

FINAL
REVIEWER COMMENTS

**External Peer Review Meeting on the
Toxicological Review of cis- and trans-1,2-Dichloroethylene
(CAS Nos. cis: 156-59-2; trans: 156-60-5; mixture: 540-59-0)**

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I. INTRODUCTION

The Integrated Risk Information System (IRIS) is an EPA database containing Agency consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances.

IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose (RfD) for non-cancer health effects resulting from oral exposure, a reference concentration (RfC) for non-cancer health effects resulting from inhalation exposure, and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program within EPA's National Center for Environmental Assessment (NCEA) has developed the "Toxicological Review of cis- and trans-1,2-Dichloroethylene," which updates assessments that were posted to the IRIS database in 1988 for the trans isomer and in 1990 for the cis isomer. Cis- and trans-1,2-dichloroethylene were nominated for assessment by EPA's Regional Offices (Regions 2, 5 and 7) and the New Jersey Department of Environmental Protection because of their frequency of detection at Federal and state hazardous waste sites. The draft document slated for the external peer review contains a chronic oral RfD for both cis- and trans-1,2-dichloroethylene, but no inhalation RfC or quantitative cancer assessment for either cis- or trans-1,2-dichloroethylene.

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II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of cis- and trans-1,2-dichloroethylene (DCE) that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

IRIS assessments for cis-1,2-DCE and trans-1,2-DCE were posted on the IRIS database in 1990 and 1988, respectively. For cis-1,2-DCE, neither an oral reference dose (RfD) nor an inhalation reference concentration (RfC) was derived. For trans-1,2-DCE, an RfD, but not an RfC, was derived. The previous assessments for cis- and trans-1,2-DCE characterized these isomers as "not classifiable as to human carcinogenicity."

The current draft health assessment includes chronic RfDs for cis- and trans-1,2-DCE and qualitative carcinogenicity assessments for both isomers. Below is a set of charge questions that address scientific issues in the assessments of cis- and trans-1,2-DCE. Please provide detailed explanations for responses to the charge questions.

General Charge Questions:

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

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for cis-1,2-DCE

1. The McCauley et al. (1990, 1995) subchronic gavage study in rats was selected as the basis for the derivation of the RfD for cis-1,2-DCE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other study that should be selected as the principal study.
2. Increased relative liver weight in male rats (McCauley et al., 1990, 1995) was selected as the critical effect for the RfD for cis-1,2-DCE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoint that should be considered in the selection of the critical effect.

3. Benchmark dose (BMD) modeling methods were applied to liver weight data to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% change in relative liver weight) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

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

(B) Oral Reference Dose (RfD) for trans-1,2-DCE

1. The 90-day immunotoxicity study by Shopp et al. (1985) was selected as the basis for the RfD for trans-1,2-DCE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other study that should be selected as the principal study.

2. Immune suppression, as indicated by the decrease of sheep red blood cell (sRBC)-specific IgM antibody-forming cells (AFCs) in the spleen in male mice, was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoint that should be considered in the selection of the critical effect.

3. BMD modeling was applied to data for suppression of AFCs in the spleen in male mice in the Shopp et al. (1985) study to derive the POD for the RfD. Has the BMD modeling been appropriately conducted? Is the BMR selected for use in deriving the POD (i.e., a change in response of 1 standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

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

(C) Inhalation Reference Concentration (RfC) for cis-1,2-DCE

1. An RfC was not derived due to the lack of available studies to characterize the health effects associated with cis-1,2-DCE administered via the inhalation route. Are there available data that might support development of an RfC for cis-1,2-DCE?

(D) Inhalation Reference Concentration (RfC) for trans-1,2-DCE

1. An RfC was not derived for trans-1,2-DCE. Has the scientific justification for not deriving an RfC been clearly described in the document? Are there available data that might support development of an RfC for trans-1,2-DCE?

