD determination should be mentioned as major sources of uncertainty; the shortcomings associated with the NOAEL/LOAEL approach lead to a great deal of inconsistency with respect to the levels of response that may be observed at NOAELs and LOAELs.

The review statements about comparative derivations (p. 99, first two paragraphs) are inadequate. There is one statement (line 8) about a POD that could have been derived from the NCI (1978) study; it appears to dismiss that POD because it is "higher" than that derived from the Kociba et al. (1974) study. That does not suffice as an uncertainty assessment. There are many reasons why the two PODs might differ, and why the "higher" POD might in fact be the better value. Those reasons include the strong possibility that the POD from the Kociba et al. (1974) study is associated with a very small risk of response (e.g., much less than the typical EPA default choice of 10% risk). That possibility arises because in the Kociba et al. (1974) study, there were 60 animals/sex/dose (a relatively large number) and there was about a 10-fold gap between the LOAEL and the NOAEL. The larger sample size would tend to make the LOAEL determination correspond to a smaller risk (compared to a study with smaller group sample sizes). The 10-fold gap would make the NOAEL tend to be associated with a substantially lower risk than the LOAEL.

The statement (lines 11-12 on p. 99) about confidence in the LOAEL is totally irrelevant. It is not the LOAEL that is the basis for the POD determination, but rather the NOAEL. The fact that the LOAEL is at or near the same level as doses that elicited responses in other studies does not tell us anything. What were those levels and what were the dose-response model-predicted response rates for the dose corresponding to the LOAEL? More importantly, what did those model-predictions say about the NOAEL and possible associated responses? That kind of information would be more relevant to assessing the uncertainty associated with the use of this one particular study.

The remainder of Section 5.3 is a general restatement of the fact that there are 3 types of uncertainty factors for modifying the POD: animal-to-human, human-variability, and database-completeness. There is nothing in these paragraphs that relates to the important uncertainties specifically associated with this particular assessment.

Section 5.5 discusses the uncertainties associated with the cancer risk estimates. This entire section appears to be little more than a generic rehash of the major themes that affect all cancer risk assessments. There is no exploration of the impacts of the choices that have been made (i.e., there is no statement that doing X instead of Y would have changed the risk estimates by such-and-such amount).

I was particularly disappointed not to see more discussion of what would happen if the POD/UF approach would have been used. I believe the entire document overly downplays the information that supports a non-linear low-dose behavior. I think the uncertainty section should be modified to acknowledge that behavior as the most-likely, high-impact alternative to the linear extrapolation and to provide some quantitative reflections on how that would affect the cancer assessment.

It also appears that the discussion about uncertainty related to the dose metric is severely lacking. Despite the fact that EPA chose not to use PBPK modeling (or any kind of PK adjustments) in their dose-response analysis, there is much that could have been done with those models or observations to inform the uncertainty assessment. The statements (p. 108, lines 1-3) about not knowing which moiety is responsible for toxicity appears to be inconsistent with the tenor of earlier observations (that it is unlikely to be a reactive intermediate, that the saturation of metabolism tends to favor the parent compound as the effective dose, etc.) and, frankly, to be a bit disingenuous. Not having an irrefutable, concrete understanding is not the same as having no information at all. It appears that some uncertainty assessment, largely quantitative in nature, could, in fact, be completed to ascertain the effect of making a *reasonable* alternative assumption about the dose metric.

I believe it is incorrect to imply that the multistage model, as used for cancer risk assessments (and included in EPA's BMDS software), is "motivated" by the MVK model (p. 107, lines 8-13). The later includes factors (e.g., cell proliferation of untransformed or initiated cell populations) that are not features of the multistage model.

A final issue relates to the use of the 10% BMR as the basis for the CSF derivation. I would prefer that the assessment (and all other assessments that derive CSFs) look at the impact of that choice on the CSF value. If the CSF is intended to characterize the slope of the dose-response at *low* doses, then reliance only on the 10% level may be insufficient. The linearity (slope) that is derived from a starting point of 10% extra risk is not necessarily the same as the slope one would obtain if one examined lower risk levels as the starting point, and in fact the CSF based on 10% risk might overestimate the low dose slope. A procedure can be implemented whereby BMDLs (and the associated CSFs) could be derived for lower and lower risks until the CSF started to increase (representing the increased uncertainty in the slope because of going beyond the low end of the data range). It is the minimum CSF derived in that manner that should be considered, and at least compared to the CSF derived using 10% risk, to assess one source of uncertainty.

I strongly disagree with the statement (p. 113, lines 1-2) that confidence in the selected study for the RfD is "medium." I have much less confidence in the numerical values that come from that study, as discussed elsewhere. As it stands, until and unless an alternative study is chosen or a much fuller uncertainty analysis is done that allays concerns about those numbers, I would conclude that confidence in the RfD derivation should be low.

The remainder of the uncertainty discussion in Section 6 (subsections 6.2.3.1 through 6.2.3.8) simply reiterates what is in Section 5.5. The same issues are present here as were discussed in the preceding remarks.

In conclusion, I would say that the key sources of uncertainty have not been adequately discussed; the choices and assumptions have not been transparently or objectively described; nor have the impacts of the uncertainties be included at all.

James V. Bruckner

Uncertainty has been adequately identified and characterized in most of the document. Uncertainties about the critical (JBRC, 1998) carcinogenicity bioassay need to be addressed.

Harvey J. Clewell III

I think the authors have done an excellent job of identifying and characterizing sources of uncertainty. The choices and assumptions in the assessment, and their impact on the risk assessment, are, for the most part, transparently and objectively described.

One important area of uncertainty that has not been given sufficient attention is the possibility that the toxicity and tumorigenicity of dioxane is due to a metabolite rather than the parent chemical. This is particularly apparent in the discussion of susceptible populations and life stages (section 4.8). If a metabolite is the active moiety, then the susceptible individuals would be those with the highest production of that metabolite, which could be a complex function of relative cyp isoform activities (e.g., 2e1 vs. 3a4).

Lena Ernstgård

The uncertainty in data is extensively and adequately discussed in the document. I think that the assumptions made in the discussion and the impact of the uncertainty are transparently and well described.

Frederick J. Kaskel

The liver and kidney toxicity observations for oral or inhalation exposure are not recent with the exception of the JBRC 1998b report which did not show evidence of kidney injury as compared to other models. The key sources of uncertainty in the review of the current experimental and clinical data bases have been identified and are transparent.

Even the Kociba 1974 study has limitations in that there were no data on incidence of liver and kidney toxicities. The absence of adequate quantitative data is repeatedly stated and indicates the need for additional investigations.

The review emphasizes that the extrapolation methods were unable to adequately establish a MOA by which 1,4-dioxane induces tumors in various organs in mice and rats. Furthermore, the multistage model in the Benchmark Dose Software did not provide an adequate fit to the data for the incidence of hepatocellular adenoma or carcinoma in female mice. Thus, extrapolation of study data to estimate potential risks to humans is fraught with uncertainties. Only two studies were available for analyses to assess the risk between occupational exposure and increased risk for cancer and these were small sample sizes.

Kannan Krishnan

The identification and characterization of sources of uncertainty, in themselves, are useful for any assessment. However, it would be relevant to more closely relate them to their sensitivity and variability. In other words, the key question of concern is: are highly uncertain parameters (inputs) of the assessment also the most sensitive or most variable ones?

Raghubir P. Sharma

Sources of uncertainty for the oral RfD have been identified as (1) factors involved in the extrapolation of animal data to human effects, (2) heterogeneity in exposed populations, and (3) deficiencies in information regarding reproductive toxicity. These sources of uncertainty are traditionally employed and have been adequately addressed. The reference data used in the determination of the oral RfD have been based on the study that provided the lowest dose exposure for the NOAEL (Kociba et al., 1974) and is an appropriate available study. The UF of 10 was used for uncertainty for extrapolating animal data to humans as PBPK models to predict human exposures were not satisfactory. This is an acceptable UF. An additional UF of 10 was used to account for the individual variability and to further provide safety to sensitive individuals. The UF of 3 for database deficiency, particularly lack of information on multi-generation reproductive effects. These key sources of uncertainty have been well elaborated in the document. The choices and assumptions made in the discussion of uncertainty have been clearly and objectively described. The impact of the uncertainty on the assessment is also clear and objective. For the estimation of the cancer slope factor, a variety of uncertainty factors have been enumerated and discussed in Chapter 5. All these uncertainty factors, including the choice of low-dose extrapolation, dose metric involving effect of metabolism, cross-species scaling, statistical uncertainty, the selection of bioassay, choice of species and gender, relevance to humans, and human population variability, have been clearly and objectively presented.

Chemical-Specific Charge Questions:

(B) Oral reference dose (RfD) for 1,4-dioxane

1. A chronic RfD for 1,4-dioxane has been derived from a 2-year drinking water study (Kociba et al., 1974) in rats and mice. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has the selection of this study been transparently and objectively described in the document? Are the criteria and rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

George V. Alexeeff

Kociba et al. (1974) reported effects in male and female rats dosed at three levels, 10-fold apart, plus a control. There were many animals used in the study, 60 per sex per dose group. In addition to weights and clinical observations, the studies collected blood samples, organ weights of all major tissues, and a histopathological examination of all rats. There was increased mortality and carcinogenicity in the high dose group. While the Kociba et al. (1974) study does provide a NOAEL and a LOAEL, summarized in Table 4-17, they did not provide quantitative incidence or severity data for liver and kidney degeneration and necrosis. The reason for choosing the Kociba study as the principal study is because the renal tubular epithelial and hepatocellular degeneration and necrosis reported in the study were clearly adverse, and of all the studies, the Kociba study identified the most sensitive effects with a NOAEL at 77-81 mg/kg-day and a LOAEL at 323-398 mg/kg-day. Further the IRIS 1,4-dioxane report indicates that the noncancer effects identified do not appear to be directly related the carcinogenic effects. In contrast, kidney effects were noted at higher doses in the NCI study (240 mg/kg-day) and the Argus study (430 mg/kg-day). The JBRC study did report liver hyperplasia at comparable levels (NOAEL=16mg/kg-day), but it is possible that the effects are preneoplastic in nature. The choice of the Kociba study as the principal study was scientifically justified and the rationale for the selection was transparent and objectively described. I do not believe another study should be chosen as the principal study for the oral RFD derivation.

Bruce C. Allen

I believe the selection of the Kociba et al. (1974) study was not the best choice. It is an older study that has, to me, the fatal flaw that it does not report the incidences of the endpoints from which the RfD has been derived.

The rationale provided is that the endpoints from the study are adverse and that they represent the most sensitive effects identified. I would argue that only having the NOAEL and LOAEL as the basis for identifying the most sensitive endpoints is an unsatisfactory basis for doing so. One has no idea what the rates of response were at the NOAEL and LOAEL doses and so it is far from clear that the endpoints from that study are indeed the most sensitive.

Moreover, it seems inappropriate to base the selection of the *study* on the sensitivity of the endpoints. Study selection should consider the design, the relevance of the exposures, and the relevance of the animal model(s). And, it should consider the adequacy of the reporting of the results, with respect to which the Kociba et al. (1974) is woefully inadequate.

Thus, I conclude that the objectivity, transparency and rationale for this selection are severely lacking. Not only is the sensitivity of the endpoints irrelevant (or less relevant) to the identification of studies that can and should be used in the risk assessment, it is also the case here that the information needed to assess relative endpoint sensitivity is not available for 1,4-dioxane. Additional comments related to this point are provided in response to the next question.

I believe the NCI (1978) or the JBRC (1998) studies would be much better choices for the principal study.

James V. Bruckner

Adequate scientific justification has been given for selection of the chronic investigation of Kociba et al. (1974). Administration of the test chemical in drinking water is the oral exposure route/regimen most relevant to humans. There were similar findings in three other chronic drinking water studies, albeit at somewhat higher doses. As described in the text, the 2-year study by Kociba et al. (1974) was well designed, in that both sexes of two rodent species consumed 3 dosage-levels (+ controls) of 1,4-D. Body weight gains and water consumption were monitored, so that ingested doses could be estimated. Hematological indices, organ/body weights and histopathological changes were carefully assessed in most organs.

