

<p align="center">Department of Defense Comments on 1,4-Dioxane Draft Toxicological Review</p>					
<p>Comments submitted by: Chemical Material Risk Management Directorate</p>		<p>Organization: Department of Defense</p>		<p>Date Submitted: 7/25/2013</p>	
<p>*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.</p>					
Comment No.	Section	Pages	Comment	Suggested Action, Revision and References (if necessary)	*Category
1	3	8-19	<p>In the "External Peer Review of the Toxicological Review of 1,4-Dioxane", Raghbir P. Sharma raises the issue of saturation of 1,4-dioxane metabolism occurring >50 ppm, therefore making lower levels exposures of this chemical unlikely to pose a quantifiable risk. James Bruckner also raises the question of a threshold for cytotoxicity/carcinogenicity due to metabolic saturation, and reiterates the data supporting 1,4-dioxane as the proximate carcinogen. These comments are different than questions related to defining the 1,4-dioxane carcinogenic MOA, and EPA has not adequately addressed these concerns within the Toxicological Review or Appendix A responses.</p>	<p>Please address reviewer concerns regarding near complete metabolism of 1,4-dioxane to a non-toxic metabolite (HEAA) at low-level exposures. Even if EPA does not consider this metabolic threshold to affect the derivation of the RfC or IUR, this information would be extremely useful for low-level environmental exposures.</p>	S
2	3.3	8	<p>The review has not taken note of a paper addressing chemical stability of 2-dioxanone that has a major impact on the metabolism discussion: Koissi et al. 2012. Chem.Res.Toxicol. 25:1022-1028. This</p>	<p>Please add the reference and include its findings in the discussion.</p>	S

			publication addresses a significant shortfall in the understanding of dioxane metabolism and its implications for carcinogenicity.		
3	4.2.2.1.2	50	In line 3 of this section, the dosing values contain a typographical error (“0, 360, 720, 1,400, 2,900, 5,800, 1,2000, and 23,000”).	Correct the comma placement in the 12,000 value.	E
4	4.5.1	70	Line 9. In mutagenicity testing, use of a closed system to control for evaporation would be likely to increase exposure. It is not clear why the phrase was added in the redline version and why it implies that controlling evaporation is a problem.	Since mutagenicity of 1,4-dioxane generally does not occur, even when evaporation is restricted, this is another factor that argues against carcinogenicity of dioxane. Discussion should reflect this fact.	S
5	4.7.3.1.1	89 and 90	Figure 4.1 does not seem to match the text lines 16-19, which states “Nannelli et al. (2005a) demonstrated that an increase in the oxidative metabolism of 1,4-dioxane via CYP450 induction using phenobarbital or fasting does not result in an increase in liver toxicity. This result suggested that the highly reactive intermediates did not play a large role in the liver toxicity of 1,4-dioxane, even under conditions where metabolism was enhanced.” If CYP metabolism does not “play a large role” we are unsure how it could be a “possible key event” worthy of the figure.	If CYP450 metabolism and resulting highly reactive metabolites “did not play a large role” in liver toxicity, then we suggest it be downplayed, a footnote added, or remove the suggestion from Figure 4.1 that CYP450 (specifically, CYP2E1 and CYP2B1/2) metabolism is a “possible key event” as the Figure 4.1 title suggests.	S
6	4.7.3.1.2	91	We believe that the same level of MOA knowledge for inhalation carcinogenicity is presented to create a parallel IUR figure as Figure 4-1 for oral carcinogenicity.	Suggest that a Figure 4.2 be added. If the MOA is unknown for inhalation, then downgrade the Fact Sheet and IUR discussion that links the “multiple study” endpoints to “nasal squamous cell carcinomas” as we don’t yet understand	S

				enough to know how those are formed.	
7	5.2.4	114	<p>A total UF of 1000 is applied to the inhalation RfC for 1,4-dioxane. Although the reviewers generally agreed with the selection of UFs, there were numerous questions and requests for clarification. Several reviewers specifically did not believe that the justification of the database UF was adequately supported. We believe that EPA could more fully respond to these requests for additional clarification and justification. EPA added the statement “The authors found statistically significant changes in fetal body weight at the highest dose group and reduced ossification of the sternbrae”, as justification for the UFD of 3. However, a reduction in maternal body weight gain (and concomitant lower food consumption) was observed in the high-dose group dams, and it is unknown if the reduced ossification of the sternbrae was indicative of a true developmental defect, or was simply a developmental delay, as suggested by Giavini et al. DoD agrees with Dr. Bruckner that these effects are “unremarkable” and have questionable toxicological significance. Further, no effects were seen in reproductive organs in numerous oral or inhalation studies. Additionally, the reference provided in the response to comments (Valcke and Krishnan 2011) that assessed the neonate versus adult 1,4-dioxane blood concentration ratio does not seem to have been considered within the uncertainty for inter-</p>	<p>Please consider whether the UFD of 3 is truly necessary given the entire 1,4-dioxane database and postulated MOAs and consider whether chemical-specific information can be used to develop 1,4-dioxane specific uncertainty factors.</p>	S/M

