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To: Dr. Deborah Cory-Slechta, Chair  
U.S. Environmental Protection Agency Science Advisory Board (SAB)  
Trichloroethylene Review Panel

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RE: Questions and clarifications related to SAB draft Review of EPA's Draft Assessment  
entitled "Toxicological Review of Trichloroethylene"

We are pleased to have had a chance to read the SAB panel's August 25, 2010 draft Review of EPA's draft "Toxicological Review of Trichloroethylene." We sincerely appreciate the time and attention that the SAB panel has taken in its review, particularly given the length and complexity of our draft assessment. We are also grateful for the opportunity to provide some questions seeking clarification of certain points in the panel's draft review.

The following table identifies areas in the draft Review that we think could benefit from some additional clarification to help us better understand and respond to the panel's recommendations. We would appreciate your conveying the following questions and clarifications to the members of the SAB Panel in advance of the scheduled teleconference on Monday, September 13, 2010. These will also form the basis of my oral remarks to the panel during the teleconference. We can also answer any questions from the panel regarding our comments at that time.

Questions and clarifications regarding SAB August 25, 2010 draft review of EPA’s draft Toxicological Review of Trichloroethylene

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<p>ii, lines 22-23</p> <p>and</p> <p>2, line 46 to 3, line 4</p> <p>and</p> <p>31, lines 11-22</p>	<p>EPA concluded that TCE-induced kidney tumors were mediated solely by a mutagenic mode of action (MOA).</p> <p>...</p> <p>However, the Panel concluded that the weight of evidence for the MOA for TCE-induced kidney tumors also involved cytotoxicity and compensatory cell proliferation and including these may more accurately reflect kidney tumor formation than does a mutagenic mechanism alone. The combination of cytotoxicity, proliferation and DNA damage together may be a much stronger MOA than any individual components.</p> <p>...</p> <p>The panel agreed that the weight of evidence supported a mutagenic MOA for TCE induced kidney tumors. However, the panel concluded that the weight of evidence did not exclude the MOA for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation and including this MOA may more accurately reflect kidney tumor formation than a mutagenic mechanism alone. Furthermore, the combination of cytotoxicity, proliferation and DNA damage together may be a much stronger MOA than the individual components.</p> <p>Recommendations:</p> <ul style="list-style-type: none"> <li>• modify the relevant text to reflect that the available data do, in fact, provide support for TCE-induced kidney tumors involving cytotoxicity and compensatory cell proliferation, possibly in combination with a mutagenic MOA, although not to the extent that support for a mutagenic MOA was provided.</li> </ul>	<p><b>Can the panel provide more specific details as to the recommendation?</b></p> <p>(i) Is the panel suggesting that EPA clarify that one or more MOA may be operative (independently or in combination) for TCE-induced kidney tumors, and that the MOA involving cytotoxicity is “not excluded” (independently or in combination with a mutagenic MOA)?</p> <p>(ii) Alternatively, is the panel suggesting a different characterization of the weight of the evidence for a MOA involving cytotoxicity and compensatory cell proliferation? For example, disagreement with the conclusion that there is inadequate experimental evidence “linking TCE nephrotoxicity and sustained cellular proliferation to TCE-induced nephrocarcinogenicity.”</p> <p>As a point of clarification with respect to (i), the draft Tox Review concludes that a mutagenic MOA “is operative” – meaning that it contributes (but not necessarily wholly) to the carcinogenic response – and deliberately avoids use of the word “solely” or similar terms for this MOA. See Page 4-210, lines 16-24:</p> <p>Although not encompassing all of the actions of TCE and its metabolites that may be involved in the formation and progression of neoplasia, available evidence supports the conclusion that a mutagenic MOA mediated by the TCE GSH-conjugation metabolites (predominantly DCVC) is operative in TCE-induced kidney cancer. This conclusion is based on substantial evidence that these metabolites are genotoxic and</p>