Harvey J. Clewell III

I believe that the use of the Kociba study was scientifically justified and feel that the selection was transparently and objectively described.

Lena Ernstgård

According to EPA, the study was chosen as the principal study because it provides the most sensitive measure of adverse effects by 1,4-dioxane. I agree, the study seems to be a well-conducted, chronic drinking water study with an adequate number of animals. The selection of this study has been transparently and objectively described in the document. I can not identify any other study that is better to be selected as the principal study.

Frederick J. Kaskel

The study is appropriate but lacks insight into potential risks for liver and kidney toxicities. The review emphasizes the limitations in this rather old and solitary investigation. The critique clearly indicates the need for additional studies using newer

models and biomarkers of injury. One can only speculate that the use of genetically engineered models would provide new and useful information into the kidney/liver toxicities.

Kannan Krishnan

The critical study is described well and the selection is justified. However, it is unclear as to why the analysis of all relevant datasets on the basis of internal dose (or human-equivalent dose) was not attempted.

Raghubir P. Sharma

The selection of this study to derive the chronic RfD for 1,4-dioxane (Kociba et a., 1974) has provided the lowest NOAEL for this chemical of all available studies. This study was well conducted and effects have been clearly described. The selection of this study has been clearly and objectively described. The criteria and rationale for this selection have been adequately discussed. No other study was found suitable for the derivation of this parameter.

(B) Oral reference dose (RfD) for 1,4-dioxane

2. Degenerative liver and kidney effects were selected as the critical effect. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed explanation. Please comment on whether EPA's rationale regarding adversity of the critical effect for the RfD has been adequately and transparently described and is scientifically supported by the available data. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

George V. Alexeeff

The choice of liver and kidney effects as the critical effects is justified since they are the primary non cancer effects in animals and humans that are associated with exposure to 1,4-dioxane . Human occupational exposures have resulted in hemorrhagic nephritis and centrilobular necrosis, while animal studies have identified renal degeneration and liver damage as endpoints. Table 4-17 identifies two subchronic oral studies. Each of these two studies identified liver damage as an endpoint, and one of them identified renal toxicity as well. Two subchronic inhalation studies were also listed in the table, and one of them reported hepatocyte swelling as an effect. Six chronic studies were also summarized in Table 4-17. Each of the six studies reported either hepatic or renal toxicity. Thus, based on available subchronic and chronic studies, liver and kidney appear to be target organs of 1,4-dioxane toxicity. These are straight-forward, classic adverse effects. Tissue degeneration and necrosis are considered adverse effects of fairly great severity and are common endpoints used in risk assessments. The other major effect of interest reported in the subchronic and chronic inhalation studies is nasal epithelial inflammation. While this may be an important effect, it has not been reported as often, and the NOAEL for the effect is just slightly higher than the one based on Kociba. Thus, there appears to be no reason to choose the nasal tissue effects over the kidney and liver effects.

Bruce C. Allen

I have stated above that I view the selection of these endpoints to be without merit. That determination was based on the fact that the study from which they were selected was deficient in the reporting of the results. There are no data to allow confirmation of the claims that the 9.6 and 94 mg/kg/day doses from that study were indeed the NOAEL and LOAEL, respectively. Moreover, because the claimed sensitivity of those endpoints is based on a comparison of NOAELs and LOAELs, the claim that they are the most sensitive is essentially unsupported. It is well known that NOAELs and LOAELs are severely affected by experimental design constraints such as number of doses, dose spacing, and the actual values of the doses. Relative sensitivity of endpoints should be judged based on their dose-response patterns, not on the very design-limited LOAEL evaluations. In this particular case, the 10-fold gap between the NOAEL and the LOAEL, in conjunction with the fact that BMDLs for endpoints from other studies fall in that gap (see Figures 5-1 and 5-2, for example), are indicative of the fact that there is no

"resolution" for making this determination about what is or is not the most sensitive endpoint.

Moreover, I do not understand the rationale that EPA has added on that other endpoints (from NCI, 1978 or JBRC, 1998) appear to be related to the carcinogenic process and thus should not be considered. What precludes basing an RfD on endpoints that may be related to the later development of cancer? If those endpoints are considered adverse in and of themselves, then they deserve consideration. If they are only "biomarkers" for later cancer development, then modeling them, and deriving an RfD that is protective against them should have the benefit of protecting against carcinogenicity. In fact, the optimal procedure for such endpoints would appear to be to use them in a harmonized or integrated cancer/noncancer assessment so that safe levels (RfDs) for such biomarkers are derived in such a way that they are protective for all potential (noncancer and cancer) adverse effects.

Based on the data presented in the review, I would select the most sensitive endpoint(s) from the NCI (1978) or JBRC (1998) studies as the basis for the RfD. There is mention of significant changes in relative liver weight in rats from the JBRC (1998) study (p. 43, lines 6-8), but no data on such changes (in liver or in any other organ) are presented. In fact, there are no continuous endpoints presented at all, which strikes me as somewhat odd. It is sometimes difficult to evaluate the dose-response trend and to appreciate what the predictions (of BMDs, for example) will be for continuous endpoints, so as a general rule, it may be preferable to model them and to see explicitly how sensitive (relative to other continuous and/or dichotomous endpoints) they might be.

James V. Bruckner

The document's authors have given a very comprehensive justification for selection of liver and kidney injury as the critical effects, including an accurate summary of adverse hepatorenal effects and corresponding doses described by different researchers. The effects in both mice and rats were quite similar from one study to another. There appeared to be a common sequence of events in the liver, beginning with centrilobular hepatocellular hypertrophy, vacuolation and proliferation of the smooth endoplasmic reticulum, progressing to hepatocellular necrosis and regenerative hyperplasia. Renal proximal tubule cells exhibited nuclear enlargement, degeneration, necrosis with hemorrhage and glomerulonephritis. It is obvious in the document that liver and kidney damage were consistently seen in published studies. Considerable detail about doses and other features of the nephrotoxicity experimental designs are presented in several different sections.

Harvey J. Clewell III

I believe that the identification of the liver and kidney effects as the critical effects was scientifically justified and feel that the selection was transparently and objectively described.

Lena Ernstgård

Several oral studies (acute, subchronic, and chronic) support liver and kidney toxicity as the critical effects. The criteria and rationale for the selection of the critical effect have been transparently and objectively described in the document.

Frederick J. Kaskel

The liver and kidney organ specific targets of 1,4-dioxane are logical in that they represent both the metabolic and excretory organs of note, and have the highest likelihood of sustaining injury. However, with regards to nephrotoxicity, the models and limited human data have barely addressed the mechanisms of injury and the clinical correlates to the histologic data. There are no measurements of renal function nor have any of the newer molecular identification of biomarkers of renal injury been applied to the models or the human studies. Advances in the field of biomarkers have not started to be used for the study of this agent.

Kannan Krishnan

The critical effect(s) appears to be justified appropriately. The selection is transparent and supported by available data. However, if all datasets are analyzed (e.g., BMD modeling) following dose adjustment (based on either on body surface scaling or PBPK-based dose metrics), it would represent a better rationale for selection of a sensitive critical effect/dataset.

Raghubir P. Sharma

The rationale for the selection of critical effects, those on liver and kidney, has been scientifically justified. The criteria and rationale for this selection have been transparently and objectively described in the document. As indicated above, this study provided the least NOAEL value and was carefully carried out to determine the dose-effects of 1,4-dioxane in male and female Sherman rats. The duration of exposure was proper for deriving an oral RfD and so was the route of administration (oral). Both non-cancer and cancer effects were determined. The study has been properly described in the document, along with all other available studies and the rationale regarding adversity of the critical effect for the RfD, and is scientifically supported by the available data. The endpoints employed were the most sensitive ones and no other endpoints are suitable.

(B) Oral reference dose (RfD) for 1,4-dioxane

3. Kociba et al. (1974) derived a NOAEL based upon the observation of degenerative liver and kidney effects and these data were utilized to derive the point of departure (POD) for the RfD. Please provide comments with regard to whether the NOAEL approach is the best approach for determining the POD. Has the approach been appropriately conducted and objectively and transparently described? Please identify and provide rationales for any alternative approaches for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

George V. Alexeeff

While a benchmark dose approach may be preferred in most instances, in this case, choosing the NOAEL approach with the Kociba data is adequate, sufficient, and possibly superior to other approaches. One reason for using a benchmark approach is to take into account the number of animals per dose group, so that small studies are not treated equivalently to large studies. In this case the Kociba study is a large study, so the NOAEL and LOAEL are about as well defined as possible. A related concern is the NOAEL does not necessarily represent a no response level since effects could be occurring at a low incidence rate. With 60 animals per dose group, the absence of the critical effect in the low dose group, indicates that the incidence of the liver or kidney effects were likely to be below 2% (1/60). This incidence rate is below the default 10% incidence rate POD used with the benchmark dose. Further, the 10-fold difference between the LOAEL and NOAEL for the relatively severe critical effect is consistent with standard risk assessment practices. That is, if there was no NOAEL, a 10-fold factor would be applied to the LOAEL. In this case, absent specific incidence data, there is no standard alternative to using the NOAEL-LOAEL approach for the point of departure. This is consistent with EPA approaches.

Bruce C. Allen

First of all, the premise of these questions is wrong. Kociba et al. (1974) did claim that the NOAEL and LOAEL for those endpoints were 9.6 and 94 mg/kg/day, respectively. But these reported claims are not *data*, so it is not true that any *data* were used to derive the POD.

I have addressed my concerns about using the Kociba et al. (1974) study and the endpoints from it as the basis for the POD derivation. A BMD approach should be used on data that have been adequately reported and for which consistent, dose-response-related estimates can be derived. There are studies and endpoints for which this can be done, as described above.

James V. Bruckner

Kociba and co-workers' NOAELs of 94 mg/kg/day for nephrotoxicity and 9.6 mg/kg/day for hepatotoxicity were clearly delineated. The latter, lower value, is the correct choice

for a POD. BMD modeling cannot be used, as dose-response/incidence data were not provided by Kociba et al. (1974).

Harvey J. Clewell III

I agree with the use of the NOAEL approach, as described in the document.

Lena Ernstgård

I have no other suggestion than the NOAEL approach for determination of POD. The approach is well described.

Frederick J. Kaskel

Considering the limitation in the field, the use of NOAEL approach for determining the POD is appropriate. The interpretation of the data is objective and clear. Alternative approaches for the determination of the POD would involve prenatal exposure in pregnant animals and effects on the offspring and the mother in experimental models as well as analyses of outcome in breastfed offspring exposed contaminated breast milk.

Kannan Krishnan

The approach is appropriately conducted (NOAEL/UF) and objectively and transparently described. However, it is unclear as to whether any attempt was made to semi-qualitatively represent the histopathological observations to facilitate a quantitative analysis.

For additional comments, please see response to Question B.2, above.

Raghubir P. Sharma

As yet, the NOAEL appears to be the best parameter to estimate the RfD or POD. Based on several doses used in this study, the NOAEL of 9.6 mg/kg-day was established for female liver and kidney effects (the NOAEL for male rats was an order of magnitude higher than this level). Same lesions have been reported in other studies; however, the NOAEL values were relatively higher (JBRC, 1998a; Argus et al., 1973; NCI, 1978). The approach has been appropriately conducted and objectively and transparently described. No alternative approaches for the determination of the POD for this chemical could be identified from the studies reported here.

(B) Oral reference dose (RfD) for 1,4-dioxane

4. EPA evaluated the PBPK and empirical models available to describe kinetics following inhalation of 1,4-dioxane (Reitz et al., 1990; Young et al., 1978, 1977). EPA concluded that the use of existing, revised, and recalibrated PBPK models for 1,4-dioxane were not superior to default approaches for the dose-extrapolation between species. Please comment on whether EPA's rationale regarding the decision to not utilize existing or revised PBPK models has been adequately and transparently described and is supported by the available data. Please identify and provide the rationale for any alternative approaches that should be considered or preferred to the approach presented in the toxicological review.

