PEER REVIEWER COMMENTS

External Peer Review on the *Toxicological Review of 1,4-Dioxane* (CASRN No. 123-91-1)

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I. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) was seeking an external peer review of the draft *Toxicological Review of 1,4-Dioxane (inhalation route of exposure only)* that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). The existing IRIS assessment for 1,4-dioxane (oral route of exposure only) was posted in 2010 and includes a reference dose (RfD), cancer descriptor, mode of action analysis for cancer, and oral slope factor.

During the development of the *Toxicological Review of 1,4-Dioxane (oral route of exposure only)* that was posted on the IRIS database in 2010, new studies (Kasai et al., 2009; Kasai et al., 2008) regarding the toxicity of 1,4-dioxane via the inhalation route of exposure became available. These new studies have been merged with the previously posted assessment (U.S. EPA, 2010) resulting in a complete assessment of the health hazards associated with both the oral and inhalation routes of exposure to 1,4-dioxane. An evaluation of the data from the new studies resulted in the derivation of a reference concentration (RfC) and an inhalation unit risk (IUR) for 1,4-dioxane, and these toxicity values are now presented in the Toxicological Review. The sections of the Toxicological Review that have been impacted by the new inhalation studies were the focus of the current external peer review. This external peer review was to evaluate only the data and qualitative and quantitative decisions relevant to the inhalation route of exposure. Although this external peer review was focused only on the sections of the Toxicological Review that were revised based on the new inhalation studies, the entire document was provided to the external peer reviewers for completeness.

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II. CHARGE TO REVIEWERS

Below is a set of charge questions that address scientific issues in the draft *Toxicological Review of 1,4-Dioxane (inhalation route of exposure only)*. Please provide detailed explanations for responses to the charge questions. EPA will also consider reviewer comments on other major scientific issues specific to the hazard identification and dose-response assessment of 1,4-dioxane. Please identify and provide the rationale for approaches to resolve the issues where possible. Please consider the accuracy, objectivity, and transparency of EPA's analyses and conclusions in your review.

(A) General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer health effects from exposure to 1,4-dioxane via inhalation?

2. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects from exposure to 1,4-dioxane via inhalation**.**

Chemical-Specific Charge Questions:

Please Note: An external peer review for 1,4-dioxane (oral route of exposure only) was completed in 2009. The conclusions of the peer review panel and EPA's responses can be found in Appendix A. This information, particularly regarding the cancer descriptor and cancer mode of action evaluation, may be useful for the review of the inhalation portion of the 1,4-dioxane assessment.

(B) Inhalation Reference Concentration (RfC) for 1,4-Dioxane

1. A 2-year inhalation bioassay in male rats (Kasai et al., 2009) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.

2. Atrophy and respiratory metaplasia of the olfactory epithelium in male rats were concluded by EPA to be adverse effects and were selected as co-critical effects for the derivation of the RfC. Please comment on whether the selection of these co-critical effects and their characterization is scientifically supported and clearly described. If a different health endpoint is recommended as the critical effect for deriving the RfC, please identify this effect and provide scientific support for this choice.

3. Benchmark dose (BMD) modeling methodology (U.S. EPA, 2000) was used to analyze the candidate endpoints identified for 1,4-dioxane. However, due to poor fit or substantial model uncertainty, BMD model results were inadequate for the following nasal lesions: atrophy (olfactory epithelium), respiratory metaplasia (olfactory epithelium), and sclerosis (lamina propria). Consequently, the NOAEL/LOAEL approach was used to

identify the POD for derivation of the RfC. Please comment on whether this approach is scientifically supported and clearly described.

4. The human equivalent concentration (HEC) for 1,4-dioxane was calculated by the application of the dosimetric adjustment factor (DAF) for systemic acting gases (i.e. Category 3 gases), in accordance with the U.S. EPA RfC methodology (U.S. EPA, 1994). This conclusion was based upon a number of factors, including the low reactivity of 1,4-dioxane, and the occurrence of systemic effects following oral and inhalation exposure to 1,4-dioxane. However, since 1,4-dioxane is water soluble and induces effects in portal-of-entry tissues, an alternative calculation of the HEC for 1,4-dioxane based on the application of the corresponding DAF for portal-of-entry acting gases (i.e., Category 1) is provided in Appendix G. Please comment on EPA's conclusion that 1,4-dioxane is a Category 3 gas, and the resulting application of the corresponding dosimetric adjustment factor (DAF) in deriving the RfC. If a different approach is recommended in the derivation of the RfC, please identify this approach and provide scientific support for the proposed changes.

5. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs appropriate based on A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002; Section 4.4.5; www.epa.gov/iris/backgrd.html) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

(C) Carcinogenicity of 1,4-Dioxane and Derivation of an Inhalation Unit Risk (IUR) for 1,4-Dioxane

1. Under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005; Section 2.5; www.epa.gov/iris/backgrd.html), the draft IRIS assessment characterizes 1,4-dioxane as "likely to be carcinogenic to humans" by all routes of exposure. Please comment on whether this characterization of the human cancer potential of 1,4-dioxane is scientifically supported and clearly described.

2. The draft assessment concludes that there is insufficient information to identify the mode(s) of carcinogenic action for 1,4-dioxane. Please comment on whether this determination is appropriate and clearly described. If it is judged that a mode of action can be established for 1,4-dioxane, please identify the mode of action and its scientific support (i.e., studies that support the key events, and specific data available to inform the shape of the exposure-response curve at low doses).

3. A two-year inhalation cancer bioassay in male rats (Kasai et al., 2009) was selected as the basis for the derivation of the inhalation unit risk (IUR). Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the IUR, please indentify this study and provide scientific support for this choice.

4. The incidence of hepatocellular adenomas and carcinomas, nasal cavity squamous cell carcinoma, renal cell carcinoma, peritoneal mesothelioma, mammary gland

fibroadenoma, Zymbal gland adenoma, and subcutis fibroma were selected to serve as the basis for the derivation of the IUR. Please comment on whether this selection is scientifically supported and clearly described. If a different health endpoint is recommended for deriving the IUR, please identify this endpoint and provide scientific support for this choice.

5. The IUR was derived based on multiple carcinogenic effects observed in rats exposed to 1,4-dioxane via inhalation. A Bayesian approach was used to estimate a BMDL10 associated with the occurrence of these multiple tumors, and then a linear low-dose extrapolation from this POD was performed to derive the IUR. Additionally, for comparative purposes only, a total tumor analysis was performed with the draft BMDS (version 2.2Beta) MSCombo model that yielded similar results (See Appendix H). Please comment on whether these approaches for deriving the IUR have been clearly described and appropriately conducted?

References:

Kasai, T; Saito, M; Senoh, H; Umeda, Y; Aiso, S; Ohbayashi, H; Nishizawa, T; Nagano, K; Fukushima, S. (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats. Inhal Toxicol 20: 961-971. http://dx.doi.org/10.1080/08958370802105397.

Kasai, T; Kano, H; Umeda, Y; Sasaki, T; Ikawa, N; Nishizawa, T; Nagano, K; Arito, H; Nagashima, H; Fukushima, S. (2009). Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal Toxicol 21: 889-897. http://dx.doi.org/10.1080/08958370802629610.

U.S. EPA. (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. (EPA/600/8-90/066F). pp. 409. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993.

U.S. EPA. (U.S. Environmental Protection Agency). (2000). Benchmark dose technical guidance document [external review draft]. (EPA/630/R-00/001). pp. 96. Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/benchmark-dose-doc-draft.htm.

U.S. EPA. (U.S. Environmental Protection Agency). (2010). Toxicological review of 1,4-Dioxane (CAS No. 123-91-1) in support of summary information on the Integrated Risk Information System (IRIS). (EPA-635/R-09-005-F). pp. 319. Washington, DC. www.epa.gov/iris/toxreviews/0326tr.pdf.

III. GENERAL IMPRESSIONS

James V. Bruckner

This draft of the *Toxicological Review of 1,4-Dioxane (With Inhalation Update)* is one of the best of its type I have reviewed. The Table of Contents, numbering of lines and pages, format and organization make it easy to read and find desired information. The accounts of the major studies' designs and findings are clear, accurate and complete. Most of the text is well written. I did notice, however, some decline in the quality of the sentence structure and punctuation in the red text describing the inhalation experiments, data and their interpretation.

I have serious problems with the interpretation of some of the experimental findings and the conclusions reached from them. One problematic conclusion is that data are lacking on the identity of the toxic/carcinogenic moiety or moieties. As outlined in my comments there is reasonable, though not absolutely conclusive evidence, that dioxane itself is the primary irritant/cytotoxicant/carcinogen. Very high repeated doses that saturate the metabolism of dioxane appear to be necessary to cause cellular injury and ensuing regenerative hyperplasia in the nasal cavity, liver and kidney. It is concluded in the document that the carcinogenic MOA is unknown, and that at least one link in the chain of events of a nasal damage-cell proliferation-hyperplasia mode of action (MOA) is missing in the findings of Kasai et al. (2009) and Kano et al. (2009). This is not the case at the termination of these 2-year studies, as I point out. Each of these events was observed in the proper sequence. The majority of *in vivo* and *in vitro* genotoxicity assays are negative, even at very high exposure levels. Thus, dioxane would not be expected to act by a genotoxic mechanism at low, environment exposure levels. The irritation and resulting inflammation caused by high concentrations will be a critical component of tumor progression. Results of *in* vitro studies indicate that dioxane may act as a tumor promoter, but not an initiator. Upgrading dioxane from a possible to a likely human carcinogen under realistic exposure conditions is not warranted.

Harvey J. Clewell III

As a reviewer of the initial oral risk assessment for dioxane, I am pleased to see that EPA has been very responsive to the suggestions of that peer review panel. The one exception is a public comment indicating that there appears to be an important error in the PBPK model as modified by the Agency. Specifically, the slowly perfused tissue compartment description does not appear to be physiologically appropriate. It was not possible to identify this problem from the documentation in the IRIS assessment, because the model equations and physiological parameters were not included. This oversight should be corrected.

This being said, I agree with the conclusion that the existing PBPK models are inadequate to perform route-to-route and cross-species extrapolation of animal studies by the oral route to support an inhalation dose-response assessment. I also agree with the conclusions regarding the inability to use Benchmark dose analysis for the nasal noncancer endpoints.

My overall impression of the present document is very positive. The additions are well written, clear and transparent. This expanded risk assessment document provides an

accurate, open-minded and balanced analysis of the literature evidence regarding dioxane's dosimetry, toxicity, and mode of action.

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 necessary. Until the question of the appropriate dose metric for dioxane toxicity and carcinogenicity (concentration of dioxane, concentration of a metabolite, or production of a reactive metabolite) 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 am concerned about a public comment suggesting that the documentation of the Agency's risk assessment calculations may not be adequate to allow them to be reproduced by an external expert, in the spirit of the Information Quality guidelines. The Agency should make every effort to assure that it is possible for stakeholders to understand how the quantitative risk assessment results were obtained and to reproduce the calculations described in the document.

The Agency should consider including information on likely human exposures to dioxane in Chapter 2 of the document. This information would provide an important perspective for the discussion of the possibility that the dose-response for the carcinogenicity of dioxane may be nonlinear. For such a possibility, the margin of exposure between tumorigenic and environmental exposures would be pertinent.

David C. Dorman

The document was well written and reviewed the available toxicological literature for 1,4-dioxane. I agree with most of the EPA author's interpretations concerning the available literature. Additional documentation of literature search methods would be appreciated.

Ronald L. Melnick

The draft document is, in general, a well-written, comprehensive review and assessment of published studies on the health effects of 1,4-dioxane. The information is clearly presented and the conclusions are generally scientifically justified and consistent with EPA policy. The inhalation updates to the toxicological review from oral exposures are treated as separate additions to each section of the overall review. In some cases this resulted in a few inconsistencies in the dose-response assessments from oral and inhalation exposures. In addition, the inhalation cancer assessment, which is based on data from a 2-year study in male rats would have benefited from information derived from the oral cancer assessment, which is based on chronic studies in both sexes of rats and mice. While the focus of this review is on the contribution of inhalation exposure to the health risks of 1,4-dioxane, it is inappropriate to ignore information from oral exposure studies in assessments of health risks associated with inhalation exposures.

While I concur with the conclusions on the limited utility of available mechanistic data in these assessments, I found the analyses and discussions on the mode of action of 1,4-dioxane-induced toxicity and carcinogenicity to be repetitive and disjointed. In some cases, mechanistic arguments were not clearly linked to available data and pertinent data were not fully addressed. It would be most helpful to include tables that show nonneoplastic lesions in the 13-week and 2-year studies as a function of dose for organs in which there was an increased tumor response. If nonneoplastic lesions do not precede neoplastic lesions or if they occur only at higher doses than those used in the cancer bioassay, then they are clearly not early key events in the carcinogenicity of 1,4-dioxane. If nonneoplastic lesions precede the tumor response in time and dose, then they may be contributory to the tumor outcome. Comparisons among toxicity/carcinogenicity studies of other chemicals would be necessary to see if nonneoplastic lesions induced by 1,4-dioxane are consistently associated with organ-specific tumor responses. Other areas for improving this draft document are described below in my responses to the "chemical-specific charge questions."

Frederick J. Miller

Overall, the document is logically organized and the material within the various sections is clearly presented. There is a fair amount of verbatim repetition of text from earlier sections as one goes through Sections 5 and 6 of the document that is a distraction; some rewording of the material would seem to be in order in the sections where earlier findings are discussed. The conclusions reached by the authors of the document are scientifically defensible relative to the selection of the critical studies and effects for derivation of the RfC. For the IUR, the text in various parts of the document seem to be contradictory of each other concerning the justification for the Kasai et al. (2009) study having only used males in the chronic inhalation bioassay (see Specific Observations section below).
The document does not have a description of ambient 1,4-dioxane exposure levels. The Agency should add material on exposure levels so readers can gain a better perspective on what margin there is between the RfC and IUR values and actual human exposures to 1,4-dioxane. At a minimum, some exposure data are available via the Internet, and the Agency should have the ability to provide reasonable estimates of current exposure levels. Two Internet sites with data are:

(1) http://www.atsdr.cdc.gov/toxguides/toxguide-187.pdf; and

(2) http://scorecard.goodguide.com/chemical-profiles/html/14dioxane.html

The information from the literature on the noncancer and cancer findings of the 1,4-dioxane inhalation studies described in the document is accurate. However, in Appendix G, the extrathoracic surface area default value for humans only accounts for the nasal cavity surface area, and, the value of minute ventilation used in the RfC calculation is not defensible for the vast majority of the population (see Specific Observations section below). While the Agency states that the calculation of the RfC in Appendix G is associated with considering 1,4-dioxane as a Category 1 gas and that the final analysis treats 1,4-dioxane as a Category 3 gas, it is still critical that the Agency use correct and reasonable default values in Appendix G.