			individual differences.		
8	5.4.4.2	129; 15-17	All possible permutations of the multistage model for cancer dose-response were tried before considering other models that may provide a better fit.	Absent biologically directed model selection, we suggest that all BMD models be examined for fit as is done with the noncancer effects.	S
9	A.3	A-20, A-24, A-29	There are several editorial issues related to citations and HERO links; pgs. A-20, A-24, A-29.	Please revise/correct.	E
10	A.3.1	A-20; 5-6	<p>“While it is important for risk assessors to understand ambient exposure levels in utilization of IRIS reference values, ambient exposure levels are dependent upon location and media and thus are not included in IRIS assessments”.</p> <p>We believe ambient levels have been used in some IRIS assessments, e.g., for the noncancer risks of dioxin. Additionally, we agree with the NAS that chemical assessments should include a problem formulation step and that some consideration of ambient air, and environmental concentrations of 1,4-dioxane would play a role in problem formulation. Section 6.4 of the ATSDR Toxicological Profile for 1,4-Dioxane (2012) contains useful information relative to environmental concentrations.</p>	We recommend that EPA check other assessments to determine whether this unequivocal statement might warrant some editing and also suggest that problem formulation be considered in future IRIS reviews.	S
11	A.3.1	A-20; 4	<p>“This departure resulted in a necessary and significant difference in approaches.” This statement is only true because IRIS only considers models other than the multistage if it cannot find any version of the multistage model (including the dropping of one or two high dose</p>	It would be useful if EPA would provide an explanation of why the “best fit” was used instead of determining if one model for a specific tumor type provided an adequate fit for systemic cancer. Since “best fit” can depend on many factors, e.g., differences in dose levels or	S

			<p>responses) that will fulfill its criteria for a sufficiently good fit. It generally seems biologically implausible for the same chemical or metabolite to have a different dose-response function for systemic tumors based on route of exposure. This is supported by EPA's response on page A-26; 1-2 that "the tumors that were observed in animals were systemic and independent of the route of exposure." How can tumors that are independent of the route of exposure have different dose-response functions depending on the route of exposure? Perhaps because the external peer reviews of oral and inhalation cancer risks were the focus of two different meetings, this issue was not apparent to the external peer reviewers.</p>	<p>days/week of exposure by route of exposure, and since models other than the multistage might have a better fit unless no multistage model can be made to fit, it would seem that "adequate fit" with biological consistency would be a better criterion.</p>	
12	A.3.1	A-21	<p>One of the peer reviewers suggested a paper on studies of metabolism of 1,4-dioxane be considered. We do not consider the response ".a report that has not undergone formal peer-review and thus, is generally not considered in the development of an IRIS assessment" adequate. We believe the publication may be able to shed some light on the MOA. Additionally the systematic review process EPA is starting to use on assessments allows for use of unpublished studies, it seems inconsistent to dismiss the paper solely on this basis.</p>	<p>Please reconsider the use of the report titled "Studies on Metabolism of 1,4-Dioxane" and include a more substantial justification if it is not used in the assessment.</p>	S/M
13	A.3.1	A-21; 6-10	<p>Even though IARC reports undergo external peer review, EPA chose not to cite it because it was</p>	<p>If the criterion is external peer review, the results of IARC and other national and international</p>	S

			<p>“produced by an organization other than the U.S. EPA”. This seems inconsistent with other IRIS documents where publications produced by states have been referenced, hexavalent chromium for example.</p>	<p>bodies should be included.</p>	
14	A.3.3	A-26	<p>EPA has inadequately responded to peer review questions regarding the carcinogenic MOA for 1,4-dioxane and the related low-dose cancer slope modeling methodology. The data limitations preventing nonlinear extrapolation have not been well described. EPA 2005 Cancer Guidelines, Section 3.3.4 state “Nonlinear extrapolation having a significant biological support may be presented in addition to a linear approach when the available data and weight of evidence evaluation support a nonlinear approach, but the data are not strong enough to ascertain the mode of action applying the Agency’s mode of action framework.” We believe this is the case for 1,4-dioxane, and by presenting the nonlinear extrapolation, EPA would be able to clearly demonstrate the data limitations.</p>	<p>Per EPA’s 2005 Cancer Guidelines, we suggest that both a linear and nonlinear extrapolation for the carcinogenic effects of 1,4-dioxane be presented.</p>	S/M
16	A.3.3	A-28;36	<p>An “invalid citation” is included in the text.</p>	<p>Please provide more information and/or correct.</p>	E
17	A.3.3	A-29;34-40	<p>This response implies that EPA has been using a model (Bayesian WinBUGS) in this and other IRIS documents that has not undergone external peer review.</p>	<p>If this inference is accurate, we recommend the model be externally peer reviewed.</p>	S
18	A.3.3	A-30; 6	<p>The statement “multistage model was not the</p>	<p>While a lack of fit means that the multistage</p>	E

			best fitting model for female mouse liver tumors” is not entirely true. The document states that no multistage model fit the female mouse liver tumor data.	model was not the “best” fit, the statement should be revised to be more precise and transparent.	
19	A.4.2	A-31; 27-28	The statement “inflammation by itself is not direct evidence of cytotoxicity.” is not supported by a reference.	Please include a reference for this statement.	S
20	A.4.2	A-34;19-23	That exposure to a concentration for 13 weeks has no overt toxicity does not mean that the MTD for a 2-year cancer bioassay has not been exceeded. For example, several NTP studies, including the first 2 for perchlorethylene, had no toxicity for 90 days but very high toxicities in less than 2 years.	Please reconsider the response to this comment and the text in this paragraph.	S