George V. Alexeeff

The document evaluated the potential application of the Leung and Paustenbach (1990), Reitz et al., (1990) and Young et al, (1977, 1978) models. It is important to note that the existence of an alternative model does not imply it is valid or appropriate to use. The document made an effort to determine if the existing PBPK models are scientifically superior to the default approach and could be used in the assessment. The document reviewed the utility of PBPK models for predicting internal dosimetry and for cross–species extrapolation of exposure response relationships. EPA concluded that use of the existing models were not superior to default approaches for dose-extrapolation. The EPA provided adequate and transparent rationale for these conclusions. For 1,4-dioxane, the primary metabolite in all species appears to be HEAA. This metabolism appears to be CYP450-mediated. Rat metabolism appears to be a saturable and an inducible process. Yet, it is apparent that 1,4-dioxane toxicity can occur at concentrations below saturation of metabolic processes. Animal data derived from Young et al. (1978a, b), Woo et al., (1977 a,b) and Mikheev et al. (1990) is available for modeling. Studies by Young et al. (1977, 1978a, b) provide the primary human data needed for modeling. The IRIS document evaluated the ability of the PBPK models to predict observations made in experimental studies of rat and human exposures to 1,4-dioxane. Only code for the Reitz et al. (1990) model is available.

The document describes a sensitivity analysis that was performed to determine the model parameter having the greatest influence on the blood level of 1,4-dioxane. The recalibrated model predictions for blood 1,4-dioxane did not come within 10-fold of the experimental values measured. Only when unrealistic tissue perfusion rates are used can an adequate fit be achieved for first order kinetics. Sweeney (2008) also attempted to adjust the model to improve the fit and found that it fit only one of two human studies. Increasing the ventilation rates does not appear to be appropriate either. Since the subjects were in a chamber is resting or sitting ventilation rate would be appropriate which would be 7 to 9 L/min (Marty et al. 2002 [Hum Ecol Risk Assessment, 8:1723-1737.], OEHHA, 2000 [Air Toxics "Hot Spots" Program Risk Assessment Guidelines Part IV Exposure Assessment and Stochastic Analysis Technical Support Document at http://www.oehha.ca.gov/air/hot_spots/finalStoc.html#download]).

Since an adequate fit could not be obtained for the model it is appropriate to not use the model. The reasoning for this is well-described in the document and supplementary data are provided in the appendix. Clearly, it is not prudent to use the PBPK model since it cannot predict even the available limited data. Its application to a risk assessment is too uncertain and would reduce the quality of the assessment. One of the difficulties of using a PBPK model is the transparency. Rarely do the models provide adequate code, nor do they clearly explain the parameters that most importantly influence the results. The document attempted to address these issues in an open manner, and identified key errors in the models. Further, since there are three noncancer endpoints of concern, liver pathology, kidney degeneration and nasal epithelium inflammation, and four cancer endpoints of concern, the current models have not provided an adequate evaluation of all these tissues. Finally, the human study is based on the results of four individuals, while the animal studies often had 3 individuals per dose group. It would be important to repeat the human studies and expand the animal studies prior to using the data in model development. At this point the PBPK models are too uncertain, appear to be inaccurate, and do not provide an understanding of the tumorgenicity and toxicity of 1,4-dioxane in animal species or humans.

Bruce C. Allen

I understand EPA's reluctance to use a model for which even the very limited human observations cannot be matched by any reasonable choice of model parameters. Thus, I concur with their use of the default dose metric options (mg/kg/day).

On the other hand, I am curious if some of the known (and apparently well-modeled) effects of saturable metabolism in experimental species might have been able to be explored for the dose-response modeling. At the very least, some consideration of the impact of such considerations should appear in the uncertainty sections. As it is, the discussion of uncertainty related to dose metrics makes it sound as if there was absolutely no clue what metric might be responsible for the effects observed or how kinetics might affect that metric. That appears to be a significant under-representation of what is known and what has been successfully captured by the PBPK modeling that has been done.

Thus, for example, if the parent compound is suspected of being the moiety responsible for the endpoints observed, and if some of the experimental doses might be subject to saturation of metabolism, then would it be possible to adjust the mg/kg/day doses in light of that knowledge. This need not necessarily be done to the same extent on the human side, since it may be safe to assume that for the human (lower-dose) exposures of interest, there is no saturation and there would be a linear relationship between this adjusted metric and mg/kg/day. Such an analysis would not be suitable as the primary RfD or CSF assessment, but if presented as part of the uncertainty evaluation, it could be quite useful.

James V. Bruckner

It is clear that competent PBPK modelers from EPA and its contractor (Syracuse Research Co.) went to great lengths to recalibrate and to try to implement existing PBPK

models for species-to-species extrapolation. It is obvious in Figures B-8 and B-9 that blood 1,4-D time-courses and cumulative urinary excretion of HEAA could not be accurately simulated, even when a biologically-implausible model parameter was inputed. It is worthy of note that Sweeney et al. (2008) could accurately predict only one of two human datasets with their recently-published PBPK model for 1,4-D. Additional human kinetics (i.e., blood parent compound and metabolite) time-course data are seriously needed, as there is some question about the quality of the few human data currently available.

Harvey J. Clewell III

I agree with the conclusion that the existing PBPK models are inadequate to perform route-to-route or cross-species dosimetry for either the cancer or noncancer dose-response assessments. However, I felt that the discussion in the appendix (sections B4 and B5) on the evaluation of the PBPK model was hard to follow. Nevertheless, I do not think that further attempts to improve the PBPK model are warranted unless they include an experimental component (as in the work of Sweeney et al. 2008). I think the main document should include a more complete description of the model refinement effort performed by Sweeney et al. 2008, but having looked over that publication, it does not give me reason to believe that a PBPK model for dioxane can be used in current risk assessments without performing additional laboratory experiments to improve the understanding of dioxane kinetics and mode of action. Until the question of the appropriate dose metric for liver toxicity and carcinogenicity (concentration of dioxane in the liver, concentration of a metabolite in the liver, or production of a reactive metabolite in the liver) can be resolved by additional experimental studies, it will not be possible to depart from default approaches for cross-species dosimetry and it will not be possible to use a PBPK model to conduct route-to-route extrapolation in the rat.

Lena Ernstgård

The PBPK model is well described in Appendix B. The decision to not utilize existing or revised PBPK models is adequately and transparently described, and is supported by the available data. I have no suggestion of an alternative approach.

Frederick J. Kaskel

The EPA's rationale regarding the decision to not utilize existing or revised PBPK models has clearly been described and is appropriate.

Kannan Krishnan

The effort expended to reconstruct and/or recalibrate the PBPK models is described adequately. In the revised model, two aspects need to be verified:

- The constant KL is defined as a first order rate and scaled on the basis of $BW^{0.7}$. It is inconsistent with the current literature. A first order constant multiplied with concentration will not give mg/hr, as calculated in the equation on page B-30, lines

23, 28, 29. However, if this parameter is considered as a clearance constant with units of L/hr, then the scaling rule used, as well as the interpretations provided, would be acceptable.

- It is unclear as to why AM is calculated on the basis of RAM and not RMEX. In fact, RMEX seems to represent the amount metabolized per unit time, which is accounted for in line 23 of page B-30.

The decision not to utilize the models for high dose to low dose extrapolation and interspecies extrapolation is not entirely satisfactory. It might be preferable to rank order the level of confidence in the model, as a function of dose measures that can be simulated with the model (e.g., amount absorbed, steady-state blood concentration, amount metabolized) of relevance to MOA.

The decision not to rely on the PBPK model based on its inability to fit to the human blood PK data is questionable. The reported human and rat PK data (for 50 ppm, 6 hr exposures) actually look much alike (See figures B5 and B6). In this regard, the adjustments made to the model by EPA and others have involved reduction of volume of distribution and increase in breathing rate. Only physiologically-irrealistic values would appear to allow the model fit to human PK data. A visual evaluation by this reviewer indicates that the rat model simulations might actually fit to the human blood PK data (uptake part of the curve, elimination phase as well as steady-state levels), raising concerns about the excessive reliance on these blood PK data in humans, to evaluate the reliability of the PBPK model for dioxane.

The urinary metabolite data associated with the two human studies might be sufficient in this case.

Since the animal data, including the non-linear kinetics, are fairly well reproduced by the PBPK model, the rationale for not using it in the conduct of high dose to low dose extrapolation is questionable. Feasibility of using an oral absorption constant to adapt the models to simulate internal dose associated with the bioassay doses may be investigated.

In this regard, it is unclear the extent to which the Agency evaluated or recalibrated the Sweeney et al. (2008) model.

Raghubir P. Sharma

The EPA's rationale for not using the PBPK approach to describe kinetics following inhalation is appropriate as the models did not agree with experimentally observed values. This was true for predicted blood concentrations (which were consistently lower) and also the urinary metabolite concentrations. Therefore, the use of existing, revised, and recalibrated PBPK models for 1,4-dioxane was not superior to default approaches for the dose-extrapolation between species. No alternative approaches that should be considered are apparent based on the available information.

(B) Oral reference dose (RfD) for 1,4-dioxane

5. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factors:

- ***An interspecies uncertainty factor of 10 was used to account for uncertainties in extrapolating from laboratory animals to humans because a PBPK model to support interspecies extrapolation was not suitable.***

- ***An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the RfD because the available information on the variability in human response to 1,4-dioxane is considered insufficient to move away from the default uncertainty factor of 10.***

- ***A database uncertainty factor of 3 was used to account for lack of adequate reproductive toxicity data for 1,4-dioxane, and in particular absence of a multigeneration reproductive toxicity study. Has the rationale for the selection of these uncertainty factors been transparently and objectively described in the document? Please comment on whether the application of these uncertainty factors has been scientifically justified.***

George V. Alexeeff

An uncertainty factor of 10 for interspecies extrapolation is fully supportable. This is the default value to account for differences in pharmacokinetic and pharmacodynamic issues. PBPK models could not be derived to address pharmacokinetic issues and possibly reduce the uncertainty factor. For humans, data on only four individuals are available limiting quantitative interspecies extrapolation. There is no clear information demonstrating that humans are less sensitive than animals for the effects of concern. The uncertainty factor of 10 for interindividual variability is the standard default values and is fully supportable. There is limited information on the variability of susceptibility in the human population. There is one human study on pharmacokinetics and the individual data are not available, only summary data were reported. There are three target organs of concern, liver, kidney and lung. Interindividual variability can be considerable in each of these organs. Finally a factor of 3 is added for database deficiencies. This factor is standard for USEPA risk assessment and is also fully justified due to the absence of sufficient studies on the developmental and reproductive toxicity of 1,4-dioxane. This is particularly justified in this case since the little evidence available suggests a greater potential sensitivity during fetal development. Other factors were not necessary based on the study chosen as the principal study. Thus, the selection of uncertainty factors was standard based on the quality of the data available. There use was clearly stated and transparent.

Bruce C. Allen

The uncertainty factors chosen for the RfD appear to be the standard default choices that accompany an assessment that does not use pharmacokinetic dose adjustments and for which no toxicodynamic considerations are applied. In so far as that is the case, I have no alternatives to suggest.

James V. Bruckner

- A full interspecies factor of 10 is justified. There are apparently no data from toxicokinetic (TK) studies that allow comparison of absorption, disposition, metabolism or elimination of 1,4-D, HEAA or other metabolites in different test species or in humans. There are limited human data, but not a valid PBPK model that will allow interspecies extrapolations or accurate forecasts of TK in humans.

- It is unclear whether the parent compound, HEAA, or another metabolite is (are) responsible for nasal irritation/proliferation, liver damage, or kidney degeneration. Since the identity of the cytotoxic moiety or moieties is unknown, it is not possible to postulate what factors, or characteristics of different populations may enhance their susceptibility. Thus, adoption of the default intraspecies uncertainty factor of 10 is necessary.

- Selection of a database uncertainty factor of 3 is dictated by the lack of a multigeneration reproduction study. This fact is clearly described in the document.

Harvey J. Clewell III

I don't understand why an animal to human uncertainty factor of 10 was used instead of using BW^3/4 scaling for kinetics and a factor of three for dynamics. I thought that BW^3/4 scaling had recently been adopted by EPA for noncancer cross-species dosimetry in the derivation of RfDs. It is certainly preferable to use BW^3/4 scaling for kinetics, rather than a factor of 10^1/2.

I agree with the intraspecies and database uncertainty factors and feel that they are scientifically justified.