The use of Haber's Law to adjust the exposure duration when converting all PODs to equivalent continuous exposure levels (i.e., by multiplying by the fraction of hours per day and the number of days per week that the critical studies used under the assumption of equal cumulative exposures leading to equivalent outcomes) is not scientifically defensible. This reviewer has shown (Miller et al., 2000) that Haber's Law is a simplification of the generalized power law family (i.e., $C^{\alpha} \times t^{\beta} = k$) and that most toxicological responses do not follow Haber's Law, which is a power law curve with $\alpha = \beta = 1$. **Moreover, whether the Agency's use of Haber's Law results in an under- or over-prediction of risk depends entirely upon the magnitude of the relationship between α and β in combination with where the true curve intersects Haber's line.** There is no acknowledgement of this uncertainty by the Agency, and the net impact of this uncertainty increases as RfC and IUR levels encompass very low exposure levels. While these points are expanded upon in the Specific Observations section below, Dr. Paul Schlosser, who was a co-author on the Miller et al. (2000) paper, is now an NCEA employee and is familiar with the kinds of analyses and caveats that need to be considered.

The Agency has an obligation to examine the nature of α and β for 1,4-dioxane inhalation noncancer and cancer endpoints to the extent that the available data will allow. At a minimum, this can be accomplished using the ten Berge $C^{n} \times t$ software available in BMD 2.2 accessible at www.epa.gov/ncea/bmds or by using the categorical regression capabilities available in BMD 2.2. If the available data are insufficient to yield insights on the values of α and β, then the Agency needs to make clear to the reader the potential direction and magnitude of the uncertainty involved with an adjustment of the POD to encompass continuous exposure

Raghubir P. Sharma

The document is a well-prepared revision of the 2009 version of the Toxicological Review of 1,4-dioxane. Because the addition of inhalation data were clearly indicated in red, this part was carefully reviewed while the rest of the draft was perused. The additions to the previous document are clear, complete, concise, and follow the required format for Toxicological Reviews of the EPA. New studies of 1,4-dioxane toxicity via inhalation (Kasai et al., 2008; Kasai et al., 2009) have been added that were not available at the time of last assessment for this chemical as posted in IRIS database (EPA, 2010). The effects of inhalation of 1,4-dioxane have been logically placed, appropriately described, scientifically interpreted, and various toxicological values (RfC, BMD, IUR) are correctly analyzed. The noncancerous and cancer health effects via inhalation to 1,4-dioxane are clearly described. The uncertainty factors are fairly well described. The rationale for selected studies, the derivation and uncertainties for RfC and IUR are adequate. References are added for the new information and corresponding appendices have been included. The appendices are clear with graphic or tabular presentation. The presentation of the information is clear, accurate, and the conclusions are sound. The fact that a saturation of 1,4-dioxane metabolism is required for its carcinogenic effects, low level exposures (<50 PPM) of this chemical in humans are unlikely to pose a quantifiable risk.

IV. RESPONSE TO CHARGE QUESTIONS

(A) General Charge Questions

Question 1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer health effects from exposure to 1,4-dioxane via inhalation?

James V. Bruckner

The document is clear, presented in a logical sequence, and as concise as the required EPA format (with its redundancy) allows. Most of the accounts of cited studies are accurate, complete and well written. The paragraphs in Section 4.6.2 describing the inhalation studies by Kasai et al. (2008, 2009) are not quite as well written. I made a number of editorial changes to pages 79 & 80, and included the marked-on text as an Appendix to my review.

The last paragraph in Section 4.6.2.1 on Mode of Action Information should be expanded and focused on the likelihood that the **parent compound** is the **proximate irritant/cytotoxicant/carcinogen.** Findings of Kociba et al. (1975) and Kasai et al. (2008) in support of this hypothesis are clearly detailed in lines 25 – 30 of page 82 of the document. Young et al. (1978) also reported that saturation of metabolism of dioxane at high dosage levels leads to toxicity in rats. Neoplasms are usually manifest in mice and rats only at high exposure levels that very likely saturate dioxane metabolism. These findings indicate that cytotoxicity and carcinogenicity may have a **threshold** coinciding with the threshold for metabolic saturation. Below this dosage threshold, most all dioxane will be oxidized to nontoxic metabolites. At higher doses, the internal dose of dioxane, the assumed proximate toxicant, builds. These researchers demonstrated that dioxane metabolism was clearly not saturated in humans inhaling 50 ppm for 6 hours. Some 99.3% of dioxane eliminated was metabolized to β-hydroxyethoxy acetic acid (HEAA), which was recovered in the urine. Thus, **usual** dioxane exposures should **not** present a significant risk of toxicity or cancer to humans. Environmental exposure levels are commonly some three orders of magnitude lower than carcinogenic doses in rodents (WHO, 2005; ATSDR, 2007).

Dioxane's major metabolite, HEAA, is not cytotoxic (Landry et al., 2011) or carcinogenic, lending further support to the premise that dioxane is the active moiety. As described in lines 14 – 82 of page 82 of the current document, inducers of CYP2B1/2, CYP2E1 and other P450 isozymes do not potentiate dioxane toxicity. Pretreatment of rats with a variety of P450 inducers did not enhance covalent binding at dioxane (Woo et al., 1977b). Dioxanone, a postulated dioxane metabolite, has been predicted by QSAR modeling to be carcinogenic. Dioxanone was not found in blood or urine of rats gavaged with a single dose of 10 or 1,000 mg dioxane/kg (U.S. Army, 2010). As described on pp. 66 – 72 of the current document, dioxane was not genotoxic in the majority of *in vitro* and *in vivo* test systems. There was no provision for metabolic activation in the few *in vitro* systems in which dioxane was toxic or mutagenic. Very high concentrations of dioxane were utilized in these positive *in vivo* assays.

The **weight of scientific evidence** in the foregoing paragraphs is clearly in favor of the parent compound (dioxane) being the proximate irritant/cytotoxic/carcinogenic moiety. Results of

the P450 induction experiments discount the role of metabolite(s), in that enhanced metabolism does not enhance cytotoxicity. It would be useful to assess the influence of P450 inhibitors on dioxane toxicity and mutagenesis. Cytotoxicity, as discussed below, may well be the primary mechanism of dioxane carcinogenesis in key tissues.

Harvey J. Clewell III

The presentation of the inhalation risk assessment is necessarily brief, given the limited number of studies available, but provides a clear and adequate description of the evidence for noncancer and cancer effects of inhaled 1,4-dioxane.

David C. Dorman

The U.S. EPA draft document is a well written and well referenced document. The draft document includes relevant tables that summarize the toxicological data – these tables are complete enough to allow independent interpretation of the data.

- Documentation of literature search terms and inclusion/exclusion criteria are lacking.
- The severity of lesions observed by Kasai and coworkers (e.g., Page 50) should also be mentioned in the main body of the text.
- There is some inconsistency in the interpretation of the toxicological significance of 'nuclear enlargement' in 1,4-dioxane exposed rodents. In most, but seemingly not all (for example see page 55 line 14), this lesion is described as having questionable toxicological significance (e.g., see page 110, lines 29-32 and multiple sites elsewhere) – however, this seems to be tissue specific – is there a justification for this? This change is used as the LOAEL in certain studies (see page 55, line 14; page 109 line 22 – and elsewhere). EPA is often citing conclusions raised by a study author; however, if their conclusion about the toxicological significance of this lesion differs then some clarifying statement is needed.
- I found the discussion on page 55 lines 10-15 confusing. The authors of the draft document are overly focusing on a single (and possibly questionable) finding solely because the incidence of the finding was 100% (50/50) – other lesions were also seen with high incidence (e.g., similar changes in the olfactory region occurred in 96% of the animals) – it would be better to discuss the collection of nasal lesions for determining the LOAEL/NOAEL rather than rely on a single observation (for example atrophy of the olfactory epithelium is generally accepted as having toxicological significance).
    - There isn't a need to state "nasal olfactory epithelium" (see Table 4-20 and elsewhere) since this epithelial subtype is restricted to the nasal cavity.
- Page 54, line 23 – this was also likely used as a method of decalcification of the nasal samples prior to sectioning.
- Page 83, Line 34-35 – the document states that no evidence of cytotoxicity was observed in the nasal cavity – this conclusion may not be supportable. Was nasal epithelial metaplasia considered an 'adaptive response" – is olfactory epithelial atrophy not considered 'cytotoxic'. The authors go on to describe these effects as

evidence of nasal toxicity (see page 85, line 28-35) – some of these processes cited – inflammation, atrophy could be broadly considered as cytotoxic. The EPA should provide definitions for this term if a more restrictive use is implied.

- The text suffers from portions that are very repetitive – for example portions of 4.7.3.1.2 (page 89) are repeated in section 4.7.3.2.2 (page 90) – this is but one example of repetitive text that becomes very distracting to the reader.
- Tables 4-27 (page 91) and 4-28 (page 93) are very confusing and do not readily demonstrate temporal relationships of interest.
- Page 113, lines 9-19 – the EPA needs to also consider lesion severity in the selection of the critical endpoint .
- Page 140. The EPA should better describe the rationale for their conclusion of overall medium confidence in their RfC.

Ronald L. Melnick

For the most part, the Toxicological Review of 1,4-dioxane was clear and concise, and the scientific evidence for both noncancer and cancer health effects from inhalation exposure were clearly presented. However, the mode-of-action analysis, particularly that related to the carcinogenicity of 1,4-dioxane, tended to ramble and did not fully utilize all of the available and relevant information. To help the reader of this document evaluate potential relationships between nonneoplastic lesions and tumor induction by 1,4-dioxane, a table on temporal and dose-related effects should be prepared (see my comment under General Impressions). Also, there were some inconsistencies between the approaches used to derive the oral reference dose and oral cancer slope factor compared to the derivation of the inhalation reference concentration and the inhalation cancer slope factor. These inconsistencies, which may have arisen because the oral and inhalation assessments were performed at separate times, need to be resolved.

Frederick J. Miller

The review of the scientific studies in animals and humans related to noncancer effects from exposure to 1,4-dioxane is clear and concise. The Table of Contents provides a logical breakdown of the information into the categories of information needed to assess hazard, dose-response, and calculation of an RfC. The cancer information is also logically presented for the two studies that are available involving chronic exposure of animals to this compound. The one area that is missing from the document is a brief description of what ambient exposure levels are so the reader can have a perspective on what margin there is between the RfC and IUR values and actual human exposures to 1,4-dioxane.

The Agency notes that there are effects on nuclear enlargement but does not use this endpoint because there is a question as to the interpretability and significance of changes in this endpoint. I would suggest that a pulmonary morphometrist be consulted to provide advice on the importance of this endpoint and our ability to interpret such changes. Logical contact persons are at UC Davis and include Drs. Dallas Hyde, Kent Pinkerton, and Charles Plopper.

Raghubir P. Sharma

The review is logical, clear, concise, accurate and objectively presented. Both noncancer and cancer hazards are discussed appropriately. The information on inhalation is clearly presented and synthesized for noncancer and cancer health effects of 1,4-dioxane via inhalation. This reviewer agrees with EPA's assumption that the MOA of 1,4-dioxane carcinogenicity is unknown. Simply a chemical being cytotoxic, leading to cell damage and hyperplasia, is not necessarily the MOA in itself. It may be worthwhile to emphasize that the MOA for carcinogenicity of 1,4-dioxane is an unknown epigenetic mechanism.

(A) General Charge Questions

Question 2. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects from exposure to 1,4-dioxane via inhalation.

James V. Bruckner

Ben-Baruch, A. (2006). Inflammation-associated immune suppression in cancer: The roles played by cytokines, chemokines and additional mediators. *Sem Cancer Biol* 16: 38-52.

Boatman, RJ and Knaak, JB. (2001). Dioxane. *Patty's Toxicology* (5th ed., pp. 177-187). John Wiley & Sons, New York.

Brown, RP, Delp, MD, Lilndstedt, SL, Rhomberg, LR, Beliles, RP. (1997). Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind Health* 13: 407-484.

Coussens, LM and Werb, Z. (2002). Inflammation and cancer. *Nature* 420: 860-867.
German Commission for Investigation of Health Hazards in the Work Area (2011). Deutsche Forschungsgemenschaft, Report No. 47, Bonn.

Hall, WC. (1990). Peritoneum, retroperitoneum, mesentery and abdominal cavity. In: *Pathology of the Fischer Rat*. Boorman, GA; Eustis, SL; Elwell, MR; Montgomery, CA, Jr; MacKenzie, WF (Eds.), pp. 63-69. Academic Press, San Diego, CA.

Haseman, JK, Hailey, JR, Morris, RW. (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update. *Toxicol Pathol* 26: 428-441.

Kundu, JK and Surh, Y-J. (2008). Inflammation: Gearing the journey to cancer. *Mutat Res* 659: 15-30.

Landry, GM, Martin, S and McMartin, KE. (2011) Diglycolic acid is the nephrotoxic metabolite in diethylene glycol poisoning inducing necrosis in human proximal tubule cells *in vitro*. *Toxicol Sci* 124: 35-44.

Monticello, TM, Morgan, KT, Uraih, L. (1990). Nonneoplastic lesions in rats and mice. *Environ Health Perspect* 85: 249-274.

Silverman, L, Schulte, HG, First, MW (1946). Further studies on sensory response to certain industrial solvent vapors. *J Ind Hyg Toxicol* 28: 262-266.

Takano, T, Murayama, N, Horiuchi, K, Kitajima, M, Shono, F. (2010). Blood concentrations of 1,4-dioxane in humans after oral administration extrapolated from in vivo rat pharmacokinetics, in vitro human metabolism, and physiologically based pharmacokinetic modeling. *J Health Sci* 56: 557-565.

U.S. Army Public Health Command (2010). Studies on Metabolism of 1,4-Dioxane, Toxicology Report No. 87-08 WR-09, Aberdeen Proving Ground, MD.

WHO (World Health Organization). (2005). 1,4-Dioxane in Drinking Water, WHO/SDE/WSH/05.08/120, Geneva.

Harvey J. Clewell III

I am not aware of any additional studies on 1,4-dioxane beyond those cited in the document.

David C. Dorman

None identified.

Ronald L. Melnick

No other studies relevant to an assessment on the health effects of 1,4-dioxane were identified in a PubMed search.