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		<p>are delivered to or produced in the kidney, including evidence of kidney-specific genotoxicity following in vivo exposure to TCE or DCVC. Cytotoxicity caused by DCVC leading to compensatory cellular proliferation is also a potential MOA in renal carcinogenesis, but available evidence is inadequate to conclude that this MOA is operative, either together with or independent of a mutagenic MOA.</p> <p>As a point of clarification with respect to (ii), the draft Tox Review did not state that a MOA involving cytotoxicity and compensatory cell proliferation was “excluded.” Instead, it concluded that there was inadequate experimental evidence to demonstrate that it was “operative” (as defined above). See page 4-203, lines 6-14:</p> <p>Evidence for the hypothesized MOA consist primarily of (1) the demonstration of nephrotoxicity following TCE exposure at current occupational limits in human studies and chronic TCE exposure in animal studies; (2) the relatively high potential of the TCE metabolite DCVC to cause nephrotoxicity; and (3) toxicokinetic data demonstrating that DCVC is formed in the kidney following TCE exposure. Data on nephrotoxicity of TCE and DCVC are discussed in more detail below, while the toxicokinetic data were summarized previously in the discussion of mutagenicity. However, there is a lack of experimental support linking TCE nephrotoxicity and sustained cellular proliferation to TCE-induced nephrocarcinogenicity.</p>
2, lines 5-8 and other places	One issue of concern was the inconsistencies between estimated levels of S-dichlorovinyl glutathione (DCVG, a glutathione conjugation pathway metabolite) produced in rats and mice by Lash et al. (1999a) as compared to Green et al. (1997a).	<p>Lash et al. (1999a) only reports data in humans, not data in rats and mice. <b>Can the panel clarify which Lash study is being compared to Green et al. (1997a)? The following Lash studies related to GSH metabolism were cited in the draft assessment:</b></p> <p>Lash, LH; Xu, Y; Elfarra, AA; et al. (1995) Glutathione-dependent metabolism of trichloroethylene in isolated liver</p>

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in text		<p>and kidney cells of rats and its role in mitochondrial and cellular toxicity. Drug Metab Dispos 23:846–853.</p> <p>Lash, LH; Qian, W; Putt, DA; et al. (1998) Glutathione conjugation of trichloroethylene in rats and mice: sex-, species-, and tissue-dependent differences. Drug Metab Dispos 26(1):12–19.</p> <p>Lash, L H; Visarius, TM; Sall, JM; et al. (1998) Cellular and subcellular heterogeneity of glutathione metabolism and transport in rat kidney cells. Toxicology 130:1–15.</p> <p>Lash, LH; Lipscomb, JC; Putt, DA; et al. (1999a) Glutathione conjugation of trichloroethylene in human liver and kidney: kinetics and individual variation. Drug Metab Dispos 27(3):351–359.</p> <p>Lash, LH; Putt, DA; Brashear, WT; et al. (1999b) Identification of S-(1,2-dichlorovinyl)glutathione in the blood of human volunteers exposed to trichloroethylene. J Toxicol Environ Health A 56:1–21.</p> <p>Lash, LH; Fisher, JW; Lipscomb, JC; et al. (2000a) Metabolism of trichloroethylene. Environ Health Perspect 108(Suppl. 2):177–200.</p> <p>Lash, LH; Putt, DA; Parker, JC. (2006) Metabolism and tissue distribution of orally administered trichloroethylene in male and female rats: identification of glutathione- and cytochrome P-450-derived metabolites in liver, kidney, blood, and urine. J Toxicol Env Health A 69(13):1285-1309.</p> <p>Lash, LH; Putt, DA; Huang, P; et al. (2007) Modulation of hepatic and renal metabolism and toxicity of trichloroethylene and perchloroethylene by alterations in status of cytochrome P450 and glutathione. Toxicology 235(1–2):11–26.</p>