Lena Ernstgård

The uncertainty factor of 10 for intra-species differences is appropriate since the PBPK model did not support intra-species extrapolation. An uncertainty factor of 10 for human variability seems to be reasonable since there is limited information on the degree to which humans vary in the disposition of or response to 1,4-dioxane. Also, the default uncertainty factor of 3 selected due to lack of a multigeneration reproductive toxicity study seems to be appropriate. The selection of these uncertainty factors have been transparently and objectively described in the document.

Frederick J. Kaskel

This factor is appropriate due to the large variation in the data. It is extremely difficult to include all of the factors that may apply to the variation in inter- and intraspecies outcomes of exposures to 1,4-dioxane by various routes, durations and doses, and this has been addressed by critical analyses.

Kannan Krishnan

- For the interspecies extrapolation, the implementation of a steady-state calculation, using PBPK model parameters, should be given consideration. In other words, the steady-state concentration would equal dose divided by clearance. Was there any attempt to conduct steady-state calculations (and not necessarily using the full-blown PBPK models), based on parameters from PBPK models? Of interest is that, at steady-state, the volume of distribution (or the tissue:blood partition coefficient) is not a sensitive parameter of the blood concentration. The use of a factor of 10 (that partially is justified on the basis of body surface scaling) might be a good approximation of internal dose, particularly if the pulmonary clearance in both rats and humans is negligible compared to hepatic clearance (which appears to be the case for dioxane), and if the hepatic clearance scales to body surface.

- The use of a factor of 10 for inter-individual differences appears to be justified appropriately and supported by available data on PK. Supporting information for the PK component might be drawn from Sweeney et al. (re: metabolism rate in humans).

- Clear articulation of the science policy for the use of a factor of 3 as the database factor is suggested.

Raghubir P. Sharma

A factor of 10 to account for uncertainties in extrapolating from laboratory animals to humans is appropriate because a PBPK model to support interspecies extrapolation was not suitable. An intraspecies (human variability) uncertainty factor of 10 is also appropriate to derive the RfD because the available information on the variability in human response to 1,4-dioxane is insufficient to move away from the default uncertainty factor of 10. An additional uncertainty factor of 3 to account for lack of adequate reproductive toxicity data for 1,4-dioxane, and in particular absence of a multigeneration reproductive toxicity study, is also appropriate. The rationale for the selection of these uncertainty factors has been transparently and objectively described, and has been scientifically justified in the document.

(C) Carcinogenicity of 1,4-dioxane

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgr-d.htm), the Agency concluded that 1,4-dioxane is likely to be carcinogenic to humans. Please comment on the cancer weight of evidence characterization. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described? Do the available data for both liver tumors in rats and mice and nasal, mammary, and peritoneal tumors in rats support the conclusion that 1,4-dioxane is a likely human carcinogen?

George V. Alexeeff

The EPA 2005 Guidelines for Carcinogen Risk Assessment calls for a single integrative step, after assessing all of the individual lines of evidence, to weigh all of the evidence in reaching conclusions about the human carcinogenic potential of a chemical. The evidence to be considered includes tumors found in humans and laboratory animals; an agent's chemical and physical properties; its structure-activity relationships (SARs) as compared with other carcinogenic agents; and studies addressing potential carcinogenic processes and mode(s) of action, either *in vivo* or *in vitro*.

The information considered in the evaluation is clearly laid out in the document. In weighing the evidence, the document (page 77) indicates 1,4-dioxane produced tumors at multiple sites in multiple animal species, and in multiple animals strains. Specifically, the document described evidence of liver carcinogenicity in 2-year bioassays conducted in three rat strains, two mouse strains and in guinea pigs. The document explained that both adenomas and carcinomas have been described in rats and mice. Further the document identified nasal tumors in three rat strains, in additional to mesothiolomas of the peritoneum, and mammary tumors. In contrast, information on human studies and mode of action were found to be inconclusive.

The chosen descriptor, ***"Likely to Be Carcinogenic to Humans,"*** is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic 'potential' to humans. As stated on page 2-55 of the EPA Cancer Guidelines, the phrase "***Likely to Be Carcinogenic to Humans"*** is appropriate when an agent has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans. In this case, 1,4-dioxane has been shown to cause tumors in eight studies as is summarized in Table 4-18. Clearly the potential to cause cancer in humans is there. The data available for both liver tumors in rats and mice and nasal, mammary, and peritoneal tumors in rats support the conclusion that 1,4-dioxane is a likely human carcinogen. There have been inadequate human studies to identify tumors in humans from 1,4-dioxane exposure.

The available human evidence (Thiess et al., (1976), Buffler et al. (1978)) is not informative and is not inconsistent with the potency estimate. The highest potency estimate in the draft assessment is 0.19 per mg/kg-day. The Thiess et al., (1976) study on page 24 states the study evaluated 74 workers. They were employed in dioxane production from 5 to 41 years. Air concentrations were estimated to range from 0.06 to

7.2 ppm, or 0.2 to 26 mg/m3. Assuming 10 m3/day of air and 70 kg worker, an intermediate value for exposure of 2 mg/m3 for 23 years (instead of full 70 year lifetime), yields a ballpark exposure 0.09 mg/kg-day (2 x 10/70 x 23/70). Multiplying the exposure times the potency yields an estimated cancer risk of 0.017 (0.19 x 0.09) or 1.1 per hundred. Since there were only 74 people in the cohort you would not expect a case of cancer to result from the dioxane exposure. Applying a similar estimate to the Buffler et al. (1978) study yields an air concentration of 90 mg/m3 (25 ppm) for 5 years. This yields a ballpark exposure 0.26 mg/kg-day (25 x 10/70 x 5/70). Multiplying the exposure times the potency yields an estimated cancer risk of 0.05 (0.19 x 0.26) or 5 per hundred. Since only 12 total deaths were reported it is hard to identify a risk of 5 in 100 from cancer.

Bruce C. Allen

This determination appears to be consistent with the guidelines. With respect to transparency, however, the terse statement about the weight of evidence (pp. 111-112) could be improved. A brief recapitulation of what the guidelines say about making this determination would be useful in that regard. That would help readers to see exactly what the basis for that determination is, to see that it is being applied objectively, and (to the extent that they accept the guidelines) that it is scientifically justified.

James V. Bruckner

The description of the criteria that 1,4-D satisfies to be classified as "likely to be carcinogenic to humans" has clearly been presented in Section 4.7.1 on page 77. The classification of "possible" human carcinogen fits just as well.

Harvey J. Clewell III

Yes, based on the available evidence of dioxane tumorigenicity and the lack of information on mode of action, I agree with the characterization of dioxane as a likely human carcinogen.

Lena Ernstgård

The scientific justification for the weight of evidence descriptor is sufficiently, transparently and objectively described. The available data for both liver tumors in rats and mice, and nasal, mammary, and peritoneal tumors in rats support the conclusion that 1,4-dioxane is a likely human carcinogen.

Frederick J. Kaskel

The cancer weight of evidence characterization is appropriate and justified base upon EPA's review of the existing scientific literature. Careful attention has been paid to the details of the data base that arrived at these conclusions in the analyses.

Kannan Krishnan

The weight of the evidence might be narrated better; it might address not only the likelihood of human carcinogenic effects, but also the conditions under which such effects may be expressed.

The presented evidence suggests that 1,4-dioxane would neither fall under the category of "carcinogenic to humans" nor under the "suggestive evidence of carcinogenic potential." Therefore, the present conclusion appears to be acceptable, provided it is qualified appropriately to reflect the totality of data.

Raghubir P. Sharma

The statement that 1,4-dioxane is a likely human carcinogen is debatable. The animal data clearly indicate that this chemical is an established carcinogen in several species and at several target tissues; however, the chemical has been shown to be a nongenotoxic carcinogen and perhaps the tissue damage is a prerequisite to the carcinogenic process. Besides, the carcinogenic response has been observed at relatively high doses (the dose for nasal carcinogenesis in male rats may or may not be appropriate for carcinogenic potential estimates as it is likely that the tumor production requires a direct contact with the nasal septal membranes). In view of this information, a potential for cancer production in humans is limited and may appear only if a continued exposure to large doses is achieved. Such exposure is possible in either accidental or intentional ingestion of this chemical, or in industrial exposures that exceed established exposure guidelines. It is unlikely in environmental situations and, therefore, the chemical is a *potential human carcinogen* and not a likely human carcinogen. However, it appears that EPA has certain established categories to describe the human carcinogenic potential and, of these, the suggested *Likely to Be Carcinogenic to Humans* is an acceptable alternate designation.

(C) Carcinogenicity of 1,4-dioxane

2. Evidence indicating the mode of action of carcinogenicity of 1,4-dioxane was considered. Several hypothesized MOAs were evaluated within the Toxicological Review and EPA reached the conclusion that a MOA(s) could not be supported for any tumor types observed in animal models. Please comment on whether the weight of the scientific evidence supports this conclusion. Please comment on whether the rationale for this conclusion has been transparently and objectively described. Please comment on data available for 1,4-dioxane that may provide significant biological support for a MOA beyond what has been described in the Toxicological Review. Considerations should include the scientific support regarding the plausibility for the hypothesized MOA(s), and the characterization of uncertainty regarding the MOA(s).

George V. Alexeeff

There is an inability to establish a genotoxic or nongenotoxic MOA for 1,4-dioxane. There is very limited evidence for each MOA. The document transparently explains why a mode of action could not be identified for any of the tumor sites. The weight of evidence clearly supports this conclusion. The document follows a Hill approach (as suggested in EPA's 2005 Guidelines for Carcinogen Risk Assessment) to consider the MOA data available. Figure 4-1 lays out the key events that should be documented if one is to consider tumor promotion, i.e., cell proliferation, as an MOA for 1,4-dioxane. Table 4-18 of the report indicates that adenomas and or carcinomas occurred in male and female mice at two dose levels below the dose levels that produced liver damage or cell proliferation. Even if possibly some events occur at one dose below the doses producing tumors, dioxane appears to be an extremely weak cytotoxic agent. The level of evidence has not satisfied information for the key events. The weight of the evidence indicates it does not act via a cytotoxic mode of action to produce carcinogenicity. Thus, the cytotoxicity-cell proliferation MOA has not been supported or established by the scientific literature. Further, if one were to presume a cytotoxic mode of action, there would be insufficient information to determine if the action was linear or not-linear. There has been insufficient examination of potential metabolites of 1,4-dioxane. One key study by Nanelli et al., (2005) found that induction of CYP450 did not increase liver toxicity of 1,4-dioxane. Similarly, Figure 4-2 suggests a possible MOA for the nasal tumors, but studies to justify the key events have not been completed. For these reasons, an MOA for any of the four tumors sites cannot be identified at this time with any certainty.

Bruce C. Allen

Mode of action is outside my area of expertise. Apparently, evaluating various pieces of information about MOA and reconciling them sufficiently to reach a conclusion is a difficult task.

However, my impression of the evaluation in this review is that it was too superficial. Although it is stated that the evidence for MOAs was considered, the discussion of the pros and cons for a proliferation-based MOA in Section 4 struck me as rather cursory.

And the rather strong concluding statements, that the evidence is inadequate to establish a MOA, appear to overly downplay what is known in relation to what might not be known.

It is difficult to envision from this document what would constitute "enough" evidence for a MOA for EPA to diverge from the default assumptions/procedures. In fact, one very useful and valuable addition to the document would be some statements about what additional pieces of evidence/analyses EPA would consider essential to make a determination about a MOA. Or, if there are already data presented that would just make it impossible for EPA to make a decision, or to decide to deviate from the default, then the review should just say so.

As noted elsewhere in this review, some additional consideration of the effect of various MOA assumptions could be included in the uncertainty discussions to provide some real substance to those sections.

James V. Bruckner

The document's authors do a reasonable job describing several hypothesized MOAs for carcinogenesis, and presenting some of the supporting data. I do not agree entirely with their conclusion, in line 14 of page 77, that "The available evidence is inadequate to establish a MOA." There will probably never be enough evidence for any chemical to be absolutely certain about its MOA. Although the MOA for 1,4-D is not clear, there is substantial experimental evidence that the MOA is non-genotoxic.