Frederick J. Miller

This reviewer is not aware of other noncancer and cancer health effects studies involving 1,4-dioxane that should be included in the document. However, there are a number of papers in the literature that are relevant to the computations of the RfC that are provided in this reviewer's specific comments; these papers support criticisms of the extrathoracic surface area default value for humans, the value of minute ventilation used in the RfC calculation, and the use of Haber's Law to adjust the exposure duration.

Raghubir P. Sharma

No relevant additional studies after inhalation exposure were found from an on-line literature search during 2008-2011 that would be appropriate for this document. Several other reports identified are either on unrelated chemicals or reviews summarizing original reports cited in this document.

Chemical-Specific Charge Questions

(B) Inhalation Reference Concentration (RfC) for 1,4-Dioxane

Question 1. A 2-year inhalation bioassay in male rats (Kasai et al., 2009) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.

James V. Bruckner

The justification for selection of the chronic study of Kasai et al. (2009), as the basis for derivation of the RfC, is clearly presented and scientifically justified.

Harvey J. Clewell III

The Kasai et al. (2009) study is clearly described and the selection of this study as the basis for the RfC is adequately supported. It appears to be a well-conducted study.

David C. Dorman

- I agree with this selection.
- Torkelson could also be discounted since assessment of nasal tissues (a known target for this chemical) was not performed.

Ronald L. Melnick

Clearly, the most comprehensive studies on the inhalation toxicity of 1,4-dioxane were the 13-week and 2-year studies conducted by Kasai et al. (2008, 2009). Advantages of using data from the 13-week study to derive the RfC are that this study had more exposure groups and smaller spacing of doses compared to the 2-year study. However, the 2-year study had more animals per group and a longer duration of exposure. The latter factor eliminates the need to apply an uncertainty factor for assessments of health risks from chronic exposures. Because most exposure-related toxicological effects extended to lower concentrations of 1,4-dioxane in the 2-year inhalation study, and two of the most sensitive effects (atrophy and respiratory metaplasia of the nasal olfactory epithelium) were not observed in male rats in the subchronic study, the selection of the 2-year inhalation study (Kasai et al., 2009) to derive the RfC is scientifically justified. Because atrophy of the olfactory epithelium was observed in female rats but not in male rats in the 13-week studies, the lack of a 2-year inhalation study in female rats may result in an underestimate of potential adverse toxicological effects from chronic exposure to 1,4-dioxane based on an RfC derived from male rat data. Consequently, an RfC should also be derived from data on toxicological effects in the olfactory epithelium of female rats exposed to 1,4-dioxane for 13 weeks (with application of an uncertainty factor to extrapolate from subchronic to chronic exposure duration).

Frederick J. Miller

The selected critical study was clearly described relative to experimental design and scientific findings. There was adequate description of the reasons why the Kasai et al. (2009) study was selected as the basis for the derivation of the RfC for 1,4-dioxane.

The reason stated by Kasai et al (2009) for using only male rats is a weak one in the opinion of this reviewer. Those authors stated female counterparts were not exposed given data illustrating the absence of induced mesotheliomas following exposure to 1,4-dioxane in drinking water, as shown in Yamazaki et al. (1994). Yet, the 13-week toxicity study that was the pilot study for design of the chronic exposure study showed that female rats were more responsive than male rats to a number of endpoints, as reflected either in a greater magnitude of change or response at a lower exposure level (e.g., see Table 4-15 in the Agency's 1,4-dioxane document).

Raghubir P. Sharma

The selection of Kasai et al., (2009) is the only study available for derivation of RfC, therefore the selection of the study is scientifically justified and appropriate. No other study is currently available for this purpose.

(B) Inhalation Reference Concentration (RfC) for 1,4-Dioxane

Question 2. Atrophy and respiratory metaplasia of the olfactory epithelium in male rats were concluded by EPA to be adverse effects and were selected as co-critical effects for the derivation of the RfC. Please comment on whether the selection of these co-critical effects and their characterization is scientifically supported and clearly described. If a different health endpoint is recommended as the critical effect for deriving the RfC, please identify this effect and provide scientific support for this choice.

James V. Bruckner

Atrophy and metaplasia of the olfactory epithelium are appropriate co-critical effects. The justification for their selection is not complete in this section of the document. Readers should be referred to the discussion in lines 12 & 13 of page 112 and lines 1 – 8 of page 113. It should be pointed out that the criteria for selection were the most sensitive effects leading to the lowest RfC.

Harvey J. Clewell III

I agree with the decision of the Agency to use olfactory atrophy and metaplasia as the critical effects for derivation of the RfC. I agree that the toxicological implications of nuclear enlargement are unclear.

David C. Dorman

I agree with this selection. This approach was scientifically supported and clearly described by US EPA. As mentioned earlier, the interpretation of the toxicological significance of 'nuclear enlargement' in 1,4-dioxane exposed rodents requires additional clarification. No other health endpoints are recommended for advancement.

Ronald L. Melnick

Atrophy and respiratory metaplasia of the olfactory epithelium are appropriate adverse effects for derivation of the RfC. Other than nuclear enlargement in the nasal epithelium, these are the only lesions that showed significant increases in response at the lowest exposure level compared to controls and maintained nearly 100% responses at the two higher exposure concentrations.

The draft document does not provide an adequate explanation for why nuclear enlargement was excluded as a critical effect (i.e., "the toxicological significance of nuclear enlargement is uncertain"). If nuclear enlargement is due to blocked nuclear division or blocked cytokinesis, then this response reflects a toxicological effect. Thus, either the rationale for not including nuclear enlargement needs to be strengthened or this effect should be included as an endpoint for which an adjusted POD is calculated.

In addition, I note here an inconsistency between adverse effects assessed from oral exposure studies versus inhalation exposure studies. Spongiosis hepatis was not

considered to be indicative of liver toxicity in the analysis of data from the drinking water studies and was therefore excluded for the derivation of the oral reference dose; however, the same lesion was included as a candidate nonneoplastic lesion in the assessment of adverse effects following inhalation exposure and was considered in the derivation of an inhalation RfC for 1,4-dioxane (page 110, Table 5-5, Table 5-6). Spongiosis hepatis is not a preneoplastic lesion for hepatocelluar adenoma or carcinoma, and therefore, for consistency should be included as a candidate nonneoplastic lesion in the assessment of the oral RfD for 1,4-dioxane. The POD for spongiosis hepatis in male rats (Kano et al., 2009) is probably lower than the POD for liver hyperplasia, a toxicity endpoint that was included as a potential POD for deriving the RfD for 1,4-dioxane following oral exposure.

Frederick J. Miller

Section 5.2 contains the description of the candidate effects for derivation of the RfC. The material in the subsections of this portion of the document followed a logical path of describing the nature of an effect, whether it had plausibility in the progression of changes leading to more deleterious endpoints, whether the effect was consistent with dose, and whether a defensible interpretation of the biological importance of the effect could be established. For example, despite statistical increases at the low- and mid exposure concentrations (50 and 250 ppm, respectively), incidences of nuclear enlargement of respiratory epithelium (nasal cavity), olfactory epithelium (nasal cavity), and proximal tubule (kidney) were not considered candidates for the critical effect given that the toxicological significance of nuclear enlargement is uncertain.

The presentation of the text leading to the selection of atrophy and respiratory metaplasia of the olfactory epithelium as co-critical effects is scientifically sound and logical, and the authors described their reasoning in a straightforward manner.

Raghubir P. Sharma

Atrophy and respiratory metaplasia of the olfactory epithelium in male rats were apparent at the lowest inhalation concentration of 1,4-dioxane and therefore the selection of these adverse effects for the derivation of the RfC is appropriate. Nuclear enlargement of nasal respiratory epithelial cells occurring in the 100-ppm-exposed males and female F344 rats was the most sensitive effect, followed by the enlarged nuclei in the olfactory, tracheal, and bronchial epithelia. 1,4-Dioxane-induced liver lesions occurred at higher exposure concentrations than the nasal lesions did, and were characterized by single-cell necrosis and centrilobular swelling of hepatocytes in males and females in a 13-week study (Kasai et al., 2008), whereas similar lesions were obtained in a 2-year study at 50 ppm of 1,4-dioxane in male rats (Kasai et al., 2009). The selection of these co-critical effects and their characterization is therefore scientifically supported and clearly described. In view of some of the discussion that ensued at the public meeting it may be worthwhile to tabulate all available studies with the concentrations of exposure and lesions observed in an additional appendix. This would of course be true for all routes of exposure if done for inhalation exposures.

(B) Inhalation Reference Concentration (RfC) for 1,4-Dioxane

Question 3. Benchmark dose (BMD) modeling methodology (U.S. EPA, 2000) was used to analyze the candidate endpoints identified for 1,4-dioxane. However, due to poor fit or substantial model uncertainty, BMD model results were inadequate for the following nasal lesions: atrophy (olfactory epithelium), respiratory metaplasia (olfactory epithelium), and sclerosis (lamina propria). Consequently, the NOAEL/LOAEL approach was used to identify the POD for derivation of the RfC. Please comment on whether this approach is scientifically supported and clearly described.

James V. Bruckner

Use of the NOAEL/LOAEL approach was necessary and statistically-justifiable in this instance.

Harvey J. Clewell III

I agree completely with the decision to use the NOAEL/LOAEL approach for the olfactory endpoints. Adequate evidence is provided to demonstrate that the BMD approach was inadequate in this case.

David C. Dorman

I agree with this choice. This approach was scientifically supported and clearly described by US EPA.

Ronald L. Melnick

If no models provide an adequate fit to the dose-response data for the selected critical effects, then it would seem appropriate to use the NOAEL/LOAEL approach to identify the POD.

Frederick J. Miller

Since the BMD modeling of the co-critical effects failed to identify any acceptable model according to Agency-established criteria regarding lack of fit and likely extent of model uncertainty, the decision to proceed with a NOAEL/LOAEL approach is defensible. The text, as well as Appendix F, clearly laid out the findings for the BMD modeling of each biological endpoint.

Raghubir P. Sharma

The use of NOAEL/LOAEL approach used to identify the POD for derivation of the RfC is fine because the modeling methodology for benchmark dose (BMD, U.S. EPA, 2000) used to analyze the candidate endpoints identified for 1,4-dioxane provided poor fit or substantial model uncertainty. The BMD model results were inadequate for the atrophy

(olfactory epithelium), respiratory metaplasia (olfactory epithelium), and sclerosis (lamina propria). For this reason the approach used is adequate and is scientifically supported and clearly described.

(B) Inhalation Reference Concentration (RfC) for 1,4-Dioxane

Question 4. The human equivalent concentration (HEC) for 1,4-dioxane was calculated by the application of the dosimetric adjustment factor (DAF) for systemic acting gases (i.e. Category 3 gases), in accordance with the U.S. EPA RfC methodology (U.S. EPA, 1994). This conclusion was based upon a number of factors, including the low reactivity of 1,4-dioxane, and the occurrence of systemic effects following oral and inhalation exposure to 1,4-dioxane. However, since 1,4-dioxane is water soluble and induces effects in portal-of-entry tissues, an alternative calculation of the HEC for 1,4-dioxane based on the application of the corresponding DAF for portal-of-entry acting gases (i.e., Category 1) is provided in Appendix G. Please comment on EPA's conclusion that 1,4-dioxane is a Category 3 gas, and the resulting application of the corresponding dosimetric adjustment factor (DAF) in deriving the RfC. If a different approach is recommended in the derivation of the RfC, please identify this approach and provide scientific support for the proposed changes.

James V. Bruckner

Utilization of human versus animal blood:air partition coefficients (PCs) as the determinant of the HEC is appropriate for a direct portal of entry effect. Changes further down the respiratory tract may be influenced to some degree by a species' respiratory rate. The higher respiratory rate of rats might be anticipated to result in deposition of a larger dose distally.

Some of the toxicologically-active chemical present in the pulmonary epithelium is likely to result from systemic delivery. Systemic uptake of inhaled volatiles is determined primarily by their blood:air PC, respiratory/alveolar ventilation rate, cardiac output (pulmonary blood perfusion rate) and metabolic rate. From information provided in the current document, the rate of metabolism of dioxane by rats and humans appears to be similar. The animal blood:air PC is slightly higher than in humans. This favors more systemic absorption by rats. The respiratory/alveolar ventilation rate and cardiac output of the rat are substantially higher than in humans (Brown et al., 1997). Thus systemic absorption, target tissue doses and adverse effects due to parent compound or metabolites should be significantly greater in rats than in humans. U.S.EPA RfC methodology (1994) takes into account just one of these factors (blood:air PC). This document is in serious need of updating and revision.

Harvey J. Clewell III

I certainly agree that in view of 1,4-dioxane's high water solubility, its lack of reactivity, and its ability to rapidly achieve a steady-state blood concentration proportional to the inhaled concentration, the HEC should be calculated using Category 3 gas dosimetry.

David C. Dorman

- The effects that EPA proposes for deriving an RfC are largely based upon possible portal-of-entry responses (olfactory epithelial atrophy, and nasal epithelial metaplasia). Although nasal responses are also seen following oral exposure (data that could be used to support the characterization of 1,4-dioxane as a Category 3 gas)

the available data suggests that these lesions arise from direct contact of 1,4-dioxane in drinking water with the animal's muzzle. EPA has not provided a compelling argument why the lesions seen in the inhalation study do not represent portal of entry responses.

- EPA largely categorizes 1,4-dioxane as a Category 3 gas based upon physicochemical properties rather than the site of action. This is consistent with EPA's working definitions.
- An alternative approach could have been to handle this chemical as a Category 2 gas – one having mixed effects, moderate water solubility, and also some systemic delivery. EPA has not provided an adequate justification for why this category was not considered/used.
- Selection of the Category also requires additional consideration of the utility of the PBPK model once any errors are corrected in the PBPK model.

Ronald L. Melnick

The fact that 1,4-dioxane induces systemic effects following inhalation or oral exposures and has been measured in blood after inhalation exposure is consistent with this chemical being a systemic acting gas (Category 3 gas). However, a comparison of dose-related effects of 1,4-dioxane in the nasal epithelium following inhalation and oral exposures indicates that nasal lesions are induced at lower administered doses from inhalation exposures. Thus, it is likely that 1,4-dioxane also induces portal-of-entry effects (Category 1 gas). Furthermore, there is no information on the extent to which this volatile chemical might have been inhaled by rats during the drinking water studies. Consequently, I recommend that both human equivalent PODs (32.2 mg/m^3 as a Category 3 gas, and 8.1 mg/m^3 as a Category 1 gas) be used to derive RfCs for 1,4-dioxane. If a single RfC is required, then the mean value or the more public health protective value should be specified. This issue needs to be discussed in the section on uncertainties in the inhalation reference concentration.