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<p>2, lines 8-10</p> <p>and</p> <p>17, lines 17-21</p>	<p>The Panel recommended that the interpretation of DCVG levels from Lash et al. (1999a) paper be made with caution, since the data from Green et al. (1997a) was more consistent with observed kidney effect differences between rats and mice.</p> <p>...</p> <p>The data of Green et al., 1997a, which measured DCVG production by <sup>14</sup>C TCE and radiochemical detection followed by mass spectrometry identification of the metabolites, had lower DCVG production levels than reported by Lash, but the level of DCVG production demonstrated that rats should be more susceptible to TCE nephrotoxicity than mice, consistent with what was observed. Thus, the values of DCVG produced in the Green et al. study may better reflect the level of DCVG produced.</p>	<p><b>Can the panel clarify the statements that “data from Green et al. (1997a) was more consistent with observed kidney effect differences between rats and mice” and that “Green et al., 1997a, ... demonstrated that rats should be more susceptible to TCE nephrotoxicity than mice, consistent with what was observed?”</b></p> <p><b>We read Green et al. (1997a) as stating that their data do not correlate with interspecies differences in carcinogenic response, for example based on the following:</b></p> <p>Green et al. (1997a) P 113 The rates of glutathione conjugation of trichloroethylene found in this study, 2.5, 1.6 and 0.19 pmol/min/mg protein for mouse, rat and human liver respectively...</p> <p>Green et al. (1997a) P 115 Perhaps more importantly, based on the data available, the [GSH conjugation] pathway does not correlate with the species differences in renal carcinogenicity observed in rats and mice, the pathway occurring to a greater extent in mice, and this species being more susceptible in vivo to both DCVC, and in the studies by Eyre et al. [20,21], to trichloroethylene.</p>
<p>2, lines 39-41</p> <p>and</p> <p>28, lines 40-42</p>	<p>At a minimum, a more complete discussion of the strengths and limitations of the analytical methodologies used should be provided to address the large discrepancies in estimates of DCVG formation.</p> <p>...</p> <p>From a strictly scientific perspective however, at a minimum, such large literature disparities call for a more complete discussion of the strengths and limitations of the analytical methodologies used than what is described in the review.</p>	<p><b>Can the panel provide specific references/sources to assist EPA in making such a comparison?</b></p>

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<p>P3, lines 15-16</p> <p>And</p> <p>P32, lines 38 - 40</p>	<p>The Panel also recommended a more robust discussion on the MOA for TCE-induced non-cancer and cancer effects on the lungs.</p> <p>• A stronger discussion on the MOA for lung non-cancer and cancer effects should be included in Section 4.7.4 (Lung), and the data for chloral hydrate should be given more emphasis.</p>	<p><b>Can the panel provide more details as to the recommendation? For instance, is the panel recommending a different weight of evidence conclusion for one or more of the hypothesized MOAs – either based on different criteria or different evaluation of the experimental evidence with respect to those criteria?</b></p>
<p>3, lines 23-24</p> <p>and</p> <p>33, lines 30-33</p>	<p>However, the Panel disagreed with EPA’s conclusion that toxicokinetic variability can be adequately quantified using existing data.</p> <p>...</p> <p>The Panel disagreed with the statement that “toxicokinetic variability in adults can be quantified given the existing data,” as the main study characterizing toxicokinetic variability in adults was small (n&lt;100) and was composed of subjects selected non-randomly.</p>	<p><b>Can the panel clarify this comment? We realize that the Charge question is confusing because the quoted statement from the Charge is not contained in the assessment itself. Is there a specific change to the draft Tox Review that the panel is recommending?</b></p> <p>For reference, the draft Tox Review (in contrast to the Charge question) concludes:</p> <p>In sum, there is some evidence that certain subpopulations may be more susceptible to exposure to TCE. Factors affecting susceptibility examined include lifestage, gender, genetic polymorphisms, race/ethnicity, pre-existing health status, and lifestyle factors and nutrition status. However, except in the case of toxicokinetic variability characterized using the PBPK model described in Section 3.5, there are inadequate chemical-specific data to quantify the degree of differential susceptibility due to such factors.</p>
<p>6, lines 20-21</p>	<p>In addition, according to the authors, DCA metabolism in the lung compartment remained highly uncertain.</p>	<p>As a point of clarification, our summary of the Hack et al. (2006) conclusions were as follows (Page 3-64):</p> <p>In addition, these authors concluded that dosimetry of DCA, conjugative metabolites, and metabolism in the lung remained highly uncertain (Hack et al., 2006).</p>