Nonlinear kinetics appear to play a significant role in 1,4-D's MOA. Cytotoxicity and ensuing carcinogenesis occur at high doses where the capacity clearance processes is apparently exceeded.

A key event in hepatocarcinogenicity is sustained cellular proliferation, or hyperplasia. Under this condition, there may be spontaneous errors in replication, fixation of DNA damage before it can be repaired, and/or promotion of initiated cells. Findings of a lack of 1,4-D-initiated free radical generation, peroxisome proliferation, DNA alkylation, DNA repair, tumor initiation, or metabolic activation to reactive metabolites in hepatocytes are all indicative of an epigenetic mechanism. It is possible that 1,4-D or a metabolite alters gene expression and stimulates proliferation, so long as it is present in sufficient quantities. The frequent observations of single cell necrosis, progressing to sustained cytotoxicity, regenerative hyperplasia, nodule formation, and carcinogenicity are indicative of a cytotoxic MOA. Although the document's authors point out a deficit of dose-response data, this does not detract from the consistent findings of this continuum of events by different investigators. Liver hyperplasia was seen by JBRC (1998a) at or below doses that which resulted in tumor formation. Liver toxicity at 13 weeks (Kano et al., 2008) preceded tumorigenesis at similar doses (JBRC, 1998a). Hepatocytomegaly was present in the livers of female rats that developed liver adenomas upon chronic 1,4-D ingestion (NCI, 1978). Hepatocytotoxicity and regenerative hyperplasia occurred at lower doses than did liver tumors in the Kociba et al. (1974) bioassay. In only one instance was evidence of liver injury not reported in rodents exhibiting liver tumors [i.e.,

in the low (77 mg/kg)] dose female mice of JBRC (1998a). The preponderance/weight of a considerable body of *in vivo* and *in vitro* experimental evidence supports: (a) an inflammation/cytotoxicity/regenerative hyperplasia MOA for liver and nasal tumors; and (b) a non-genotoxic MOA. An automatic default to a linear cancer risk assessment model under these circumstances is not dictated by the science. I do not believe it is consistent with the spirit of EPA's *Guidelines for Carcinogen Risk Assessment* (2005), where the weight-of-scientific evidence is to prevail.

Sustained renal proximal tubular cell degeneration and necrosis are also hallmarks of 1,4-D exposure in mice and rats. Rhinitis and inflammation of the nasal cavity are reported in rats, by Sweeney et al. (2008), to be due to direct exposure of the nasal mucosa to 1,4-D in the animals' drinking water. It is well established that chronic inflammation and several inflammatory mediators contribute significantly to carcinogenesis by a variety of mechanisms.

Lastly, 1,4-D has not been found to be genotoxic in the large majority of *in vitro* assays or *in vivo* mammalian test systems. Van Delft et al. (2004) classified the chemical as a non-genotoxic carcinogen, on the basis of gene expression profiling in cultured human hepatoma HepG2 cells.

If one considers the "weight of scientific evidence," as directed by EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, information currently available strongly supports a cytotoxic, proliferative regeneration MOA for liver and nasal tumors. The MOA for other tissues in which tumors occur remains to be established. Nevertheless, I believe there are sufficient data at the present time to support adoption of a non-genotoxic MOA, for the primary/critical tumor. Therefore, a biologically-based (non-linear) cancer risk assessment model should be utilized.

Harvey J. Clewell III

I agree with the conclusion that the MOA for dioxane carcinogenicity is likely to be nonlinear, but that there is inadequate evidence to support a specific MOA hypothesis with any confidence, so that a default linear low-dose extrapolation approach is (unfortunately) necessary. However, I was surprised to see the apparent acceptance of a mode of action involving direct effects of dioxane rather than a metabolite. I don't think the available evidence is sufficient to rule out a toxic metabolite that is produced disproportionately at higher concentrations where high affinity, low capacity cyps like 2e1 are saturated but other low affinity, high capacity cyps like 3a4 are not. My bet would be on the dialdehyde. The recently published inhalation study of Kasai et al. should provide an opportunity to evaluate the relationship between the plasma concentrations of dioxane and the observed preneoplastic lesions and toxicity in the liver, which could help to distinguish between a parent chemical and metabolite effect.

Lena Ernstgård

A detailed discussion of 1,4-dioxane's hypothesized MOAs is performed in the document. The weight of evidence presented support that a MOA could not be determined for any tumor types. The rationale for this conclusion is transparently and objectively described.

Frederick J. Kaskel

The scientific evidence reviewed supports the EPA's conclusion that a MOA(s) could not be supported for any tumor types observed in animal models. The rationale is clearly described in the review.

Kannan Krishnan

The summary statement could contain a narrative + descriptor to more fully reflect the information presented in the mode of action section. The conclusion that the available data do not support any hypothesized MOA is different from the statement that the data do not support a mutagenic MOA. The document states that the genotoxic mode of action is not well supported; and that enhanced cell proliferation is a plausible mode. However, the summary statements and choice of models are only based on statements that reflect – inadequate, unknown, inconclusive or conflicting data on MOA. This style of presentation might be improved to better reflect the data and discussions on pages 60-68. This should then be tied to the conclusion and choice of an extrapolation model for the assessment.

Raghubir P. Sharma

Although no MOA for 1,4-dioxane carcinogenesis is established, it is likely an epigenetic carcinogen. The genotoxicity evaluation for this chemical is largely negative and metabolism is not likely to be responsible for such processes. A direct repeated contact of tissues with the parent chemical, leading to tissue destruction, appears to be a requisite for the carcinogenic process. The resulting damage and perhaps the subsequent repair process may involve abnormal expression of yet unknown oncogenes or suppression of protective genes.

(C) Carcinogenicity of 1,4-dioxane

3. A two-year drinking water cancer bioassay (JBRC, 1998a) was selected as the principal study for the development of an oral slope factor (OSF). Please comment on the appropriateness of the selection of the principal study. Has the rationale for this choice been transparently and objectively described?

George V. Alexeeff

Three chronic animal bioassays provided incidence data with the potential of calculating a potency value for liver tumors in rats and mice. For the tumors sites of nasal cavity, three studies were available. For the tumor sites of the mammary gland and peritoneum, only one rat study was available. One reason provided for choosing the JBRC (1998a) drinking water bioassay as the principal study was that the study used four dose groups including a control. This is a strong reason for choosing this study. Another reason provided is that the study resulted in an increase in tumors at lower doses that the other available studies. This is an important consideration since one must consider the most sensitive species/study in conducting a risk assessment. A final reason provided is the more complete documentation provided for both carcinomas and adenomas in the JBRC (1998a) drinking water bioassay. The Kociba study only reported carcinomas and this may have resulted in an underreporting of tumor incidence. Thus, the document transparently and objectively describes the choice of bioassay for dose-response assessment.

Bruce C. Allen

I believe that the choice of the JBRC (1998) study is appropriate. It is a recent study of two species and both sexes in those species. It tested a sufficient number of dose groups and, in comparison to the other available studies, used doses that were more appropriate for the low-dose risk estimation.

With respect to transparency, however, the text (p. 100) could benefit from an itemized listing of the pros and cons associated with each study. And, to the extent possible, the study evaluation should be separated from the evaluation/selection of the endpoints within those studies. This has not been done in the current review document. At the stage of the study selection, one should focus on the merits of the study itself, not on the "sensitivity" of the endpoints observed in the study.

In relation to this choice, vis-à-vis the choice of the Kociba et al. (1974) for the RfD derivation, I have to wonder why the Kociba et al. study was considered more suitable for the noncancer risk assessment but the JBRC (1998) was considered better than the Kociba et al. study for the cancer assessment. On a study level (ignoring sensitivity of endpoints) it is difficult for me to reconcile those two opposite conclusions about the merits of the two studies.

James V. Bruckner

A complete rationale for selection of the JBRC (1998a) bioassay is not given. This bioassay was apparently chosen, because it yielded the lowest LOAEL, or POD (i.e., combined liver adenomas and carcinomas) in female mice. This may have been a well-done study, but there is no indication in the text of whether it was conducted under GLP-like conditions, peer reviewed or published and its data available publically for examination. The NTP (1978) bioassay is not appropriate to use, as it involved oral bolus dosing. Kociba et al. (1974) reported an apparent NOAEL (121 mg/kg/day) for liver tumors in rats, which is obviously higher than the LOAEL of 77 mg/kg/day in female mice of JBRC (1998). It is quite likely that Kociba and co-workers' LOAEL would have been lower, had they employed both sexes of mice and combined benign and malignant liver tumors.

Harvey J. Clewell III

I believe that the use of the JBRC study was scientifically justified and feel that the selection was transparently and objectively described.

Lena Ernstgård

The choice to select the two-year drinking water cancer bioassay (JBRC, 1998a) as the principal study for the development of an oral slope factor is transparently and objectively described.

Frederick J. Kaskel

This study is an appropriate investigation to develop an OSF and the rationale has been clearly and objectively described in the review. All considerations have been addressed by the analyses.

Kannan Krishnan

Yes. However, more details including the following aspects would be essential for a transparent and objective description of this study:

- Rationale for selection of doses, including MTD determination, if performed as part of this study;
- Temporal information on body weight for individual treatment groups;
- Temporal information on survival (mortality); and
- Dosing details.

Raghubir P. Sharma

Although this study is an unpublished one, it is assumed that the detailed report has been evaluated by the EPA. A draft of the report was recently provided by EPA for review. The JBRC is a reputable institution and the results of both JBRC and NCI have not been published in scientific journals. Besides, most of the $BMDL_{10\ HED}$ from both studies are comparable (Table 5-8), the lowest value in the JBRC study was for female Cjr:BDF_1 mice. Therefore, the choice of this value for deriving an oral CSF seems reasonable. Rationale for this choice has been clearly and objectively described in the report.

(C) Carcinogenicity of 1,4-dioxane

4. Combined liver tumors (adenomas and carcinomas) in female Cjr:BDF$_1$ mice from the JBRC (1998a) study were chosen as the most sensitive species and gender for the derivation of the final OSF. Please comment on the appropriateness of the selections of species and gender. Please comment on whether the rationale for these selections is scientifically justified. Has the rationale for these choices been transparently and objectively described?

George V. Alexeeff

The study design strongly supports use of the female Cjr:BDF$_1$ mice JBRC (1998a) data set. The study included 50 animals per dose group, four dose levels, exposed for 2 years, by an appropriate exposure route and vehicle. Clinical and histopathological evaluations were thorough in the study. The decrease in survival was caused by liver tumors at the high doses, indicating the dosing regimen was appropriate. The survival rate and tumor incidence was consistent with historical laboratory and species results. There were no reports of disease or ill health.

Further, the OSF is derived using the combined incidence of liver adenomas and carcinomas in mice. It is appropriate to combine benign and malignant tumors in the analysis, especially when the benign tumors have the capacity to progress to the malignancies with which they are associated. This is scientifically appropriate, and is consistent with the approach of the National Toxicology Program and the International Agency for Research on Cancer. This approach is mentioned on page A-5 of the EPA's 2005 Guidelines for Carcinogen Risk Assessment. The method is protective of public health and considers available scientific evidence. The choice of the female mouse is appropriate since it is represents the most sensitive species and gender. Also, as indicated in Table 5-6, each dose group indicates a statistically significant increase in tumors when compared pair-wise to the control group using the Fisher's Exact test, as well as, the full study representing a statistically significant positive dose-related trend. Consequently, this is a very strong dataset on which to base an oral potency slope, and rationale for its use has been transparently and objectively described.

Bruce C. Allen

The selection of the endpoint, sex, and species that give the highest OSF is a policy decision that is health protective and can be supported from that perspective. In that light, the choice made in this Toxicological Review is appropriate, because there is no suggestion that this endpoint, sex, or species is irrelevant to the task of predicting what risks might be associated with human exposures to 1,4-dioxane.

As in the response to the previous question, I would suggest that that specific rationale be stated clearly and succinctly in a paragraph separate from the other considerations of study selection. Once the principal study(ies) have been identified, it could be stated what endpoints (in particular sexes and species) were chosen for dose-response modeling (because responses for them appeared to be related to exposure level) and that, among

those, the final OSF will be determined by the one that gives the greatest OSF value. Such a concise statement would be much more transparent and could be clearly applied in an objective manner.