Frederick J. Miller

The classification of 1,4-dioxane as a Category 3 gas is primarily due to the fact that none of the available or recalibrated PBPK models for this gas were found to fit adequately the experimental data. However, one of the presenters at the review meeting on March 19th pointed out that they had found multiple errors in the PBPK modeling code. As such, it is imperative that the Agency corrects these errors and revisits whether the revised PBPK model now provides an adequate fit to the human experimental data. This could end up changing the category classification and approach (i.e., the Agency may end up needing to treat 1,4-dioxane as a Category 2 gas). Because of the extrapulmonary effects of 1,4-dioxane, a classification as Category 3 or 2 is more appropriate than a classification of Category 1. The Agency described the characteristics of 1,4-dioxane relative to reactivity and solubility, as well as the extrapulmonary effects, such that a Classification of Category 3 is reasonable if the revised PBPK models still do not adequately fit the experimental data.

It would also be useful if the Agency would include in an appendix to any IRIS document the code for the PBPK models used so others can reproduce the results that the Agency has presented.

Since the calculated animal blood:air partition coefficient for 1,4-dioxane is higher for rats than the human value, the Agency used a DAF = 1 in accordance with the Agency's RfC methodology. Further, application of PBPK models to gases that have similar physicochemical properties and induce similar nasal effects as 1,4-dioxane also estimate DAFs $\geq$ 1, which provides additional support justifying the use of the default value of 1.

However, this reviewer notes errors in the Agency's calculations of the DAF in Appendix G as well as the inappropriate use of Haber's Law to adjust the exposure duration when determining the point of departure (POD). These issues are dealt with in my specific comments as well as in my description above of the overall adequacy of the information and methods used by EPA in this document.

Raghubir P. Sharma

The approach used is appropriate. The human equivalent concentration (HEC) for 1,4-dioxane calculated by the application of the dosimetric adjustment factor (DAF) for systemic acting gases (i.e. Category 3 gases) in accordance with the U.S. EPA RfC methodology (U.S. EPA, 1994) seems correct. This consideration based upon the low reactivity of 1,4-dioxane, and the occurrence of systemic effects following oral and inhalation exposure to 1,4-dioxane seems appropriate. The rationale that since 1,4-dioxane is water soluble and induces effects in portal-of-entry tissues, an alternative calculation of the HEC for 1,4-dioxane based on the application of the corresponding DAF for portal-of-entry acting gases (i.e., Category 1) is provided in Appendix G. EPA's conclusion that 1,4-dioxane is a Category 3 gas, and the resulting application of the corresponding dosimetric adjustment factor (DAF) in deriving the RfC is reasonable. No other approach for the derivation of RfC can be suggested at this time as the methodology used seems scientifically justified and this point is clearly outlined in the draft.

(B) Inhalation Reference Concentration (RfC) for 1,4-Dioxane

Question 5. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs appropriate based on A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002; Section 4.4.5; www.epa.gov/iris/backgrd.html***) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.***

James V. Bruckner

The intraspecies UF of 10, interspecies factor of 3, LOAEL to NOAEL UF of 10 and additional UF of 3 for lack of a multigeneration reproduction study are all warranted. It should be noted, however, that reproductive toxicity and teratogenicity indices monitored by Giavini et al. (1985) in rats were unremarkable/negative. Reduced ossification of sternebrae and lower fetal body weight were observed only at the highest dosage (1,000 mg/kg/day) group, in which there was reduced maternal body weight gain.

Harvey J. Clewell III

I fully agree with the Agency's application of uncertainty factors in the derivation of the RfC. They are clearly consistent with Agency guidance.

David C. Dorman

I do not believe that the justification for the database UF has been adequately supported. I agree with the selection of the other UFs.

Ronald L. Melnick

The UFs of 10 for interindividual variability and 3 for animal-to-human extrapolation to account for pharmacodynamic differences between species are consistent with EPA policy for selecting UFs.

Because the incidence of atrophy of the olfactory epithelium was 80% at the LOAEL (50 ppm, the lowest level tested) compared to 0% in the control group, application of an uncertainty factor of 10 to estimate a NOAEL may be insufficient to derive a level of daily inhalation exposure (RfC) that would likely not cause appreciable risk in sensitive subpopulations experiencing lifetime exposure to 1,4-dioxane. Furthermore, because atrophy of the olfactory epithelium was observed in female rats, but not in male rats in the 13-week studies, the lack of a 2-year inhalation study in female rats may result in an underestimate of potential adverse toxicological effects from chronic exposure to 1,4-dioxane based on an RfC derived from male rat data. Some discussion on the reliability of the 10X factor to extrapolate to a LOAEL is needed with consideration of the level of response at the LOAEL compared to controls, and the fact that the LOAEL is based on male rat data while female rats were more sensitive to this effect in the 13-week studies.

The selection of an UF of 3 instead of an UF of 10 for database deficiencies was not adequately justified. The lack of an exposure group below 50 ppm (an exposure associated with an 80% incidence of atrophy of the olfactory epithelium) and the lack of a chronic study in female rats (the more sensitive gender to toxicity of the olfactory epithelium in the 13-week study) should be considered as part of the database deficiency.

Frederick J. Miller

The Agency describes 5 UFs and their values that are included to adjust the POD to establish the RfC. Of these 5 UFs, the case for and the value of the UF are clear cut for 3 of the UFs: the use of a UF of 10 for going from a LOAEL in the critical study to a NOAEL, an interspecies UF of 3 to account for potential pharmacodynamic differences between rats and humans, and the use of a UF of 1 for subchronic to chronic exposure because the critical study was a chronic exposure of rats.

One could debate the use of a UF of 10 for interindividual differences among human subjects in the dosimetry of and sensitivity to 1,4-dioxane. The UF is typically broken down into a value of 3 for dosimetric differences and 3 for sensitivity in response differences among humans. This reviewer has found that the dosimetric difference among human subjects for particles when establishing Human Equivalent Concentrations is 1.3 rather than 3, and the situation for gases is likely to be similar. However, in the absence of specific uptake data for 1,4-dioxane in human subjects, this reviewer cannot fault the Agency for using a UF of 10. However, this type of question is one that could be easily addressed in studies by NHEERL's Environmental and Public Health Division.

The discussion of and justification for the use of a UF of 3 for data base deficiency due to a lack of a multigenerational reproductive toxicity study is weak in the opinion of this reviewer. The Agency's document has the statement "The oral toxicity database included a single prenatal developmental study that indicated the developing fetus may be a target of toxicity." as the only defense of this UF. However, when one looks as the oral study, the only hint of something relevant was that embryotoxicity occurred only at the highest dose level, as reflected in a reduced fetal weight. The abstract of the cited paper is included below:

> "The industrial solvent dioxane (1,4-diethylene dioxide) was evaluated for teratogenic potential in Sprague-Dawley rats. The compound was administered on days 6-15 of gestation by gavage (0, 0.25, 0.5 and 1.0 ml/kg/day). A slight maternal toxicity, as evidenced by reduced weight gain, was observed with 1.0 ml/kg. Animals were killed and subjected to uterine examination on day 21 of pregnancy. There were no differences between control and dioxane-treated groups in implantation numbers, live fetuses, postimplantation loss or major malformations. Embryotoxicity, manifested by reduced fetal weight, occurred only at the highest dose level."

Since reproductive toxicology is not an area of expertise for this reviewer, I would defer to those more qualified to judge the reasonableness of the UF for database deficiency due to a lack of reproductive toxicity data via the inhalation route of exposure.

Raghubir P. Sharma

The rationale for the selection of the uncertainty factors applied to the POD for the derivation of the RfC is appropriate. The basis for UFs is based on *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), and this point is clearly described. No alternative approaches for the determination of the POD for this chemical could be identified from the studies reported here.

(C) Carcinogenicity of 1,4-Dioxane and Derivation of an Inhalation Unit Risk (IUR) for 1,4-Dioxane

Question 1. Under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005; Section 2.5; www.epa.gov/iris/backgrd.html***), the draft IRIS assessment characterizes 1,4-dioxane as "likely to be carcinogenic to humans" by all routes of exposure. Please comment on whether this characterization of the human cancer potential of 1,4-dioxane is scientifically supported and clearly described.***

James V. Bruckner

I do not believe the overall strength of scientific data is strong enough to classify dioxane as a "likely human carcinogen." The category "possible human carcinogen" is more appropriate. There is no evidence of increased cancer incidence in dioxane-exposed humans, though the number and power of epidemiology studies are limited. Numerous *in vivo* and *in vitro* genotoxicity experiments are largely negative, despite the use of high concentration or doses of the chemical. These results suggest that dioxane is, at most, a weak genotoxicant. Very high chronic oral or inhaled doses are required to produce tumors in rodents. These doses saturate dioxane metabolism, resulting in delayed systemic clearance of the parent chemical. There is substantial evidence, described elsewhere in my review, that the parent compound is the proximate irritant, cytotoxicant and carcinogenic moiety. As described above, pharmacokinetically, rats should be more susceptible to inhaled dioxane than humans.

I am concerned that high dioxane exposure produces several types of tumors in two species of animals. Tumors occur systemically and at the initial site of contact. The nasal carcinomas and mesotheliomas of the peritoneum invade underlying tissues. Nevertheless, I doubt that low, environmentally-encountered levels of dioxane pose a significant cancer risk to humans.

The German Commission for the Health Hazards of Chemical Compounds in the Work Area (2001) (Deutsche Forschungsgemeinschaft) places dioxane in their Category 4: "Having carcinogenic potential for which a non-genotoxic mode of action is of prime importance and under these conditions no contribution to human cancer risk is expected". The current MAK and ACGIH TLV, established for the protection of workers, are 20 ppm. ACGIH (2001) designates dioxane as A3 – "Confirmed animal carcinogen with unknown relevance to humans". IARC and WHO have classified dioxane as Group 2B, or "possibly carcinogenic to humans". WHO (2005) notes that "only a possible weak genotoxic potential has been suggested …" and that "if it is considered that 1,4-dioxane is not genotoxic in humans at low doses, the TDI approach can be used for derivation of the guideline value". In light of the foregoing, I believe that EPA should continue to designate dioxane as a **possible human carcinogen.**

Harvey J. Clewell III

Given the evidence of multiple tumor sites by multiple routes of exposure and considering the lack of information on the mode of action for 1,4-dioxiane

carcinogenicity, I think the characterization as likely to be carcinogenic to humans is justified.

David C. Dorman

- My assessment of the criteria used by EPA and as applied to the inhalation data are as follows:

Criteria	Adequacy
Plausible association between exposure and cancer in humans	Inadequate data to support this descriptor
Positive response in more than one species, sex, etc	Incomplete data to support this descriptor – bioassay performed in one species (rat). Lack of homogenous responses between the available bioassays (Kasai and Torkelson)
Additional biological concerns (e.g., early onset, high degree of malignancy)	Inadequate data to support this descriptor
Rare tumor response	Inadequate data to support this descriptor
Positive assay strengthened by other lines of evidence	Questionable

- EPA has not justified their selection of "likely to be carcinogenic to humans" for the inhalation route. I don't disagree with this selection; however, a more transparent application of the Guideline's "criteria" would be beneficial.
- This assessment would be appropriate if one also considers the oral data where a more extensive database is available.

Ronald L. Melnick

1,4-Dioxane induced tumors at multiple sites, in multiple species, in multiple studies, and by inhalation or drinking water exposures. Thus, the conclusion that 1,4-dioxane is "likely to be carcinogenic to humans" by all routes of exposure is consistent with recommendations in EPA's cancer risk assessment guidelines for choosing a weight-of-evidence descriptor for human carcinogenic potential.

Frederick J. Miller

According to the Agency's illustrations of data and situations where the classification of "Likely to Be Carcinogenic to Humans" that appears in the 2005 Cancer Guidelines, the case for using this description was adequately made in the document under review. However, in the opinion of this reviewer, the probability of 1,4-dioxane causing any cancer at realistic inhalation exposure levels is virtually nonexistent because ambient exposure levels are about 500,000-fold lower than the lowest exposure level used in the Kasai et al. carcinogenesis study and because 1,4-dioxane is not genotoxic in the vast majority of mammalian assays and does not affect DNA repair. Thus, the supporting text

should state that the "Likely to Be Carcinogenic to Humans" would apply under conditions of lifetime exposures to 1,4-dioxane at many orders of magnitude greater than those associated current ambient levels.

Support for my indication that ambient exposure levels are vastly lower than the exposure levels used in the animal studies comes from an ATSDR website listing levels at 0.028 to 0.11 ppb (http://www.atsdr.cdc.gov/toxguides/toxguide-187.pdf) and another website that reports EPA data to be 0.12 ppb (i.e., 0.44 mg/m3) over the period 1974 to 1984 (http://scorecard.goodguide.com/chemical-profiles/html/14dioxane.html). Moreover, beyond the IUR, these values convert for the RfC calculation to a range of 0.0001 to 0.000432 mg/m^3, which is 4 to 5 orders of magnitude below the point of departure for the RfC.

Raghubir P. Sharma

The characterization that 1,4-dioxane is carcinogenic in humans by all routes of exposure is appropriate. The animal data clearly indicate that this chemical is an established carcinogen in several species and at several target tissues, even though the chemical has been shown to be a nongenotoxic carcinogen and the tissue damage may be a prerequisite to the carcinogenic process. Exposure to this chemical is possible in either accidental or intentional ingestion or in industrial exposures that exceed established exposure guidelines and therefore likelihood exists that it has a potential to cause cancer in humans.

(C) Carcinogenicity of 1,4-Dioxane and Derivation of an Inhalation Unit Risk (IUR) for 1,4-Dioxane

Question 2. The draft assessment concludes that there is insufficient information to identify the mode(s) of carcinogenic action for 1,4-dioxane. Please comment on whether this determination is appropriate and clearly described. If it is judged that a mode of action can be established for 1,4-dioxane, please identify the mode of action and its scientific support (i.e., studies that support the key events, and specific data available to inform the shape of the exposure-response curve at low doses).

James V. Bruckner

I **strongly disagree** with the conclusion that there is insufficient information to identify a mode(s) of carcinogenic action for dioxane. As I outlined in my Specific Observations in Section III of my review, both Kasai et al. (2009) and Kano et al. (2009) observed and reported each stage of the regenerative hyperplasia MOA in the respiratory and olfactory epithelium after 2 years of dioxane exposure, namely: chronic irritation and cytotoxicity; regenerative cell proliferation; squamous hyperplasia; squamous metaplasia; and neoplasia. Inflammation, a critical component of tumor progression, was also prominent. The nonneoplastic changes and tumors were both seen in the highest dosage group in each chronic study. These doses undoubtedly saturated dioxane metabolism, resulting in the build up of high internal/target organ doses of the parent compound.