(E) Carcinogenicity of cis- and trans-1,2-DCE

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that there is *inadequate information to assess the carcinogenic potential* of cis- and trans-1,2-DCE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

III. GENERAL IMPRESSIONS

James V. Bruckner

This is one of the longest and probably the most detailed EPA IRIS draft document I have reviewed. The experimental design and findings of each study are presented in exquisite detail, often numerous times throughout the document. This makes the draft document long and tedious to read. It may be preferable to be more selective and focus this degree of attention on the major studies (i.e., key studies and candidate key studies) of each category.

The descriptions of metabolic and toxicological investigations were clear, and appeared to be accurate. The conclusions and logic supporting them were sound.

The most striking and unique biological effect of DCE is its ability to inhibit cytochrome P450s (CYPs). Therefore, in sufficiently high doses, DCE inhibits the CYP-mediated activation of a wide variety of volatile organic compounds (VOCs). Barton et al. (1995) discovered that pre-exposure of rats to 40 ppm *trans*-1,2-DCE for 1.5 hours resulted in marked inhibition of trichloroethylene and vinyl chloride metabolism by competitive inhibition. Lilly et al. (1998) subsequently reported that: (1) *cis*- and *trans*-1,2-DCE inactivated CYP2E1 in rats; and (2) the *trans* isomer was more potent than the *cis* isomer. CYP2E1 inactivation was only mentioned in EPA's Toxicological Review in subsection 3.5 (page 14), in conjunction with its influence on results of PBPK modeling. A new subsection entitled "Interactions with Other Chemicals" should be added under Toxicokinetics. It should be emphasized that the DCEs may be protective against cytotoxic and mutagenic/carcinogenic actions of VOCs and other chemicals which undergo CYP2E1-catalyzed metabolic activation.

Robert A. Howd

This is a credible, well-documented risk assessment, that makes reasonable conclusions about the available toxicity data for both *cis* and *trans* 1,2-DCE. The technical writing is of high quality. Choices of critical studies and endpoints are appropriate, and the methods used for calculation of benchmark responses appear to be an improvement from the previous approaches. The uncertainty factors are consistent with the standard approaches. Differences from other risk assessments and resulting estimated health-protective doses are well within my comfort zone for responsible professional judgment.

This is one of the best-prepared drafts I have reviewed. My technical criticisms (see below) are minimal for such a lengthy, detailed document. None of the technical comments represents any serious issues. However, I continue to disagree with the U.S. EPA policy to not use data derived by the oral route for estimation of inhalation hazards for systemic effects (and vice-versa).

Ralph L. Kodell

In general, the information is presented accurately and clearly. Although it might not lead to a change in the choice of critical effects on which the oral RfDs are based, I believe that additional toxic effects should be considered as potential critical effects for both cis- and trans-1,2-DCE. I have identified those below in response to specific charge questions. The BMD modeling has been appropriately conducted and explained. The composite uncertainty factor for trans-1,2-DCE is justified. However, based on information gained during the panel discussion, I believe that the UFA factor for cis-1,2-DCE should be reduced, thereby reducing the composite factor. The rationale for not deriving an inhalation RfC for cis-1,2-DCE is clear. But, based on the panel discussion, I believe that the unpublished study of Kelly et al. (1999) should be evaluated as a possible principal study from which to derive a POD for an RfC for trans-1,2-DCE. The justification for the conclusion that there is inadequate information to assess the carcinogenic potential is clear.