Specific, more-extensive comments about the details of the modeling of various endpoints (which may have an impact on the choice of the endpoints to use for OSF derivation) are provided in Section III below.

James V. Bruckner

Is there adequate scientific justification for combining benign and malignant liver tumors? Has it been established with reasonable certainty in a stop study that liver ademomas, given time, progress to hepatocellular carcinomas in mice? This should be addressed and pertinent references cited. What is the background incidence of these liver tumors in historical control Cjr:BDF_1 male and female mice?

Harvey J. Clewell III

I believe that the identification of the liver adenomas and carcinomas as the basis for the potency estimates was scientifically justified and feel that the selection was transparently and objectively described.

Lena Ernstgård

The choice of the selected species and gender are transparently and objectively described.

Frederick J. Kaskel

The use of these tumors in this study is appropriate for the derivation of the final OSF. The data has been clearly reviewed and presented objectively.

Kannan Krishnan

The scientific basis for the selection of the female *Cjr:BDF_1* mice is unclear. Has this strain or the specific tumor type in this strain been used as the basis of other risk assessments by the Agency? The rationale for the choice of this strain/sex as the most appropriate one compared to all other ones is clearly not articulated. In this regard, there is also the question of whether, the metabolic capability and sensitivity of this mouse strain appropriately reflects that of humans? Also, do we know as to how other chemicals evaluated using this strain compare with outcome of liver tumors in other strains? That would help address the concern about the extent to which the data from other stains should be considered in the process.

Raghubir P. Sharma

The selection of species and gender is appropriate as this was the most sensitive of all species and genders tested in various studies. Indeed, the lowest carcinogenic value was reported for male mice (Table 4-14 last row) for adenoma or carcinoma (66 mg/kg-day in males vs. 77 mg/kg-day for females); however, the difference is only marginal and may not influence the final outcome for BMD or OSF. The choice has been well justified and the rationale for this choice has been transparently and objectively described. It should be noted that historical background incidence for carcinogenesis in this species was not available for review.

(C) Carcinogenicity of 1,4-dioxane

5. Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described? Regarding liver cancer, a linear low-dose extrapolation approach was utilized to derive the OSF. Please provide detailed comments on whether this approach to dose-response assessment is scientifically sound, appropriately conducted, and objectively and transparently described in the document. Please identify and provide the rationale for any alternative approaches for the determination of the OSF and discuss whether such approaches are preferred to EPA's approach.

George V. Alexeeff

In this circumstance, the use of a linear low-dose extrapolation approach is sound and appropriate. As indicated on page 104, the weight of the evidence is inadequate to establish a MOA(s) by which 1,4-dioxane produces tumors in the liver, mammary gland, nasal cavity and peritoneum. Often there is proposed to be a simple dichotomy of MOA for cancer development, genotoxic or not. Further, it is often suggested that these two MOAs result in either a linear or nonlinear approach to cancer risk assessment. However, in most instances we have little understanding of how an agent causes cancer. That is the case for 1,4-dioxane. The weight of the evidence does not support a nonlinear MOA. The fact that genotoxicity has not been established with standard tests does not automatically suggest that a non-linear approach to cancer risk assessment is appropriate. It has been suggested by others that a cell proliferation mechanism, using a non-linear extrapolation would be appropriate. Table 4-18 of the report indicates that adenomas and or carcinomas occurred in the female mice at the 77 mg/kg-day and 323 mg/kg-day dose levels although liver damage and cell proliferation did not occur at these doses. Similar results were reported for the male mice in the study. Under these circumstances, the EPA Guidelines for Carcinogen Risk Assessment suggest that a linear approach is a default health protective option. Consequently, the document uses the standard default option of calculating the CFR for 1,4-dioxane via a linear extrapolation from the POD calculated by curve fitting the experimental dose-response data. The POD is the 95% lower confidence limit on the dose associated with a 10% response rate. This is transparently described on page 104 of the document.

It is important to note that even if a nongenotoxic mechanism were operating for 1,4-dioxane, it may not be best evaluated by threshold modeling. Recently the National Academy of Sciences stated: "Effects of exposures that add to background processes and background endogenous and exogenous exposures can lack a threshold if a baseline level of dysfunction occurs without the toxicant and the toxicant adds to or augments the background process. Thus, even small doses may have a relevant biologic effect" (National Research Council (2008) Science and Decisions: Advancing Risk Assessment Committee on Improving Risk Analysis Approaches Used by the U.S. EPA).

One alternative approach that could be considered is to combine tumors in the JBRC 1998 study if the individual data are available. That way, the risk would not simply be to avoid liver tumors from 1,4-dioxane , but any tumors from 1,4-dioxane. This approach was considered for the JBRC rat data since tumors occurred at multiple sites. In that case, a multi-site potency was increased over one based solely on liver data for the rat. For the mouse, tumors were confined to the liver, so there is no multi-site effect. However, the mouse study was still the most sensitive. Thus, the mouse data still provide the appropriate health protective and scientifically valid approach.

Bruce C. Allen

Comments about the justification of the linear low-dose approach, as it relates to the judgments about MOA, have been provided above.

Should EPA want to pursue approaches other than the linear extrapolation, they could consider an integrated assessment of the cancer and the noncancer endpoints. It was noted above that many of the noncancer endpoints were excluded from analysis for the RfD because they were stated to be associated with the occurrence of tumors, or "preneoplastic" (p. 88, line 17). If that is the case, then one could consider an assessment that linked (i.e., modeled the relationship between) 1,4-dioxane exposure and those preneoplastic changes, and then modeled the relationship between those preneoplastic changes and tumor occurrence. This two-step "cause-effect" modeling approach could then still be based on cancer risk for defining PODs (e.g., could use a 10% extra *tumor* risk level for identifying a POD) but the dose associated with that POD would be mediated through its effect on producing the preneoplastic changes necessary to give the cancer risk level of interest. In other words, one would not need to define a BMR based on specified changes in the preneoplastic lesions; rather, the BMR would continue to be defined in terms of cancer risk and the linear low-dose or UF-based extrapolation (whichever was considered most appropriate based on knowledge about how the preneoplastic lesion was affected by 1,4-dioxane exposure) would be applied to doses associated with those cancer-based BMRs.

Finally, as noted in an earlier comment, if linear low-dose extrapolation is to remain the approach of choice, examination of the effects of choosing BMRs other than 10% is recommended. Since it is the low-dose slope that is of interest, and because the straight line that connects 10% risk to 0% risk may overestimate that low-dose slope, one should calculate BMDLs for lower risk levels. Those will tend to estimate the low-dose linear behavior more closely than will the slope using 10% response. Counterbalancing that tendency, however, will be the tendency for the BMDLs to be more uncertain as the risk level is reduced below the data points. Thus, with a series of additional BMRs examined, one can identify the risk level for which the low-dose slope is "best" estimated – i.e., using the risk that is low enough to capture low-dose linearity but not so low that the uncertainty about that slope gets too large. At the very least, such an analysis should be included in the uncertainty discussion.

James V. Bruckner

The basic adjustments and extrapolation method of deriving a cancer slope factor are clear and adequately described. As previously discussed above under C.2., I believe the weight of scientific evidence clearly supports a non-genotoxic MOA. This is reflected by the results of majority of the numerous *in vitro* and *in vivo* assays. Other solid evidence for ongoing hepatocellular toxicity/proliferation and for nasal chronic irritation/inflammation and cytoxicity is discussed above. The lack of absolute certainty about the stimulus for hepatocellular proliferation in the absence of cytotoxicity, or causes of high-dose, low-incidence mammary gland tumors and/or mesotheliomas, is not sufficient cause for defaulting to linear extrapolation. This action is far too common in my opinion.

Harvey J. Clewell III

I agree with the conclusion that the MOA for dioxane carcinogenicity is likely to be nonlinear, but that there is inadequate evidence to support a specific MOA hypothesis with any confidence, so that a default linear low-dose extrapolation approach is (unfortunately) necessary.

Lena Ernstgård

Scientific justification for deriving the quantitative cancer assessment is transparently and objectively described. Regarding liver cancer, the approach to dose-response assessment by doing a linear low-dose extrapolation to derive the OSF is objectively and transparently described in the document. I have no other alternative suggestion.

Frederick J. Kaskel

The justification for deriving a quantitative cancer assessment has been transparently and objectively described. The rationale for the linear low-dose extrapolation approach for the analysis of the risk for liver cancer is supported by the data.

Kannan Krishnan

The scientific justification for the use of the linear extrapolation model is not clearly provided. There would appear to be a disconnect between the mode of action summary and the choice of the linear extrapolation model. Similarly, the available pharmacokinetic information does not support the use of a linear extrapolation approach. The high dose to low dose extrapolation could be conducted on the basis of dose metrics derived with animal PBPK models – that might result in the use of a combination of linear and non-linear models. Regardless of whether that approach results in a change in the numbers or not, it would add to the scientific basis, objectivity and confidence in the extrapolation approach and the outcome of the assessment.

Raghubir P. Sharma

The rationale for a linear low-dose extrapolation to derive the CSF is not clear. However, it may be in accordance of the current EPA policy. The reason cited for this decision is a lack of clear MOA; however, for a non-genotoxic carcinogen, non-linear approaches may be more appropriate. Trials of many different non-linear models on the available data may be considered if these provide a better fit as does a linear approach. The rationale, therefore, for the approach used is not objective, clear or transparent. Unless the EPA regulations require that in the absence for a known MOA a linear low dose approach must be followed, other non-linear models may be at least considered for this purpose.

V. SPECIFIC OBSERVATIONS

George V. Alexeeff

Page 4, lines 8-37. It seems that more information on environmental exposures would be helpful to set the context.

Page 19, lines 2-5. Clarify who conducted the sensitivity analysis.

Page 26, lines 26-27. When it states "EPA identified..." it needs to clarify that the identification is happening for the purposes of this assessment and is not a citation of another EPA study.

Page 27, lines 1-7. It would be helpful to add a sentence indicating what an expected positive result would be.

Page 32, line 3. It would be helpful to add a summary table of the NOAELs and LOAELs for subchronic oral toxicity. That is, include species, exposure, adverse effect, and indicated NOAEL or LOAEL.

Page 33, lines 16-28. The evaluation should include the incidence rate. Hoch-Ligeti et al. clearly states that five groups of 30 rats were used in the analysis. This results in an incidence rate of 0/30, 1/30, 1/30, 2/30, and 2/30. A trend test could be conducted for significance. When analyzed by the Mantel-Haenszell test, the result is highly significant at $p<0.001$ (California Department of Health Services, 1989).

Page 50, line 18; page 77, line 14. The document does not provide a clear summary of all the tumors found in a tabular form. A table should be added indicating the species, strain, exposure and tumors found significant either by pair-wise comparison or by trend test.

Page 63, lines 5-7. Please clarify the sentence which currently sounds circular. 1,4-Dioxane was included as an example nongenotoxic carcinogen and was then found to be a nongenotoxic carcinogen.

Page 69, lines 1-10. Clarify the significance of peroxidase activity and indicate if it was concluded to be a free radical generator.

Page 77, line 3. Needs to include a clear summary sentence of tumors found in animal studies. The sentence on page 106 lines 23-25 is a good example.

Page 78, lines 34-35. Unclear. Why the emphasis on air concentration? Many compounds induce nasal tumors following oral exposure.

Page 85, lines 19-22. Nasal tissues were not examined in the Torkelson et al., (1974) study; it is unclear why the study is mentioned here.

Page 90, lines 24-26. It is unclear what is meant by the statement. How does 38.5 support 9.6?

Page 101, lines 1-11. I think it would be helpful to derive comparative oral potency values from all tumor sites.

Page 103, Table 5-7. Need to clarify the tumor site being evaluated.

Page 105, lines 7-9. More information on the results of multiple tumor sites should be added here. While the results are unlikely to change the bottom line, the info in Tables D-16 and D-17 is very helpful. It could be clarified that additional tumor sites were observed in the rat but not the mouse. Even if we consider all tumors in the rat, we still find that the oral potency derived from the mouse tumor site is more sensitive.

Page 106, lines 23-26. This is a great summary sentence. It is not clear why only JBRC, (1998a) is cited.