Cytotoxicity is also manifest at high dioxane doses in the liver and kidney, two other organs in which tumors were found in rats/mice. Hepatic single cell swelling and necrosis and hydropic change in renal proximal tubular cells were evident at 13 weeks at the highest exposure levels in the male and female rats dosed orally (Kano et al., 2008). Thus, cytotoxicity preceded the development of adenomas/carcinomas in each organ. Male and female mice also showed the hepatocellular changes at 13 weeks at doses lower than that which caused liver tumors by 2 years. Hepatorenal toxicity was manifest in rats in the 2-year inhalation bioassay at the same exposure level that produced hepatocellular adenoma and carcinoma and renal cell carcinoma Kasai et al. (2009). Results of experiments by Stott et al. (1981) support the cytotoxicity-regeneration hyperplasia MOA. Rats given 1,000 mg dioxane/kg/day in their drinking water for 11 weeks exhibited hepatic centrilobular swelling and increased hepatic DNA synthesis, indicative of cellular regeneration. No such effects were seen at 10 mg/kg/day. Hepatocytoxicity and regenerative hyperplasia occurred at lower dioxane doses than did liver tumors in the bioassay of Kociba et al. (1974). These data support the existence of a dosage threshold for cytotoxicity that coincides with saturation of the metabolism/detoxification of dioxane. *In vitro* and *in vivo* experiments revealed no evidence of chemically-induced DNA repair/damage in hepatocytes from rats given up to 2% dioxane daily in their water for a week. ^{3}H-Thymidine incorporation, an index of hepatocellular proliferation, was enhanced. Nasal epithelial cells from the rats showed neither DNA damage nor proliferation (Goldsworthy et al., 1991). **There is very little evidence in a rather robust data base to support the premise that dioxane-induced tumors arise by any mechanism other than a non-genotoxic mode of action.** There is only limited evidence of genotoxicity at very high concentrations/doses. Results of studies of rat liver and mouse skin indicate that dioxane does not initiate carcinogenesis, but can promote it (Bull et al., 1986; Lundberg et al., 1987). The **weight of scientific evidence** clearly supports a **cytotoxicity/inflammation/regenerative hyperplasia MOA operative at**

very high exposure levels. In this case a **threshold** cancer risk assessment model would be most appropriate to calculate an oral slope factor and IUR.

Harvey J. Clewell III

I agree with the Agency's 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 necessary.

David C. Dorman

I agree with the EPA's conclusion regarding the lack of an established MOA for this chemical. Although there is some data in support of a "cytotoxic" mode of action this database remains highly dependent upon histological evaluations at a limited range of exposure doses and timepoints. Studies examining cell proliferation and other relevant endpoints (e.g., gene expression) in support of a cytotoxicity/cell proliferation mode of action are largely lacking. The EPA should consider expanding their discussion to further address these and other deficiencies.

Ronald L. Melnick

I agree there is inadequate evidence to establish a mode-of-action for the carcinogenicity of 1,4-dioxane. The hypothesis that cytotoxicity occurring at doses above metabolic saturation followed by regenerative cell proliferation is a mode-of-action for 1,4-dioxane-induced tumors in the liver or nasal cavity is not supported by experimental evidence. However, I also found that this section was repetitive, tended to ramble, and the analysis did not address limitations in cited studies or make use of all available and pertinent data. Several examples are noted here:

1) Because 1,4-dioxane is a volatile liquid, the draft document should comment on whether or not precautionary steps (e.g., use of closed systems) were taken in the *in vitro* studies to prevent volatile loss of the test agent during incubations. Negative *in vitro* studies that did not take appropriate precautions to prevent volatile loss of 1,4-dioxane should be viewed as unreliable. Similarly, some comment is needed on the reliability of a skin paint initiation/promotion study for this volatile liquid.

2) The mechanistic section does not adequately discuss the potential for 1,4-dioxane to induce micronuclei in hepatocytes. The two studies that evaluated this endpoint in exposed mice were both positive (Morita and Hayashi, 1998; Roy et al., 2005). In addition, the only study on hepatic DNA strand breaks in rats was positive (Kitchin and Brown, 1990). The mechanistic section needs greater discussion and analysis on chromosomal/DNA damage as a potential genotoxic mode-of-action. The statements (page 66) that 1,4-dioxane was not genotoxic in the majority of in vivo mammalian assays and studies of micronucleus formation in mouse hepatocytes following *in vivo* exposures are inconsistent with the above cited results. It is not clear why these effects were only mentioned briefly under "other possible modes of action" when the

cytotoxicity/regenerative hyperplasia mode-of-action was not supported by experimental data.

3) In the discussions on the potential role of cytotoxicity/cell proliferation in the hepatocarcinogencity of 1,4-dioxane, critical deficiencies in the available database should be noted in the draft document, e.g., doses were generally not equivalent to the bioassay doses, the cited studies lacked information to compare dose-response relationships between measured endpoints and incidences of hepatocellular neoplasms, and none of the studies examined responses in mice - the more sensitive species to the induction of liver tumors by 1,4-dioxane.

4) The document should address dose-response relationships for toxic effects in the 13-week studies as predictors of tumor incidence at the same site. If reliable predictions are not possible, then the cytotoxicity/cell proliferation argument is not valid. Certainly, the large increase in hepatocellular neoplasms in female mice that received 66 mg/kg of 1,4-dioxane in drinking water for 2 years is not a predicted outcome based on the lack of toxic effects in the liver at a comparable dose in the 13-week drinking water study. When comparing temporal relationships between putative key events in 13-week studies and tumor outcome in the 2-year studies or when evaluating biological plausibility and coherence, it is essential that doses be specified to confirm or contradict potential relationships. As I noted in the section General Impressions, it would be most helpful to include tables that show nonneoplastic lesions in the 13-week and 2-year studies as a function of dose for organs in which there was an increased tumor response. If nonneoplastic lesions do not precede neoplastic lesions or if they occur only at higher doses than those used in the cancer bioassay, then they are clearly not early key events in the carcinogenicity of 1,4-dioxane. If nonneoplastic lesions precede the tumor response in time and dose, then they may be contributory to the tumor outcome. However, certain lesions, e.g., focal squamous hyperplasia seen in the 2-year study but not in the 13–week study, may reflect the continuum of proliferative changes that occur in the normal morphological progression to squamous cell carcinomas. Comparisons among toxicity/carcinogenicity studies of other chemicals would be necessary to see if nonneoplastic lesions induced by 1,4-dioxane are consistently associated with organ-specific tumor responses. In this regard, the document should note that nasal lesions, including inflammation, hyperplasia, and metaplasia, were frequently seen in inhalation studies conducted by the NTP with no evidence of nasal carcinogenicity (Ward et al., 1993 [Environ. Health Perspect. 101 Suppl 5, 125, 1993]; Haseman and Hailey, 1997). The EPA should explore the possibility that slides from the NCI studies on 1,4-dioxane are available and in adequate condition to evaluate possible linkages between toxic effects and tumor outcome in the drinking water carcinogenicity studies in rats and mice. The draft document should also note that the Kociba et al. (1974) study reported renal degeneration, necrosis, and regenerative proliferation (page 34) in exposed rats, but no increase in the incidence of kidney tumors.

5) Regarding the interpretation of initiation-promotion studies, the document should note that no studies have been conducted focusing specifically on the mouse liver. This deficiency prevents any firm conclusion on whether 1,4-dioxane acts strictly as a tumor promoter. The bioassay data demonstrate that 1,4-dioxane is a complete carcinogen.

6) In several instances (pages 73, 82, 87, 127, 131, 138, and Figure 4-1), the draft document suggests that "liver toxicity did not occur unless clearance pathways were saturated and elimination of 1,4-dioxane from the blood was reduced" or that "liver carcinogenicity is related to the accumulation of parent compound following metabolic saturation." These claims should be eliminated from the document because they are not supported by reliable data and they are probably wrong. Though changes in elimination kinetics after single iv dosing have provided information on plasma concentrations associated with metabolic saturation, blood time-course data in rats after an iv dose do not provide adequate information on exposure levels associated with metabolic saturation following 13 weeks of drinking water or inhalation exposures. The latter routes of exposure are not comparable to single iv bolus administration, because 1,4-dioxane doses are received by rats over extended durations each day. Because 1,4-dioxane induces P450 enzymes that substantially enhance its metabolic elimination, a single dose study does not provide adequate information on plasma concentrations associated with metabolic saturation following repeated long-term exposures. An increase in blood levels of 1,4-dioxane with increasing dose measured at a single time-point after inhalation exposures does not demonstrate metabolic saturation; blood time-course data are needed to make any reliable conclusions on this subject. Furthermore, there was no blood time-course data presented for mice exposed to 1,4-dioxane by inhalation or oral administration.

Frederick J. Miller

The document clearly discusses the data that might support hypothesized modes of action by which 1,4-dioxane might cause tumors and consistently concludes that the data fall short of supporting the particular mode of action under consideration. The conclusion that there is insufficient data to identify a mode or modes of action of 1,4-dioxane to cause cancer is an appropriate one. That being said, TERA provided information on a sequence of events for a potential mode of action that could well be integrated into the current text.

Raghubir P. Sharma

No mode of action for 1,4-dioxane carcinogenesis is established; it is likely an epigenetic carcinogen. The genotoxic 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. To the knowledge of this reviewer, no data exist that will provide accurate extrapolation of specific data to low doses. Therefore, the conclusion that there is insufficient information to identify the mode of action of 1,4-dioxane carcinogenesis is appropriate and clearly described in the document. It may be appropriate to emphasize that the MOA for 1,4-dioxane carcinogenicity is an unknown epigenetic mechanism that requires cytotoxic and subsequent cell proliferation response in the target tissue.

(C) Carcinogenicity of 1,4-Dioxane and Derivation of an Inhalation Unit Risk (IUR) for 1,4-Dioxane

Question 3. A two-year inhalation cancer bioassay in male rats (Kasai et al., 2009) was selected as the basis for the derivation of the inhalation unit risk (IUR). Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the IUR, please indentify this study and provide scientific support for this choice.

James V. Bruckner

The bioassay of Kasai et al. (2009) is clearly described and scientifically supported. It is the most appropriate basis for derivation of the IUR.

Harvey J. Clewell III

The Agency documents the adequacy of the Kasai et al. (2009) study to provide a sound basis for the derivation of the inhalation unit risk for 1,4-dioxane.

David C. Dorman

I agree with this choice. This approach was scientifically supported and clearly described by US EPA.

Ronald L. Melnick

The 2-year inhalation study of 1,4-dioxane in male rats by Kasai et al. (2009) is the only comprehensive inhalation study available for this chemical. The only other 2-year inhalation study of 1,4-dioxane (Torkelson et al., 1994) used a single exposure concentration, did provide an adequate rationale for the selection of that exposure level, and amazingly did not conduct histopathological evaluations of nasal tissue.

Frederick J. Miller

The reasons for basing the derivation of the IUR on the bioassay conducted by Kasai et al. (2009) were well described and justified. The selected study is the only one with exposure-response data from chronic inhalation of 1,4-dioxane.

Raghubir P. Sharma

The study by Kasai et al., (2009) is the only study available to date regarding long-term inhalation exposure to this chemical and hence the selection of this study is scientifically justified. The data presented in this study are sufficient to provide the derivation of inhalation unit risk (IUR) and calculations leading to the derivation of IUR are clearly narrated in the document.

(C) Carcinogenicity of 1,4-Dioxane and Derivation of an Inhalation Unit Risk (IUR) for 1,4-Dioxane

Question 4. The incidence of hepatocellular adenomas and carcinomas, nasal cavity squamous cell carcinoma, renal cell carcinoma, peritoneal mesothelioma, mammary gland fibroadenoma, Zymbal gland adenoma, and subcutis fibroma were selected to serve as the basis for the derivation of the IUR. Please comment on whether this selection is scientifically supported and clearly described. If a different health endpoint is recommended for deriving the IUR, please identify this endpoint and provide scientific support for this choice.

James V. Bruckner

I do not agree with utilization of all of the tumor types as the basis for IUR derivation. Zymbal gland tumors are generally limited to male rats. Peritoneal mesothelioma, subcutis fibroma, and mammary fibroadenoma are very commonly observed, spontaneous tumors in control F344 rats (Hall, 1990; Hasemann et al., 1998). Hepatocellular adenomas and carcinomas have been seen in 42 – 70% of control male B6C3F1 mice. Spontaneous liver tumors are relatively rare, however, in rats.

Harvey J. Clewell III

The experimental evidence, coupled with the lack of mode of action information, supports the use of these several tumor sites as a joint basis for derivation of an inhalation unit risk.

David C. Dorman

I agree with these choices. This approach was scientifically supported and clearly described by US EPA. One tumor type considered in the EPA's assessment is subcutis fibroma – the tumor incidence (2% in controls) reported in the cancer bioassay is consistent with other reports. The histological characterization of the tumor in the original report is incomplete, this is of concern since this tumor can arise from mammary tissues. The tumor response data also did not demonstrate a clear dose-response relationship. Likewise, Kano and coworkers (2009) stated the following regarding interpretation of this lesion in their chronic oral bioassay: "Dose-dependent increases in the incidences of squamous cell carcinomas in the nasal cavity, fibromas in the subcutis and fibroadenomas in the mammary gland was also noted in male rats, although the increases in these tumor incidences were not statistically significant." Another concern is whether sufficient data are available to justify the pooling of certain tumor types – I did not find this to be fully justified.

Ronald L. Melnick

Statistically significant increases in tumor rates were observed at each of the sites listed above in the 2-year inhalation study of 1,4-dioxane in male rats (Kasai et al., 2009). Therefore, all of these endpoints are pertinent for the derivation of the IUR of this multi-site carcinogenic agent. Though humans do not have a Zymbal gland, tumor induction at

this site should be included in this analysis because events leading to that response may occur at other sites in humans and because the known human carcinogen, benzene, induced Zymbal gland tumors in rats and mice. Because of the potential progression of hepatocellular adenoma to carcinoma the incidences of these lesions were appropriately combined.

One disturbing issue related to the determination of the IUR from the Kasai et al. (2009) study in male rats is that no inhalation cancer data are available for 1,4-dioxane in mice and tumor incidence data from the drinking studies of Kano et al. (2009) showed that mice were more sensitive than rats to the hepatocarcinogenic effects of this chemical (Table 5-12 of the 1,4-dioxane draft document). This species difference in sensitivity to 1,4-dioxane should be addressed as a source of uncertainty resulting in a potential underestimation of the inhalation unit risk value.