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7, lines 12-15	The Panel observed that there remained a significant amount of variability between animals that did not seem to be accounted for in the final model. According to the draft document, this variability was assumed to be captured in the prior distributions for model parameters.	<p><b>Can the panel provide clarification as to where the statement that “this variability was assumed to be captured in the prior distributions for model parameters” was made? We cannot locate what the panel is referring to.</b></p> <p>For example, on page 3-81 to 3-82:            Informative prior distributions reflecting the uncertainty in the population mean and variance, detailed in Appendix A, were updated from those used in Hack et al. (2006) based on an extensive analysis of the available literature.</p> <p><b>Perhaps the comment is referring to the “residual error” estimates? See page 3-96, lines 14-17:</b></p> <p>In addition, the “residual error” estimate for each measurement (see Table 3-41) provides some quantitative measure of the degree to which there were deviations due to intrastudy variability and model misspecification, including any difficulties fitting multiple dose levels in the same study using the same model parameters.</p> <p>See also page A-60, lines 11-14:            In all cases except one, the likelihood was assumed to be lognormal, which requires specification of the variance of the “residual error.” This error may include variability due to measurement error, intraindividual and intrastudy heterogeneity, as well as model misspecification.</p>
14, lines 2-3	** EPA uses 32 cases and RR=1.14 which is the entire cohort and not the TCE sub-cohort. This should be explained.	<p><b>Can the panel clarify this recommendation? For instance, is the panel suggesting that our explanation in Appendix C is unclear and/or that it should be reiterated elsewhere in the document?</b></p> <p>For reference, we discussed this RR selection in Appendix C, page C-28, lines 7-14:            In their published paper, Morgan et al. (1998) present only</p>

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		<p>SMRs for overall TCE exposure, although the results from internal analyses are presented for exposure subgroups. RR estimates for overall TCE exposure from the internal analyses of the Morgan et al. (1998) cohort data were available from an unpublished report (Environmental Health Strategies, 1997); from these, the RR estimate from the Cox model which included age and sex was selected, because those are the variables deemed to be important in the published paper. The internal analysis RR estimate was preferred for the primary analysis, and the published SMR result was used in a sensitivity analysis.</p>
14, lines 5-6	<p>*** Note that in the meta-analysis figures for kidney the confidence interval was changed from that reported in the paper. This should be explained. Hopefully the weights are correct.</p>	<p><b>Can the panel clarify this recommendation? For instance, is the panel suggesting that our explanation in Appendix C is unclear and/or that it should be reiterated elsewhere in the document?</b></p> <p>For reference, Appendix C, Table C-6 [pages C-26 to C-27] shows the actual SE(logRR) used to calculate the weights. In addition, Appendix C, page C-3, lines 14-20 explains the discordant confidence intervals in the figures:</p> <p>Figures were generated using the Comprehensive Meta-Analysis software. Note that for these figures, this software recalculates CIs for the studies based on the SE inputs, and the resulting CIs are not always identical to those reported in the original studies, in particular those based on Poisson distributions. However, the recalculated CIs are merely outputs and are not the basis for any calculations in the software; SEs were obtained as described above, and these SEs and the log RRs constitute the inputs for the meta-analysis calculations.</p> <p>As an editorial note – the SIR estimate of 1.16 is from Axelson et al. (1994), not Anttila et al. (1995), and “Anttila” is misspelled in <u>SAB draft table</u>.</p>