Page 109, lines 1-5. This section should reference all the tumors identified and species found to have tumors.

Bruce C. Allen

p. 7, line 5: "occluded" is misspelled, as is "unoccluded."

p. 39, line 16: The table cited in this sentence shows the results for mice, not rats as stated.

p. 46, line18: I believe the reference should be to Tables 4-10 and 4-11 rather than 4-11 and 4-12.

p. 47, Table 4-10: Why were the denominators shown for the nasal cavity tumors but not for the other tumors?

p.63, Table 4-16a: I think this table title should indicate that it includes only the in vitro test summaries (as Table 4-16b indicates that it has mammalian in vivo).

p. 99, line 8: What does the word "sample" mean in this context? Was it just one of many that could possibly have been derived and used? If so, why was that one picked?

p.99, line 28: change "is" to "are" (subject of that clause is "data").

p. 100, Table 5-5: the page break would be better between species/strain/gender rather than in the middle of the data for the female mice from the NCI study.

p. 105, Table 5-8: Footnote c to this table is appears to be inconsistent with the text (pp. 104-105). First, the text does not indicate that the male mouse hepatocellular cancer data were best fit by the log-logistic model, but the footnote does indicate that. Second, the

text (especially p. 104 lines 29-32) makes it appear that it was unnecessary to drop the highest dose when the log-logistic model was fit to the female mouse liver cancer data; but the footnote says that it was dropped. Appendix D results suggest that in fact the highest dose was not dropped for the final results in any of the log-logistic models. Third, it is at least unusual to specify a "degree of polynomial" for the log-logistic model as is done in the footnote.

p. 106, lines 11-13: It is stated that the previous cancer assessment obtained a CSF of 0.011 using the nasal squamous cell carcinoma data for male rats from the NCI (1978). But this CSF is different from the CSF shown in Table 5-8 for that same data set. What was done differently here?

p. 109, line 10: The start of that line should read "stages *are* unavailable." (The subject of that sentence is "data.")

p. C-2, Table C-2: Here and elsewhere, the log-probit model has been restricted to have a slope >1. This is not necessary and in fact is not the current recommendation of EPA's statistical working group. The log-probit model slope can be allowed to be less than 1 without producing curves that are "supra-linear" at low doses. For any choice of slope, at some low enough doses, the dose-response becomes linear or sublinear.

p. C-4, lines 1-6: Why was the alternative BMR of 20% calculated? There seems to be no reason for that (there is no reason given here or elsewhere) and it never appears to have been used.

p. C-4, lines 7-10: These lines talk about Table C-1 and the renal endpoint, but it is in the section on liver hyperplasia. The last sentence here should have the verb "were" rather than "was."

p. C-5, line 5; p. C-21, line 53: In both of these sentences, you might want to avoid the use of the word "significantly" as it may imply to some that there is a statistical test involved in this AIC comparison, which is not the case. In fact, what is being done is simply finding the lowest AIC, whether it looks to be "substantially" lower than other AICs or even just a "little bit" lower. There is no degree of difference that is a decision point.

p. C-21, line 56: There are no "highlighted" models in the table referred to. Moreover, the phrase "essentially equivalent" is rather loose here. How similar is "essentially equivalent?" Here the difference between the highest and lowest of the "essentially equivalent" AICs is 0.02. What if the difference had been 0.03 or 0.04? Because this seemed to imply that the model-predicted BMDLs should be averaged (a practice I was unaware of and had not encountered before), it would be good to say how the guidance defines "essentially equivalent."

Most important specific observation:

p. D-3, Section D.1.2: There are a number of problems with this section. I do not understand why the procedure as described here was at all necessary and why some of the model options were even considered. Specific comments on that section and the subsequent results that are presented in Appendix D are as follows:

It is problematic to equate the multistage model to a simplified MVK model. The MVK model considers cell proliferation among specific cell subpopulations that the multistage model does not. The MVK model has only been set up to consider two transitions, whereas this section purports to consider up to 8 stages.

This mistaken belief that the multistage model and the number of degrees in its polynomial is closely related to the MVK and the number of transitions (at least, that is the connection that I infer from this discussion), has lead to a problematic model selection procedure.

First, the model selection process that is based on the smallest AIC is statistically invalid, *as it is applied here*. The most straightforward way to explain this is by reference to one set of model runs, say those for female rat, liver tumors (Table D-3). In that table, it is claimed that the 2nd degree MS model is the "best-fitting model" presumably because it has the lowest AIC. But note that the AIC is a combination of 2 components – (-2 * log-likelihood) + (2 * #parameters). The pure quadratic MS model has only 2 parameters (background rate and the coefficient for d^2, where d is dose). In contrast, the Weibull model has 3 parameters (background rate, coefficient for d^p, and p itself). However, importantly here, the process which EPA has followed by "shopping" for the best MS model, is just like an inefficient way of doing what the Weibull model does efficiently, i.e., finding a set of parameters for the equation, $1 - \exp\{\alpha+\beta*d^p\}$ that maximizes the likelihood. EPA's approach is less efficient because they have to manually check all the integer powers between 1 and 8, and because they are restricting themselves to integer powers (which, again, appears to be a mistaken consequence of linking the power of the MS model to some specific number of stages in the carcinogenic process). But the main point is that the Weibull model should not be penalized (by counting its three parameters in the AIC calculation) relative to any of the tested MS models, which as a group can be seen to have just as many parameters (a background, a coefficient for d^p, and some power, p).

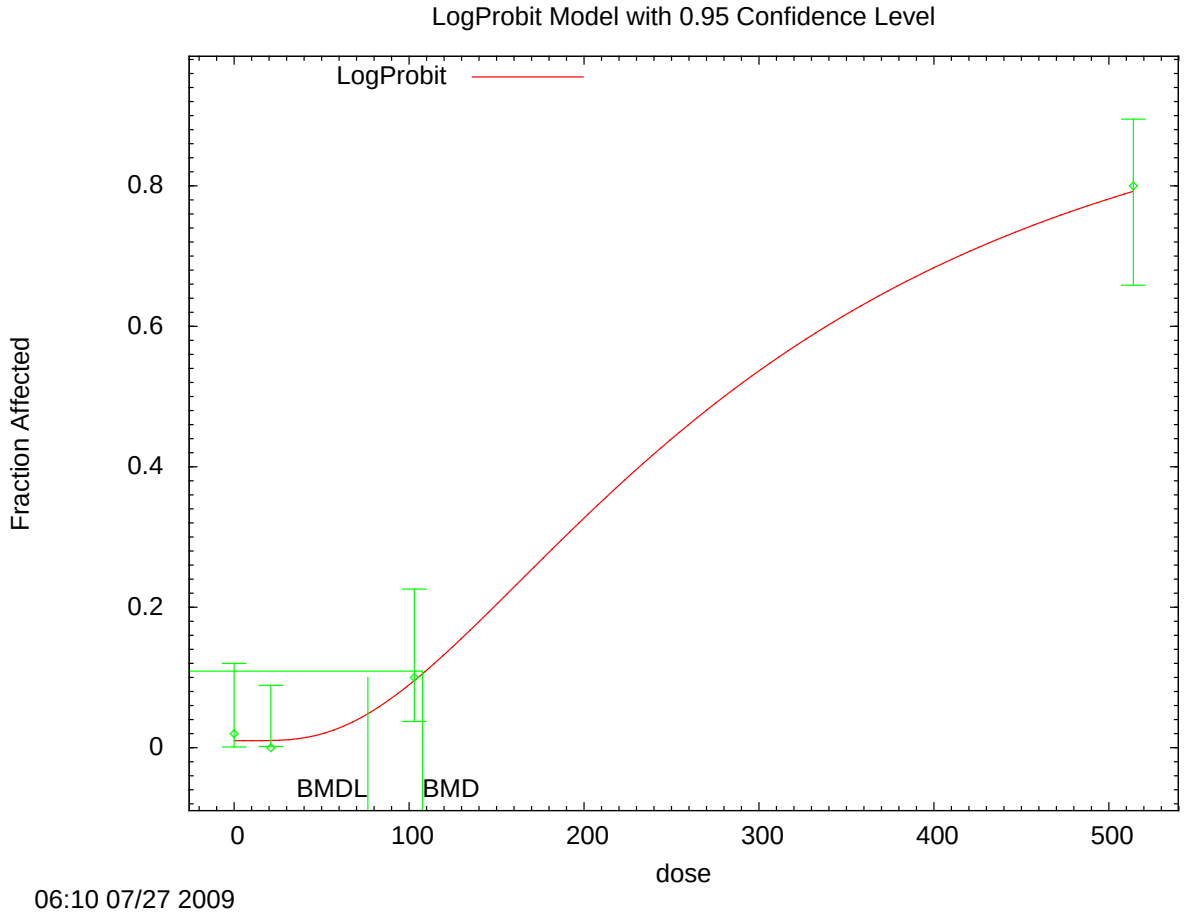
In the particular case of Table D-3, considering that there is another parameter associated with the choice of the purely quadratic MS model, the AIC for that model would be greater than that for the Weibull model. This is not at all surprising given that the Weibull-estimated power was 1.837, which is close but not quite equal to the power of 2 in the purely quadratic MS model. In this particular instance, however, the log-probit model provides the best fit of all the models and should be the choice for this endpoint.

I have never encountered, in previous assessments, this procedure of testing out various MS model configurations and restricting attention to "pure" model forms (pure quadratic,

pure cubic, etc.) and some small number of variants (e.g., the linear-quadratic, quadratic-cubic, etc of Table D-3). I see no reason to do that. For the purposes of a cancer (or noncancer) risk assessment, the MS model should be considered to be just like any other model in the BMDS suite – just another empirical model that might describe the observed dose response and therefore might provide some (rough) estimates of behavior over all doses (including lower ones). In that light, simply fitting the MS model that has some high-enough degree (up to the typical limit of N-1, where N is the number of dose groups) and which is allowed to include lower-order terms if the fit dictates, should be satisfactory. If the fitting suggests that a "pure" MS model is best, then lower-order terms will not be included. Importantly, however, when the bounds (BMDLs) are calculated, the optimization allows those lower order terms to enter back in and contribute to the BMDL estimation. This seems appropriate because one would rarely know enough, or have reduced the uncertainty to the point, that lower-order terms (the linear term in particular) can be explicitly excluded from describing the dose-response even when the observed data do not need such terms to best describe their pattern. The consequence, recognized or not, of the EPA procedure as illustrated by Table D-3 is that their chosen best model does *not* allow a linear term in the BMDL estimation. That seems inappropriate.

It also seems to be somehow inconsistent with the contention that the linear low-dose extrapolation approach is the best one for 1,4-dioxane. I realize that whatever model is fit to the data, EPA will draw a straight line from a POD to the origin, ignoring the actual low-dose model shape. But this strikes me as conceptually dissonant when the model selection procedure has ended up explicitly excluding low-dose linear behavior, even when considering the uncertainty about the model-based estimates.

I would also like to explore in this example set of considerations something touched on elsewhere in my comments. That is the use of the 10% extra risk level to define the presumed low-dose linear slope. Given the above critique of the model selection procedure and the conclusion that the log-probit model should be selected as the best one based on the AICs, the slope issue is quite apparent. Without converting to HEC doses, the BMDL for 10% risk from the log-probit model is 76.4, and the EPA procedure would estimate the low-dose linear slope to be 0.1/76.4 = 0.0013. But look at the log-probit curve that is fit to these female rat liver data:



That curve has a notable change in slope in the range of doses below those corresponding to 10% risk. In fact, calculating the BMDLs for other, lower risk levels and computing the low-dose slope for each of them results in the following:

Extra Risk Level	BMDL	Estimated Slope
0.1	76.4	0.001309
0.01	28.9232	0.000346
0.001	14.0414	7.12E-05
0.0001	7.7219	1.3E-05
0.00001	4.58897	2.18E-06
0.000001	2.87842	3.47E-07

Now, one may feel uncomfortable considering a risk of 10^{-6}, but the corresponding BMDL is not that far from the range of the experimental doses (2.8 compared to the lowest experimental dose of 21). But even for higher risks of 0.01 or 0.001 (with BMDLs solidly within or just slightly below the experimental dose range), the slopes are about 4- to 18-fold less than the slope obtained if one uses the 0.1 (10%) risk level for that estimation. This result is illustrative of the difficulties that might be obtained when only a single risk level (and a relatively high one at that) is used to approximate low-dose behavior.