Frederick J. Miller

The selection of the incidence of the tumors listed above arose from the findings of the bioassay study of Kasai et al. (2009). These tumors showed statistically significant incidences that were observed in a dose-related increase. The document clearly described the nature of the tumors, their incidence in the Kasai et al. (2009) study, and the selection of these tumors is scientifically supported.

Raghubir P. Sharma

After inhalation exposure to 1,4-dioxane lesions of hepatocellular adenomas and carcinomas, nasal cavity squamous cell carcinoma, renal cell carcinoma, peritoneal mesothelioma, mammary gland fibroadenoma, Zymbal gland adenoma, and subcutis fibroma were observed in the study by Kasai et al., (2009). These have been selected to serve as the basis for the derivation of the IUR and therefore their selection is scientifically justified. Similar lesions, particularly in hepatic and renal tissues, have been reported by other authors after oral exposure to 1,4-dioxane. Tissues in respiratory and adjacent organs would likely be noted after inhalation exposure to 1,4-dioxane as a direct contact of chemical with the tissues is apparently a requisite to cancer production. No other health points will therefore be appropriate for the derivation of inhalation unit risk. Although the Zymbal gland is anatomically limited to rats, the observation that tumors are found in this organ in rats suggests the potential of 1,4-dioxane to induce carcinogenicity in multiple unspecified tissues in various animals or humans. The inclusion of the Zymbal gland observation in overall risk assessment may be non-relevant only if the concentrations of 1,4-dioxane that produce tumors in the Zymbal gland were significantly lower than those that produce tumors of other tissues in rats.

(C) Carcinogenicity of 1,4-Dioxane and Derivation of an Inhalation Unit Risk (IUR) for 1,4-Dioxane

Question 5. The IUR was derived based on multiple carcinogenic effects observed in rats exposed to 1,4-dioxane via inhalation. A Bayesian approach was used to estimate a BMDL10 associated with the occurrence of these multiple tumors, and then a linear low-dose extrapolation from this POD was performed to derive the IUR. Additionally, for comparative purposes only, a total tumor analysis was performed with the draft BMDS (version 2.2Beta) MSCombo model that yielded similar results (See Appendix H). Please comment on whether these approaches for deriving the IUR have been clearly described and appropriately conducted?

James V. Bruckner

These approaches are clearly described and appropriately conducted, as far as I know. My knowledge of the methodology involved in these calculations, however, is limited.

Harvey J. Clewell III

The two cancer dose-response approaches used for 1,4-dioxiane are clearly described and appropriately conducted. I am very impressed with the Agency's use of the Bayesian approach and consider it to be superior to alternative options. I am concerned by a public comment indicating that it was not possible for an external expert to reproduce the Agency's calculations. The Agency should make every effort to assure that the documentation of both the Bayesian and MSCombo analyses is sufficient to assure that stakeholders can verify the results.

David C. Dorman

Appears justified; however, the quantitative methods used are outside of my area of expertise.

Ronald L. Melnick

Based on the multiple carcinogenic effects of 1,4-dioxane in male rats, the multi-tumor analysis described in the draft document is an appropriate approach to calculate total tumor risk. Because results of both the Bayesian analysis and the BMDS MSCombo model were similar (BMCL10: 31.4 and 32.3, respectively), the IUR derived by the multi-tumor analysis seems to be reasonable. However, it should be noted that survival was significantly reduced in the high exposure group compared to controls and the cancer dose-response modeling did not use survival-adjusted incidence values. The IUR estimates might have been slightly larger if survival-adjusted tumor rates had been available. If day of death data were available for the oral carcinogenicity studies, then survival adjusted tumor rates should have been used to derive the oral CSF.

One deficiency in the inhalation cancer database for 1,4-dioxane is the lack of an inhalation bioassay of this chemical in mice. This deficiency is important because the

potency of liver tumor induction by 1,4-dioxane administered in drinking water is greater in mice than in rats. The apparent greater cancer potency of 1,4-dioxane following oral exposure compared to inhalation exposure may be due in large part to the lack of mouse cancer data. Certainly, differences in sensitivity between rats and mice to 1,4-dioxane need to be addressed in Section 5.5 on uncertainties in the inhalation cancer risk value and in Chapter 6 on characterization of hazard and dose response.

Another inconsistency between the cancer assessments by the two different routes of exposure is that the derivation of the oral CSF in rats was not based on total tumor risk even though neoplasms were induced at 3 separate sites in male and female rats. To eliminate this inconsistency, a total tumor analysis is recommended for male and female rats exposed to 1,4-dioxane in drinking water. Based on the oral CSFs shown in Table 5-12, it is not likely that the total tumor analyses for rats will produce a more potent CSF than that derived for liver neoplasms in mice.

Frederick J. Miller

Since the MOA for 1,4-dioxane inhalation exposure causing tumors could not be established, the linear extrapolation default approach from the POD was invoked per EPA Cancer Guidelines. The material presented at the meeting by Dr. Michael Doursen of TERA provided a clearer picture of the potential components of the MOA for 1,4-dioxane, and this material should be integrated by the Agency into the MOA discussion. The choice of a 10% increase in tumor incidence over background for modeling purposes is reasonable. The Bayesian approach for estimating the BMDL10 was clearly described and is statistically and scientifically defensible. The use of the AIC to select the best fitting model is well accepted by the statistical community when modeling tumor incidence data arising from exposure-response studies.

While a total tumor analysis was included that yielded similar results to the individual models, the document did not make clear what limitations and assumptions are typically involved when one models the risk of developing any combination of the tumor types. However, this reviewer was pleased to note that the Agency made use of the method described by EPA scientists (i.e., Kopylev et al., 2009) to employ a MCMC approach to estimate composite risk under the assumption of independence. The treatment of the multiple tumor data would be stronger if a couple of paragraphs were added that present the case for treating the 1,4-dioxane tumors sites as independent relative to tumor formation.

As with the RfC, this reviewer does not endorse the Agency's rote procedure of invoking Haber's Law to make adjustments to the length of exposure per day and the number of days per week. For the vast majority of biological endpoints and chemical compounds, the exponent on time (i.e., duration of exposure) in the power law family of curves is less than one while the exponent on concentration is greater than one. In the derivation of the IUR, the Agency is automatically assuming that both exponents are equal to one (i.e., Haber's Law) without any discussion of the reasonableness of this assumption or support from experimental data.

Raghubir P. Sharma

The approaches for deriving IUR have been clearly described and appropriately conducted in the document. In the case of 1,4-dioxane, the biological support to identify key events and how these may be related to the mode of action for carcinogenesis is not yet available. The mode of action for carcinogenesis of this chemical is therefore unknown. The approach that the IUR was derived based on multiple carcinogenic effects observed in rats exposed to 1,4-dioxane via inhalation is therefore appropriate. A Bayesian approach used to estimate a BMDL10 associated with the occurrence of these multiple tumors, and then a linear low-dose extrapolation from this POD was performed to derive the IUR is scientifically appropriate. The use of total tumor analysis for comparative purposes performed with the draft BMDS is also proper.

V. SPECIFIC OBSERVATIONS

James V. Bruckner

Page	Line # or Paragraph	Comment or Question
9	Line 4	The U.S. Army (2010) concluded from a recent study in rats that 1,4-dioxane-2-one (dioxanone) was not a primary metabolite of dioxane, as it was not detected in blood or urine.
10	Line 18	Takano et al. (2010) also recently studied the induction of CYP450 isozymes by dioxane. Male Sprague-Dawley rats were injected i.p. daily for 3 days with dioxane (dosage unclear). CYP2B and CYP2E1 activities in the animals' liver microsomes were significantly increased, but CYP2C activity was substantially reduced.
13	Lines 5 & 6	It is stated here in the document that elimination of 1,4-dioxane from the plasma of rats appeared to be linear for i.v. doses of 3 - 30 mg/kg. Young et al. (1978b), however, reported evidence of metabolic saturation at i.v. doses > 10 mg/kg. Please reconcile the dosage at which saturation was initially manifest in rats.
13	Lines 33-36	It would be worthwhile to point out that the plasma half-life of dioxane in humans inhaling 50 ppm is ~ 1 hour (Young et al., 1977). Thus, dioxane is rapidly and extensively metabolized and eliminated at this exposure level. This half-life is very similar to that reported in rats (Young et al., 1978b).
17	Line 16	Takano et al. (2010) recently published a simplified PBPK model for dioxane. A short description of it should be added, although this model is not likely to be useful in the current risk assessment.
79	Lines 29-36, p. 79-81, lines 1-4	See recommended editorial changes in pages 79-81. These are included in an Appendix to my review.
81	Lines 10-13	What is meant here in saying that metabolism of dioxane "could not be accurately depicted?"
82	Lines 7-9	The words "High doses of 1,4-dioxane were …" should be used at the beginning of this sentence.
82	Line 15	CYPB1/2 should be CYP2B1/2.
82	Line 17	The word "toxic" should be replaced with "oxidative."
82	Lines 24 & 25	The words "accumulation of" should be deleted from this sentence. The word "related" should be replaced with "responsible."
84	Lines 21-23	It is not clear how oral and inhalation kinetic and carcinogenicity data are relevant to dermal exposure. As detailed in lines 8-11 of page 6 of the current document, Marzulli et al. (1981) found that percutaneous absorption of dioxane by monkeys was quite low (2-4%). It appears that insufficient internal doses would be achieved upon dermal exposure to cause cancer in primates. Therefore, it <u>cannot</u> be concluded that dioxane is likely to be carcinogenic in

Page	Line # or Paragraph	Comment or Question
		humans by all routes.
89	Lines 10-14	Nannelli et al. (2005) did postulate that induction of CYP2E1 in target tissues might generate oxygen radical species that could contribute to injury.
90	Lines 13-16	It is stated that "accumulation of dioxane as a precursor event of nasal tumor formation is not supported, because the parent compound was only measured in one subchronic study in which no evidence of nasal cytotoxicity, cell proliferation or nasal tumors was reported". Kasai et al. (2008) did see degenerative changes (atrophy, vacuolation) and decreased cell numbers in the olfactory epithelial and sensory cells. There was not a sufficient duration of dioxane exposure for tumors to develop. The same exposure regimen was utilized by Kasai et al. (2009) in their chronic study. Squamous cell hyperplasia and metaplasia of the nasal and olfactory epithelium accompanied by inflammation and atrophy were manifest at 2 years, often at a lower vapor level than carcinoma.
92	Lines 1-12	The conclusion that "the role of cytotoxicity as a required precursor event is not supported by the data from any of the reviewed studies" is **inconsistent/not supported** by the data described in this paragraph.
95	Lines 6-9	The word "inconclusive" should be replaced by "negative," which were the findings of Buffer et al. (1978) and Thiess et al. (1975). The EPA concluded the studies were inconclusive, because their cohort sizes and numbers of reported cases were small. It is stated in lines 8 and 9 that genotoxicity has not been evaluated in animal studies. Numerous *in vivo* genotoxicity studies are listed in Table 4-24.
95	Line 13, p. 96, lines 1 & 2	Please state whether anatomical differences in the upper respiratory tract would be anticipated to make humans more or less susceptible to dioxane-induced nasal lesions, rather than simply state that uncertainty is introduced.
97	Lines 5 & 6	As related above, numerous animal *in vivo* genotoxicity studies have been conducted and the results published. As listed in Table 4-24, most of these findings were negative.
97	Lines 8-15	It is stated **incorrectly** here that at least one event is missing in the sequence of events observed by Kasai et al. (2009) and Kano et al. (2009) that constitute/support a nasal injury-proliferative regeneration MOA for nasal carcinogenesis. It is stated that Kasai et al. (2009) did not report cytotoxicity. These investigators saw squamous cell metaplasia, which results from squamous epithelial **injury** and subsequent proliferation. It is a common response of the nasal passages of rodents upon chronic exposure to cytotoxic irritants (Monticello et al., 1990). Boatman and Knaak (2001)

Page	Line # or Paragraph	Comment or Question
		described marked sensory irritation in response to dioxane. Vapor concentrations > 200 ppm of dioxane are required to cause eye, nose and throat irritation (Silverman et al., 1946). Inhalation tumorigenic concentrations were many fold greater. Kasai et al. (2009) observed **inflammation** in the respiratory and olfactory epithelial, as well as degenerative changes (i.e., cellular vacuolation and atrophy) in the olfactory epithelium and vacuolation and sclerosis of the lamina propria. These are definitely manifestations of cytotoxicity. Furthermore, it is stated in the document that Kano et al. (2009) did not report hyperplasia in the nasal cavity. This is not accurate. These researchers state the following on page 2780 of their paper: "Proliferative and prenoplastic lesions (squamous cell hyperplasia and squamous cell metaplasia) in the nasal cavity were observed in the 5,000 ppm-dosed group". Nasal squamous cell carcinoma was found only at this highest exposure level. It is clear (i.e., there is reasonable confidence) from the foregoing that Kasai et al. (2009) and Kani et al. (2009) both saw the same, complete sequence, or continuum of events of a cellular injury-regenerative proliferation MOA. Kasai et al. (2009) concluded that nasal tumors resulted primarily from injury of mucosal epithelial cells and subsequent regenerative hyperplasia. Thus, I do not agree with the EPA's conclusion in line 15 of page 97 that the MOA cannot be established. It should also be recognized that the chronic inflammation observed is a critical component of tumorigenesis (Ben-Baruch, 2006; Coussens and Werb, 2002; Kundu et al., 2008).
108	Lines 3-6	It should be stated that the epidemiology studies of Buffer et al. (1978) and Theiss et al. (1976) were negative. An explanation should be given for concluding the studies were inconclusive.
109	Line 18	It is an over simplification to state that the various lesions were (vapor) concentration-dependent. Examination of the data in Table 3 of Kasai et al. (2008), for example, reveals most/all of the 10 rats/group exhibited nuclear enlargement of the respiratory epithelium at all concentrations. The severity of the change was the same (slight) at the four lower vapor levels, but moderate at the two highest levels. Conversely, the number of rats with nuclear enlargement of the olfactory epithelium was concentration-dependent, but the severity of the change was not.
109	Line 32	Substitute "changes" or "lesions" for "incidences."
109	Line 34	Delete the word "respiratory."
113	Lines 33-36	The absence of an anterior to posterior gradient for severity of nasal effects in the 2-year study of Kasai et al. (2009) is cited as evidence that dioxane is not a directly reacting gas. Kasai et al.