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14, lines 7-8	**** Note: for Zhao a 0 lag is given since it is used in the other studies. The lag results in a RR=1.72 which is a different type of 20 yr lag than in Raaschou-Nielsen which EPA did not use.	<b>Is the panel asking EPA to clarify the rationale for selecting the “20 yr lag” result from Zhao et al. (2005) and not selecting the “20 yr lag” result from Raaschou-Nielsen et al. (2003)?</b>
24, lines 38-40	Recommendation: • EPA shall provide a more balanced description of the TCE’s adverse health effects on both kidney and liver since the role of the liver as a target tissue should not be underestimated.	It is not clear how the immediately preceding text is related to the recommendation. <b>Can the panel provide more specific details as to what is the panel is recommending?</b>
31, lines 28-40 and page 32, lines 1-4	The Panel agreed that there was inadequate support for PPAR $\alpha$ agonism and its sequellae being key events in TCE-induced human liver carcinogenesis. The EPA’s hazard assessment stated that, in humans, “Primary hepatocellular carcinoma and cholangiocarcinoma (intrahepatic and extrahepatic bile ducts) are the most common primary hepatic neoplasms (El-Serag, 2007; Blehacz and Gores, 2008).” (4.5.2. Liver Cancer in Humans). The Panel noted that these type of tumors appear to be independent of a PPAR $\alpha$ dependent MOA. In support of this, induction of peroxisome proliferation in human liver carcinogenesis is not a common feature of exposure to PPAR $\alpha$ agonists. Recommendations: • Inclusion of additional discussion of the fact that common forms of liver cancer seen in humans are not seen in rodent models of TCE liver cancer where hepatocellular carcinomas are seen primarily in a PPAR $\alpha$ dependent-manner. ... The data from these animal models suggest that activation of PPAR $\alpha$ is an important but not limiting factor for the development of mouse liver tumors and that additional	<b>Can the panel clarify what is meant by this text and the recommendation with respect to the following points?</b> • First, with respect to the statement that “common forms of liver cancer seen in humans are not seen in rodent models,” it is our understanding that “primary hepatocellular carcinoma,” which is the most common form of liver cancer in humans, is the same type of liver cancer as the hepatocellular carcinomas seen in rodents exposed to TCE or to PPAR $\alpha$ agonists. <b>If the panel disagrees, it would be useful to understand the basis for that view.</b> • <b>Can the panel clarify the statement that “In support of this, induction of peroxisome proliferation in human liver carcinogenesis is not a common feature of exposure to PPAR<math>\alpha</math> agonists?”</b> Is the panel concluding both that peroxisome proliferation is a causal event in liver carcinogenesis in experimental animals and that its apparent lack occurrence in human liver carcinogenesis indicates a PPAR $\alpha$ -independent MOA in humans? Alternatively, is the panel concluding that human epidemiologic data on

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	molecular events may be involved.	<p>PPAR<math>\alpha</math> agonists suggest a lack of liver cancer hazard in humans from exposure to PPAR<math>\alpha</math> agonists? For reference, these issues were discussed in the draft Tox Review as follows:</p> <p><u>Page 4-311, lines 31-34</u>: However, it should be noted that peroxisome proliferation and in vivo markers such as PCO are not considered causal events (Klaunig et al., 2003; NRC, 2006), and that their correlation with carcinogenic potency is poor (Marsman et al., 1988).</p> <p><u>Page E-340, lines 7-9</u>: Inferences regarding the carcinogenic risk posed to humans by PPAR<math>\alpha</math> agonists have been based on limited epidemiology studies in humans that were not designed to detect such effects.</p> <p><u>page E-342, lines 17-20</u>: Guyton et al. (2009) further explore the status of the PPAR<math>\alpha</math> epidemiological database and describe its inability to discern a cancer hazard from the available data. Thus, while existing evidence for liver cancer in humans is null rather than negative, there remains a concern for oncogenicity and many obstacles for determining such effects through human study.</p> <ul style="list-style-type: none"> <li>• <b>Can the panel clarify the relationship between the statements that “hepatocellular carcinomas [in rodents exposed to TCE] are seen primarily in a PPAR<math>\alpha</math> dependent-manner” and “activation of PPAR<math>\alpha</math> is an important but not limiting factor for the development of mouse liver tumors?”</b></li> </ul> <p>For reference, this issue was discussed in the draft Tox Review as follows (page 4-314, line 29 to 4-315, line 5):</p> <p>In summary, TCE clearly activates PPAR<math>\alpha</math>, and some of the effects contributing to tumorigenesis that Klaunig et al. (2003) and NRC (2006) propose to be</p>

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		<p>the result of PPAR<math>\alpha</math> agonism are observed with TCE, TCA, or DCA treatment. While this consistency is supportive a role for PPAR<math>\alpha</math>, all of the proposed key causal effects with the exception of PPAR<math>\alpha</math> agonism itself are nonspecific, and may be caused by multiple mechanisms. There is more direct evidence that several of these effects, including alterations in gene expression and changes in DNA synthesis, are mediated by multiple mechanisms in the case of TCE, and a causal linkage to PPAR<math>\alpha</math> specifically is lacking. Therefore, because, as discussed further in the MOA discussion below, there are multiple lines of evidence supporting the role of multiple pathways of TCE-induced tumorigenesis, the hypothesis that PPAR<math>\alpha</math> agonism and the key causal events proposed by Klaunig et al. (2003) and NRC (2006) constitute the sole or predominant MOA for TCE-induced carcinogenesis is considered unlikely.</p>