Other comments on Appendix D:

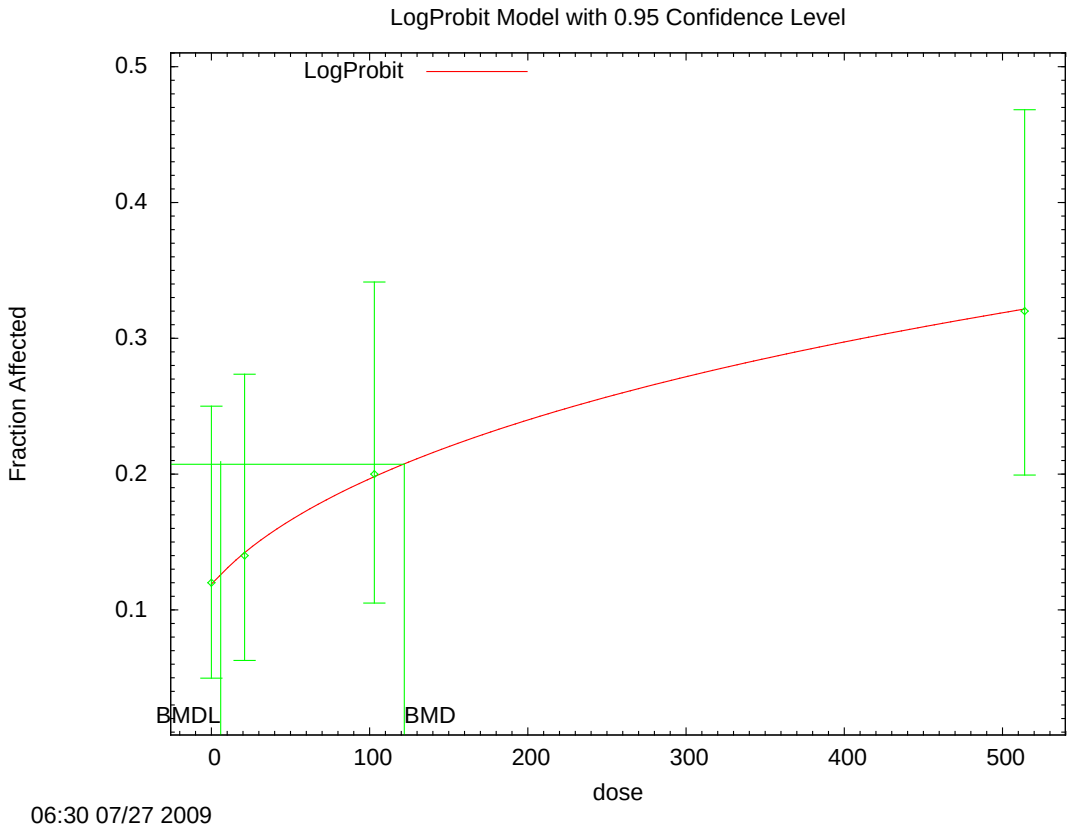
p, D-2, lines 23-26: It is *always* necessary to add incidences without double counting. This sentence, following after the previous one, makes it sound like this is only necessary when the counts are high.

p. D-3, lines 1-17: This whole listing seems entirely unnecessary. Plus, it is not true (as claimed in lines 4-5) that modeling was done on all the combinations shown – no modeling of the "neither" or the "only X and not Y" was done.

p. D-4, line 15: It is only the adenoma response in mice that was non-monotonic. And there is no "retain[ing] some of this character" in the combined adenoma and carcinoma responses; they are uniformly monotonic.

p. D-4, line 17-18: I believe the references should be to Sections D.5 and D.6.

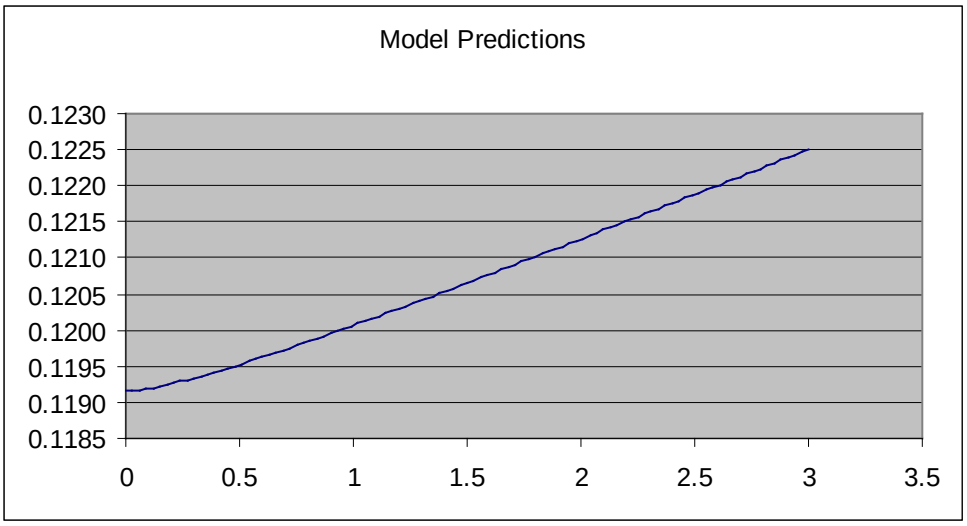
p. D-5, lines 4-6 (and various tables, e.g., starting with Table D-9): The log-probit slope parameter need not be restricted to be greater than or equal to 1 to provide non-supralinear low-dose behavior (finite slopes near zero dose). This fact has been noted by EPA's statistical working group which has recommended that the default option for running the log-probit model be to impose no restriction on the slope. For the data set whose modeling results are shown in Table D-9, the log-probit model with unrestricted slope is shown here:



That shape suggests supra-linearity, but in fact it is not. The log-probit parameters estimated for this data set are:

background	0.119157
intercept	-3.0812
slope	0.374659

And if one plots the log-probit function at the lower end of the dose range using those parameter values, one obtains a plot as follows:



This part of the curve displays a very slight sublinear pattern. Any log-probit model fit by BMDS will have that shape (for low enough doses). The value for CSF estimation of using model predictions associated with this range of doses and risks has been touched on in earlier comments.

p. D-5, lines 7-10: It is not quite true that the MS-Combo program "automatically includes a linear term." If, for example, the MS models for the two tumors being combined did not have linear terms, the MLE estimate for the combined BMD would not include a linear term. What is true is that the MS-Combo program is not configured to allow "pure" models (e.g., pure quadratic or pure cubic) for the combination of tumors when estimating BMDLs. As discussed above, the "forced" used of such models for even a single tumor is dubious; forcing such models for a combination of tumors, for which there must be even greater uncertainty, seems even worse.

James V. Bruckner

None for once. This is an exceptionally well-organized and written document.

Harvey J. Clewell III

Page 8, lines 24-28: What were the doses and dosing route in Woo et al. 1978 and 1977c?

Page 69, lines 12-22: What were the doses and dosing route in Nannelli et al.?

Page 14, line 35 to page 15, line 2; page 16, lines 5-9; and page 17, lines 11-14: I'm not sure why there would be a need to simulate human metabolism above saturation. It's surely not relevant to environmental exposures.

Page 18, lines 12-26: This paragraph is really hard to follow. I'm not sure how to fix it, but as it stands it just confuses me.

Page 69, lines 12-22: What were the doses and dosing route in Nannelli et al.?

Page 69, lines 22-26: What is the citation for this previous comparison? Was the comparison with pharmacokinetic data performed in Kociba et al. or Nannelli et al.?

Page 75, line 24: Were liver and kidney damage and acute vascular congestion of the lungs severe at all concentrations, or was severity concentration dependent?

Page 113, lines 12-14: This sentence makes no sense. I think it should be something like: "A POD in the range of observation was estimated from dose-response analysis of the experimental tumor incidence."

Page B-10, Table B-1 and Page B-11, Table B-2: "Metabolic dissociation constant" should be "Metabolic affinity constant."

Lena Ernstgård

Page 3, line 1, "pleasant odor" is very subjective; I suggest ethereal odor.

Page 18, line 29, Something is missing after the word "1,4-dioxane."

Page 23, line 19, "Ernstagård" should be "Ernstgård."

Page 23, line 23, "included ratings for discomfort, breathing difficulty" should be "included ratings for discomfort in eyes, nose, and throat, breathing difficulty."

Page 46, line 18, (Tables 4-11 and 4-12) should be (Tables 4-10 and 4-11).

Page 60, line14, "in in," delete one "in."

Page 118, Reference "Ernstård" should be "Ernstgård."

Frederick J. Kaskel

This extensive review has successfully addressed the major investigations in the field. Although hampered by limited number of studies, varying doses and durations of exposure to 1,4-dioxane, the EPA review has attempted to draw scientific conclusions from the observations in order to derive risks for toxicity and cancer that apply across and within specifies in animals to humans.

Specifically, I am concerned that the renal toxicities have not been characterized in the literature and have limited clinical correlations. The lack of data regarding possible mechanisms of injury, either tubular or glomerular are a major concern in that these may be different and have outcomes that lead to either acute kidney injury (AKI) and/or chronic kidney progression. If mechanisms can be identified, the possibility of targeting preventive treatment is likely in view of the advances in our understanding of AKI. In

addition, there have not been any application of the newer biomarkers to this field either experimentally or clinically, and this warrants investigation. Much evidence exists linking the functional correlation of biomarkers of tubular injury with NGAL and KIM both experimentally and clinically.

Finally, as a pediatrician, I am interested in the risk for toxicity and cancer in pre- and postnatal exposure to 1,4-dioxane in models and humans. There is limited data on an adverse gestational effect on ossification and fetal body weight and this should be followed up with additional studies. Also, the potential risks of contamination of breast milk with the agent have not been evaluated and are an important consideration in the constitution of infant formula with contaminated water.

The long term risks for cancer in the developing animal or human has not been evaluated and the design of long-term studies needs to be assessed. The current National Children's Study is an example of a 20 year follow up for exposures.

Kannan Krishnan

Page 8, lines 9-10: The dose level should be mentioned here.

Page 12, line 9: "lactating humans": rewording suggested.

Page 12, line 11: rewording suggested ("modeling was conducting…").

Page 89, line 2: …blood dioxane Vd between….needs to be reworded.

Raghubir P. Sharma

There are a few minor editorial suggestions as indicated below.

Page 6, line 29: determination of the absorbed fraction of inhaled 1,4-dioxane.

Page 27, line 10: It should [be] noted…(insert *be*).

Page 30, line 21: serum biochemistry in treated rats did not differ…..Should rats be changed to mice as the discussion in this paragraph refers to studies in mice?

Page 39, Table 4-6: Footnote denotations "*d*" and "*f*" in the last two columns under females are referred as *p* values of 0.008 and 0.001, respectively. These are values with no chemical treatment (controls); it is not clear what they are different from? If it refers to a trend of change for this parameter, it should be so stated.

Page 49-50, Tables 4-13 and 4-14: These two tables are apparently from the same study. The NOAEL on page 49, line 1 is identified for mice as 77; however, in Table 4-14 the incidence for adenoma or carcinoma in male mice is indicated as significant at 66 mg/kg-day (footnote *c* [$p \leq 0.01$]). From Table 4-14 it is not

clear what adenoma or carcinoma refer to (what tissue, as it is apparently other than those in liver)?

Page 52, lines 5-7: "Clinical chemistry changes consisted of a decrease in BUN (control—23 ± 9.9; 111-ppm dioxane—19.8 ± 8.8) and an increase in ALP activity (control—34.4 ± 12.1; 111-ppm dioxane—29.9 ± 9.2) and total protein (control—7.5 ± 0.37; 111-ppm dioxane—7.9 ± 0.53) in male rats (values are mean ± standard deviation)." Both decrease in BUN and increases in ALP and protein are questionable considering the standard deviations as indicated. Please check the differences again. Also replace "dioxane" with "1,4-dioxane" as elsewhere in the document.

Page 52, line 19: Replace "dioxane" with "1,4-dioxane" as elsewhere in the document.