Page	Line # or Paragraph	Comment or Question
		(2008), however, stated that the incidence and severity of nuclear enlargement decreased distally through the respiratory tract of rats inhaling dioxane for a shorter period (13 weeks). Boatman and Knaak (2001) characterized dioxane as an irritant gas to mucus membranes. Kasai et al. (2009) reported inflammation of the respiratory and olfactory epithelia. It is likely that all of the respiratory epithelium eventually became involved, because the lesions progressed distally (deeper) during the prolonged duration (2 years) of the latter study. Dioxane is water-miscible, though not water-soluble. It is stable in water.
113	Line 38	See my previous comments on evidence that the parent compound is the proximate irritant/cytotoxicant.

Harvey J. Clewell III

No specific observations provided.

David C. Dorman

Page	Line # or Paragraph	Comment or Question
55	Line 5	Change to … which was determined by the study authors to be unaltered…
81	Line 4	Reference needed.
81	Line 11	Define what is meant by "rat model" – animal model? Kinetic model?
89	Lines 13-14	Needs to be reworded – for example … neither characterized this pathway nor identified possible reactive…
97	Lines 5-7	Was confusing – as written it could imply that the nasal tumors present in exposed people were not evaluated – this does not appear consistent with the available data.
		Although not part of the revised document (inhalation update), there are some areas where additional clarification are warranted: • Redundant text found on pages 13 of the draft document. • Lines 34-35 on Page 13 confusing. • Inconsistent use of exposure metrics (e.g., ppm, mg/kg, mmol/kg (e.g., page 65, lines 13-15), etc) is distracting at best. • Avoid use of the term autopsy (see page 31, line 12) when referring to animal studies – the appropriate term is necropsy (or derivative). • Reference missing – line 34, Page 45 • The presence or absence of nasal cavity tumors was not

		evaluated (page 52, line 14-15) by Torkelson (page 53, line 16) – so the statement that no tumors were observed at this site was misleading. The authors could state that no gross evidence of tumors was seen. • Is the statement "The true NOAEL was likely to be higher" (page 53, line 12) needed? One concern is that portal of entry responses were not evaluated since nasal tissues were not examined in this study. • Page 66, Line 1 – should also cite Table 4-23. • Page 75, line 32 – consider including pneumonia and rhinitis as well?

Ronald L. Melnick

Page	Line # or Paragraph	Comment or Question
6	34	Describe how covalent binding was determined.
8	1 and 11	What was the group size (N) in these studies?
12	Figure 3-3	Since metabolism of 1,4-dioxane can occur in multiple tissues (liver, kidney, respiratory tissues), this figure should not represent metabolism as occurring only in the liver.
15	27-29	Some comment is needed on the practice of changing experimental data to fit the model. EPA needs to develop guidelines for PBPK modeling.
16	33	Provide a citation for the in vitro values.
42, 46	Tables 4-8, 4-9, 4-12, 4-13	Though statistical significance wasn't reported in the document that provided these data, EPA should provide its own analysis.
49	10-11	…during week 12 (delete 13) at 1 hour post-exposure (delete postmortem).
66	9	Define "weakly genotoxic." Does this refer to potency?
67	32-34	Specify the species used in these studies.
68	10-13	List some examples of similar chemicals that support these predictions.
82	29	Change 'accumulation' to 'concentration.'
83;89	32;3	Change 'cell proliferation' to 'hyperplasia.'
84	1	Change 'accumulation' to 'increase.'
87	24-26	Should note that these studies were in rat hepatocytes; there are no mechanistic studies demonstrating cytotoxicity and cell proliferation in mouse hepatocytes at doses that induced hepatocelular neoplasms.
90	11-12	Should note that liver hyperplasia was not seen in mice at dose levels that resulted in tumor formation.

Page	Line # or Paragraph	Comment or Question
91	Table 4-27	What is the purpose of listing metabolism? Is this meant to identify studies that used saturating doses? Surely, metabolism of 1,4-dioxane occurred in all studies. Also, hyperplasia should be distinguished as focal or diffuse. Focal hyperplasia at the end of a 2-year study may be part of the continuum of the process leading to carcinomas. The superscript [c] for adenoma or carcinoma should be changed to statistically significant increase. An increase in tumor incidence that does not reach statistical significance is not "no evidence."
99;138	1;13	Nasal toxicity was also a noncancer health effect.
101	13	Add 'tubule degeneration' after 'cortical.'
105-108	Figure 5-1 – 5-4	Specify the sex and strain of animal and the author of the study which was the source of these data.
116	Figure 5-5	Add 'F344 rats' to the figure title.
138	22-23	Squamous cell hyperplasia is not a degenerative lesion; it is part of the continuum of lesions leading to squamous cell carcinoma. What was the evidence of "preneoplastic cell proliferation" in the nasal cavity in the 2-year inhalation study?
139	21	Delete the word 'conflicting.'
139	27	Change 'are uncertain' to 'lack supporting data.'
140	16	Why is confidence in the Kasai et al study considered to be medium?
143	26	It is not apparent from the Kasai et al. (2008) study that male rats were more sensitive than female rats to effects of 1,4-dioxane following inhalation exposure. The 2-year drinking water studies show similar sensitivity for male and female rats.

Frederick J. Miller

5	13	Assuming 100% lung absorption is not correct for any gas or vapor. However, total respiratory tract absorption of 1,4-dioxane may be close to 100% as reflected by Young et al. (1977) finding over 99% of inhaled 1,4-dioxane appearing in the urine as HEAA after assuming negligible exhalation (see p.8, line 3). This is contrary to his reporting that 65% is exhaled in his empirical model. The poor fits of the PBPK models are due in part to assuming 100% lung absorption when in fact a good portion of the inhaled 1,4-dioxane is removed in the extrathoracic region and is rapidly circulated throughout the body. Moreover, at the review meeting, a public commenter pointed out that they found several errors in the PBPK model code that the Agency had used. These errors need to be corrected and the whole topic of PBPK models needs to be revisited.
B-2	26	The Young empirical model has 65% of 1,4-dioxane exhaled, which is at odds with other study results.
B-3	11	The use of use of 7 L/min for minute ventilation is too low. Young should have used a number in the range of 7.5 to 10.5 L/min.
	Fig. B-4	The right-hand panel has more curves. Recommend eliminating the ones that do not correspond to the curves in the left-hand panel, as the current figure is confusing to the reader.
	All Figures	All figures containing logarithmic scales should have the tick marks so the reader can better determine the values in the plots.
	Figure B-6	The legend for the figure appears on the next page rather than on the page where the model results are shown.
B-9	13	The lack of agreement is obvious because the single and 13-week exposure regimens were highly different relative to the levels of P450 enzymes induced and operative for metabolism.
B-9	14	Assuming a lung dead space volume of 33% is a gross over-estimation; it is less than 10% (e.g., Table 3 of Overton et al. (Toxicol Sci 64:122–134, 2001) gives for Reference Man a tracheobronchial volume of 94.7 ml with a total lower respiratory tract volume of 1113.35 ml, which would yield a lung volume dead space of 8.5 %).

	Table B-1	There is no indication if the error bars correspond to standard deviations or standard errors of the mean. The text implies standard deviations but the original papers do not give this information. In addition, the sample sizes of 14 in the Leung and Pastenbach (1990) and 5 in the Sweeney et al. (2008) studies should be added to the table. This reviewer would prefer that the Agency have used the weighted means of the variables estimated experimentally instead of the "Biologically plausible" values. Lastly, is the PSA value of EPA of 166 a typo? If not, defend why you used a value an order of magnitude lower.
B-14	1	Replace "was" with "were."
B-14	27	Perturbing model parameters by only ± 1% of nominal values is not sufficient for deriving sensitivity coefficients even with scaling the sensitivity coefficients to the nominal value to eliminate the influence of parameter units.
B-16	7	Remove the first comma on this line.
49	21	For the hematological and clinical chemistry variables measured in the Kasai et al. study, Dunnett's test or a Chi-Square test was used to compare treated groups against controls. However, Williams Test (parametric) or Shirley's test (nonparametric) should have been used because there is the expectation of a monotonically increasing or decreasing relationship among treatment groups. These tests identify the first dose such that at that dose and all higher ones, the mean response is different from controls. Moreover, Williams Test has been shown to be more powerful statistically than Dunnett's test. See Williams, D. A. (Biometrics 28:519-531, 1972) and Shirley, E. (Biometrics 33:386-389, 1977).
93	Table 4-28	Footnote "c" contains no text.
95	Last ¶	The text here says the occupational studies show inconclusive results regarding increased tumor risk, but, in earlier sections, these studies were described as finding no statistical increase in tumor incidence. The Agency needs to be consistent on this point.
F-18		For atrophy, why wasn't the highest dose group deleted as was done for Respiratory Metaplasia? The Agency's approach does not appear to be consistent.
112	Table 5-6	It would make it easier for the reader to follow the computations and put the findings into perspective with the experimental data if a column were added to show the POD_{adj} in ppm.

112	3	Haber's Law has been shown to not be applicable to a great number of toxicological responses, and Miller and colleagues (2000) have shown that this Law is merely a special case of the generalized power law family. For a full discussion of this point see their paper (Haber's rule: a special case in a family of curves relating concentration and duration of exposure to a fixed level of response for a given endpoint. Miller et al. Toxicology 149:21–34, 2000). Basically, when there is no threshold, one arrives at Haber's Law when $\alpha = 1$ and $\beta = 1$ in the generalized power law equation given as $C^{\alpha} \times t^{\beta} = k$, where C is concentration, t is duration of exposure, and k is a fixed level of effect. When a threshold exists, then the form is $(C-C_o)^{\alpha} \times t^{\beta} = k$, where Co is a constant representing a threshold concentration below which no response is measureable. Whether the use of Haber's Law provides an automatic margin of protectiveness is entirely dependent upon the values that α and β take on in the power law family of curves. When $\alpha < \beta$, the slope in Figure 9 is < 1 in Miller et al. (2000), so that to the left of the point where the true line crosses Haber's line, the true line lies below Haber's line, implying the use of Haber's Law would over-predict the exposure duration required for a given effect, and hence under-predict risk. In contrast, to the right of the crossing point for $\alpha < \beta$, the true line will lie above Haber's Law, so Haber's Law will predict that the effect will occur in a shorter time than it actually takes; thus, Haber's Law will under-predict the exposure duration needed for a given effect, and hence will over-predict risk. For $\alpha > \beta$, the true line will be steeper than Haber's line, so it will lie above Haber's line to the left of the crossing point and below Haber's line to the right, in which case the over- or under-prediction of risk by use of Haber's Law will be just the opposite of what was stated above. In fact, most toxicological responses relating C and t have $\alpha > 1$ and $\beta < 1$, showing that exposure concentration is more important than length of exposure in producing adverse effects.

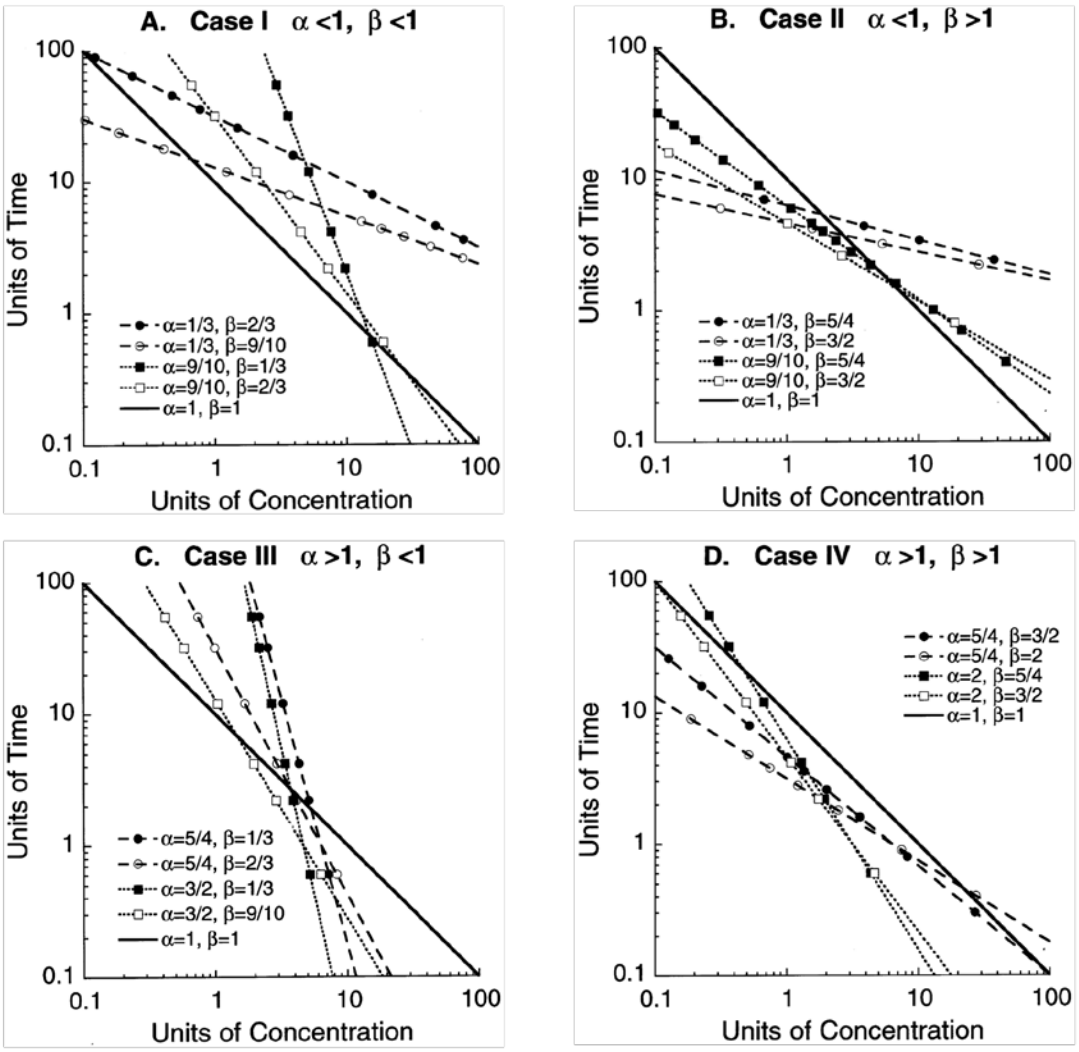

Fig. 9. Log time–log concentration plots for the general power law family, $C^{\alpha}\, t^{\beta} = k$. The panels depict the four combinations of α and β. Included for reference is the line of identity (Haber's rule) corresponding to $\alpha = \beta = 1$. See text for discussion of identifiability and overparameterization for $C^{\alpha}\, t^{\beta} = k$ in two-dimensional plots unless one of the parameters is fixed (here k was fixed at 10 for all plots).

Page	Line #	Comment or Question
G-2	6-12	The formula for the RGDR uses default SA values of 15 cm^2 and 200 cm^2 for rats and humans, respectively, for the surface area of the extrathoracic region (ET). While 15 cm^2 is a reasonable default value for the ET surface area in an adult rat, the default value of 200 cm^2 is not reasonable for an adult human. The 200 cm^2 is only taking into account the SA of the nasal cavity, which includes from the nares to the posterior end of the nasal septum. Garcia et al. (J. Aerosol Med. & Pulmonary Drug Delivery 22(2):139-155, 2009) studied 4 healthy adult noses and found an average of 201.2 cm^2 with a std. dev. of 26.8 for this portion of the ET; Using the same noses but a different mesh, Garcia et al. reported a mean of 201.7 cm^2 with a std. dev. of 26.9 in 2009 (J. Aerosol Med. Pul. Drug Delivery. 22(2):139-155). The nasopharynx, oropharynx, and larynx SA need to be added to this value to obtain a SA estimate of the ET region for humans. This additional SA is approximately 100 cm^2 based on analyzing the serial step section data in Table 3 in Cheng et al. (Aerosol Sci. Technol. 31:286-300, 1999), Ménache et al. (Inhal. Toxicol. 50:475-506, 1997), and other data in the open literature (i.e., the oropharynx and larynx are about 50 cm^2). The SA of the nasopharynx is also about 50 cm^2 as reported by Chew [Chew, C.T. (online citation) Chapter 19: Nasopharynx (the postnasal space) http:famona.sezampro.rs/medifiles/OTOHNS/SCOTT/scott419.pdf]. Thus, the default value for the SA of the ET for humans is about 300 cm^2. Using this value in the computation of the RGDR gives a value of 0.368 rather than 0.25 and results in a POD of 11.85 mg/m^3 rather than 8.9 mg/m^3. Now this is using a minute ventilation for humans of 13.8 L/min, which is also scientifically indefensible (see the paragraph below). A more defensible range for minute ventilation is 7.5 to 10.5 L/min. If these values are used together with the 300 cm^2 surface area value, the RGDR would be 0.484 to 0.677 and the POD range would be 15.7 to 21.8 mg/m^3. The default value of 13.8 L/min for minute ventilation over the course of an entire day in humans is not physiologically defensible for the vast majority (i.e., > 98%) of the population. The Agency has been using a value of 20 m^3 of air inhaled per day, which gives the 13.8 L/min value. It was derived from an aggregation of the minute ventilation over some daily activity pattern relative to total volume of air inhaled and then partitioned up as if that were the minute ventilation for each minute of the day. It is an extreme value achievable by few individuals and should not be the basis for the population estimate. For at least 90 to 95% of the day, adult humans will have minute ventilation rates between 7.5 and 10.5 L/min. The actual ET uptake mass will differ significantly

Page	Line #	Comment or Question
		compared to using a daily value of 13.8 L/min. If the Agency wants to capture the variability in exercise level and duration, then a probabilistic sampling from activity pattern data should be conducted. The net effect of using the physiologically unreasonable value of 13.8 L/min is to lower inappropriately the POD by about a factor of 2 to 3.
114	5	A UF value of 1000 appears in the RFC derivation formula, but the UF values in the text following the equation result in a value of 900. Using 1000 gives a value of 8.9 ppb and is 10% lower than the computationally correct value of 9.9 ppb based upon the discussion in the text. The Agency needs to be consistent and not have the equation imply one thing and the supporting text another.
116	Figure 5-5	At a minimum, the respiratory metaplasia and atrophy bars are not plotted correctly. Using a logarithmic scale, the value 0.032 for these endpoints is not located correctly in the figure.
126	23	Again, the conversion of POD levels for tumor incidence invoking Haber's Law as an assumption.
143	18	The material is somewhat contradictory to the text appearing on page 98, line 22. In the subchronic exposure, female rats were reported by Kasai et al. (2008) to be more susceptible to 1,4-dioxane inhalation than male rats as far as kidney damage was concerned. Yet on this page the Agency states that Kasai et al. (2008) showed male rats were more sensitive than female rats to the effects of 1,4-dioxane by inhalation and that this was the justification for using only male rats in the chronic exposure inhalation study.

Raghubir P. Sharma

No specific observations provided.

APPENDIX A – ORIGINAL DOCUMENT SHOWING COMMENTS BY JAMES V. BRUCKNER

was not considered indicative of noncancer effects. The activity of serum enzymes (i.e., AST, ALT, LDH, and ALP) was increased in rats and mice exposed to 1,4-dioxane, although only in groups with high incidence of liver tumors. Blood samples were collected only at the end of the 2-year study, so altered serum chemistry may be associated with the tumorigenic changes in the liver.

Hematological changes were reported in the JBRC (1998) study only. Mean doses are reported based on information provided in Kano et al. (2009). Observed increases in RBCs, hematocrit, hemoglobin in high-dose male mice (677 mg/kg-day) may be related to lower drinking water consumption (74% of control drinking water intake). Hematological effects noted in male rats given 55 mg/kg-day (decreased RBCs, hemoglobin, hematocrit, increased platelets) were within 20% of control values. A reference range database for hematological effects in laboratory animals (Wolford et al., 1986) indicates that a 20% change in these parameters may fall within a normal range (10th–90th percentile values) and may not represent a treatment-related effect of concern.

Rhinitis and inflammation of the nasal cavity were reported in both the NCI (1978) (mice only, dose ≥ 380 mg/kg-day) and JBRC (1998) studies (≥ 274 mg/kg-day in rats, >278 mg/kg-day in mice). The JBRC (1998) study also demonstrates atrophy of the nasal epithelium and adhesion in rats and mice. Nasal inflammation may be a response to direct contact of the nasal mucosa with drinking water containing 1,4-dioxane (Sweeney et al., 2008; Goldsworthy et al., 1991) or could result from systemic exposure. Regardless, inflammation may indicate toxicity due to 1,4-dioxane exposure. A significant increase in the incidence of pneumonia was reported in mice from the NCI (1978) study. The significance of this effect is unclear, as it was not observed in other studies that evaluated lung histopathology (Kano et al., 2008; JBRC, 1998; Kociba et al., 1974). No studies were available regarding the potential for 1,4-dioxane to cause immunological effects. Metaplasia and hyperplasia of the nasal epithelium were also observed in high-dose male and female rats (JBRC, 1998); however, these effects are likely to be associated with the formation of nasal cavity tumors in these dose groups. Nuclear enlargement of the nasal olfactory epithelium was observed at a dose of 83 mg/kg-day in female rats (Kano et al., 2009); however, it is unclear whether this alteration represents an adverse toxicological effect. Nuclear enlargement of the tracheal and bronchial epithelium and an accumulation of foamy cells in the lung were also seen in male and female mice give 1,4-dioxane at doses of ≥ 278 mg/kg for 2 years (JBRC, 1998).

4.6.2 Inhalation

Two subchronic (Kasai et al., 2008; Fairley et al., 1934) and two chronic inhalation studies (Kasai et al., 2009; Torkelson et al., 1974) were identified. Nasal, liver, and kidney toxicity were the primary noncancer health effects of inhalation exposure to 1,4-dioxane in ~~animals~~ rodents. Table 4-26 presents a summary of the noncancer results for the subchronic and chronic inhalation studies of 1,4-dioxane toxicity in laboratory animals.

Of the inhalation studies, nasal tissue was only ~~collected~~ evaluated in rat studies conducted by Kasai et al. (2009; 2008). ~~Damage to~~ Adverse effects in nasal tissue ~~was reported~~ were observed frequently in these studies, and statistically significant ~~observations~~ changes were noted at concentrations as low as 50 ppm. Nasal effects included deformity of the nose and

histopathological ~~lesions~~ changes characterized by enlarged epithelial nuclei (respiratory epithelium, olfactory epithelium, trachea, and bronchus), atrophy (olfactory epithelium), vacuolic change (olfactory epithelium and bronchial epithelium), squamous cell metaplasia and hyperplasia (respiratory epithelium), respiratory metaplasia (olfactory epithelium), inflammation (respiratory and olfactory epithelium), hydropic change (lamina propria), and sclerosis (lamina propria). In both studies, a concentration-dependent, statistically significant ~~change in~~ incidence of enlarged nuclei of the respiratory epithelium ~~was considered the most sensitive nasal effect~~ were reported by the study authors; however, the toxicological significance of nuclear enlargement is uncertain.

At high doses, liver damage was characterized by ~~cell~~ hepatocellular degeneration which varied from swelling (Kasai et al., 2008; Fairley et al., 1934) to necrosis (Kasai et al., 2009; Kasai et al., 2008; Fairley et al., 1934), spongiosis hepatis (Kasai et al., 2009), nuclear enlargement of centrilobular cells (Kasai et al., 2009) and basophilic and acidophilic cell foci (Kasai et al., 2009). ~~Altered~~ GST-P positive cell foci are commonly considered preneoplastic changes and would not be considered evidence of noncancer toxicity when observed in conjunction with liver tumor formation (Bannasch et al., 1982). Since exposure to 1,4-dioxane resulted in tumor formation in the liver, these lesions are not considered as potential noncancer toxicity.

At concentrations ranging from 200 ppm to 3,200 ppm, altered liver enzymes (i.e., AST, ALT, ALP, and γ-GTP), increased liver weights, and induction of GST-P ~~was~~ were also observed (Kasai et al., 2009; Kasai et al., 2008). Changes in the activity of serum enzymes were mostly observed in exposed rat groups ~~of~~ at high 1,4-dioxane concentrations (Kasai et al., 2009; Kasai et al., 2008). Induction of GST-P positive hepatocytes was observed in female rats at 1,600 ppm and male and female rats at 3,200 ppm following 13 weeks of exposure to 1,4-dioxane. GST-P is considered a good enzymatic marker for early detection of chemical hepatocarcinogenesis (Sato, 1989). Although, GST-P positive liver foci were not observed in the 2-year bioassay, the focally and proliferating GST-P positive hepatocytes noted in the 13-week study suggests eventual progression to hepatocellular tumors after 2 years of exposure and therefore would not be considered a potential noncancer effect.

The lowest concentration reported to produce liver lesions was 1,250 ppm, characterized by ~~necrosis of~~ vapor tic necrosis of centrilobular cells, spongiosis hepatis, and nuclear enlargement in the Kasai et al. (2009) study. However, as previously stated, the toxicological significance of nuclear enlargement after 2 years of exposure the lesions were lesions is uncertain.

Kidney effects were reported less frequently in these inhalation studies and were generally observed at higher exposure concentrations than nasal and liver effects. Kidney damage was described as patchy degeneration of cortical tubules with vascular congestion and hemorrhage (Fairley et al., 1934), hydropic change of proximal tubules (Kasai et al., 2009; Kasai et al., 2008), and as nuclear enlargement ~~of~~ in proximal tubules cells (Kasai et al., 2009). Changes in serum chemistry and urinalysis ~~variables~~ indices were also noted as evidence of renal damage. In a 13-week inhalation study of male and female rats (Kasai et al., 2008) kidney toxicity was only observed in female rats exposed to 3,200 ppm of 1,4-dioxane (i.e. hydropic change in the renal proximal tubules), which suggests ~~a possible increased~~ greater susceptibility of female rats to renal damage following inhalation exposure ~~to~~ of 1,4-dioxane.

Other noted noncancer effects in laboratory animals included acute vascular congestion of the lungs (Fairley et al., 1934); changes in relative lung weights (Kasai et al., 2008); and decrease in body

weight gain (Kasai et al., 2009; Kasai et al., 2008). Following a 13-week exposure, higher 1,4-dioxane plasma levels were found in female ~~rats as compared to~~ than male rats (Kasai et al., 2008). 1,4-Dioxane was ~~observed~~ measured in plasma along with systemic effects following subchronic inhalation exposure to 1,4-dioxane in rats.

Table 4-26 Inhalation toxicity studies (noncancer effects) for 1,4-dioxane

Species	Dose/duration	NOAEL (ppm)	LOAEL (ppm)	Effect	Reference
Subchronic studies					
Rat, mouse, rabbit, and guinea pig (3-6/species/group); unknown strains	0, 1,000, 2,000, 5,000, or 10,000 ppm for 7 days. Days 1-5, two 1.5 hour exposures; day 6, one 1.5 hour exposure; and day 7, no exposure	NA	1,000	Renal cortical degeneration and hemorrhage; hepatocellular degeneration and necrosis	Fairley et al. (1934)
F344/DuCrj rat (10/sex/group)	0, 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm 6 hours/day 5 days/wk, for 13 wk	NA	100	Respiratory epithelium: nuclear enlargement of epithelial cells	Kasai et al. (2008)
Chronic studies					
Wistar rat (288/sex)	111 ppm for 7hours/day, 5days/wk, for 2 years	111 (free standing)	NA	No significant effects were observed on BWs, survival, organ weights, hematology, clinical chemistry, or histopathology	Torkelson et al. (1974)
F344/DuCrj male rat (50/group)	0, 50, 250, or 1,250 ppm for 6 hours/day, 5 days/wk for 2 years	N/A	50	Respiratory epithelium: nuclear enlargement of epithelial cells, atrophy, and metaplasia	Kasai et al. (2009)

4.6.2.1 Mode of Action Information

The metabolism of 1,4-dioxane in humans was extensive at low doses (<50 ppm). The linear elimination of 1,4-dioxane in both plasma and urine indicated that 1,4-dioxane metabolism was a nonsaturated, first-order process at this exposure level (Young et al., 1977; 1976). Like humans, rats extensively metabolized inhaled 1,4-dioxane; however, plasma data from rats given single i.v. doses of 3, 10, 30, 100, or 1,000 mg [^{14}C]-1,4-dioxane/kg demonstrated a dose-related shift from linear, first-order to nonlinear, saturable metabolism of 1,4-dioxane (Young et al., 1978a; 1978b). Conversely, using the Young et al. (1978b; 1978a) rat model, the metabolism of 1,4-dioxane in rats that were exposed to 400, 800, 1,600, and 3,200 ppm via inhalation for 13 weeks could not be accurately depicted due to a lack of knowledge on needed model parameters and biological processes (See Section 3.5.3 and Appendix B). It appears, following prolonged inhalation exposure to 1,4-dioxane at concentrations up to 3,200 ppm, that metabolism is induced (Appendix B).