Janice Longstreth

I believe that the information provided is accurate and that the presentation is clear, but I disagree in large measure with most of the conclusions drawn from the information. First, it is unclear to me that any of the studies reviewed (with the possible exception of the Freundt study) demonstrate any real hazard from exposure to either cis- or trans-1,2-DCE. As is pointed out in Table 4-12, for most of the major noncancer subchronic studies considered, a LOAEL could not be derived. Indeed, with the exception of the Shopp et al. study, where EPA chose a LOAEL in contradiction to the authors' conclusions, the only study with a bona fide LOAEL, i.e., supported by the authors' conclusions, was the Freundt et al. study, which histopathologically characterized fatty degeneration of the liver. (However, this study was not subsequently used for RfC derivation). That being said, I recognize that it is important to various EPA procedures, e.g., site risk assessments, that there be "blessed numbers" for those chemical which are frequently encountered. My preference however, would be to fall back to the simpler more transparent approaches (i.e., NOAELs/LOAELs and UFs) for chemicals like the DCEs, where there is not particularly strong evidence of hazardous behavior so great precision is not so important, while applying the more sophisticated and presumably more precise (but also more opaque) approaches such as BMD modeling to chemicals with the robust data base needed for such an approach and a clearer evidence of hazard which would benefit from additional precision in the development of "blessed numbers."

Michael I. Luster

Overall, the review provides a concise and complete review of the toxicity of cis- and trans-DCE in humans and animals. The document clearly distinguishes toxicity studies of cis- vs. trans-DCE, experimental vs. human, chronic vs. subchronic/acute and oral vs. inhalation exposure. The synthesis section is well thought out and detailed. I do have some minor suggestions for improvement and feel there is a need to better describe the

adverse effects that may result from the selected critical effects. Specific comments are detailed below.

Cis- and trans-DCE are commonly used industrial chemicals that have been in use for some time. The report indicates correctly that there is not an overwhelming amount of animal or human toxicity data in the literature. However, I believe this is not due to the chemical being intentionally understudied as much as preliminary studies undertaken have indicated that the material was not particularly toxic or genotoxic and, hence, there was little impetus or need to conduct chronic exposure studies. Thus, for risk assessment purposes, it is true as stated, that the published information available is limited. As such, it might be useful for a public document to indicate that, based on the toxicity data available from the general literature, the relative potency for these materials appears not to be particularly high and is likely responsible for limited chronic or reproductive effects.

IV. RESPONSE TO CHARGE QUESTIONS

General Charge Questions:

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

James V. Bruckner

This toxicological review is certainly not concise. The descriptions of so many studies are so long and detailed that it is difficult for the reader to discern and retain the more important findings. There is also considerable redundancy, apparently the result of EPA's format requirements. Nevertheless, the authors manage to reconcile minor differences from one study to another and to objectively synthesize accurate conclusions from the plethora of investigations.

Robert A. Howd

Yes, it is logical, clear and concise, presenting the data and analyses in a reasonable manner, with well-supported conclusions.

Ralph L. Kodell

In general, the Toxicological Review is logical, clear and concise. There are a few instances in which I think more clarity is needed, as indicated in my specific comments below. For noncancer hazards, EPA has accurately, clearly and objectively represented the scientific evidence. However, I disagree slightly with the Agency's synthesis of that information. Specifically, I believe the Agency should consider additional toxic endpoints for deriving candidate PODs for setting oral RfDs for cis- and trans-1,2-DCE, and should consider the unpublished study of Kelly et al. (1999) for a possible RfC for trans-1,2-DCE. For cancer hazards, EPA has indicated that there is no scientific evidence that would allow an assessment of the carcinogenic potential of cis- and trans-1,2-DCE.

Janice Longstreth

Given that the goal of the Review is to "provide scientific support and rationale for the hazard and dose-response assessments in IRIS pertaining to chronic exposure," I would conclude that the document is logical, clear and concise, but incomplete. EPA has synthesized the scientific evidence for noncancer and cancer hazard at a superficial level but has not done a very good job of digging below the surface of what has been reported in the literature. To give just a couple of examples:

- The McCauley et al. study used corn oil as the gavage vehicle for cis-1,2-DCE, yet nothing was mentioned in the discussion of this study of the well documented concerns that corn oil may exacerbate the hepatotoxicity of chloroalkenes

(Raymond & Plaa 1997) which might lead one away from an increase in liver weights as the critical effect.

- In reviewing the information for trans-1,2-DCE, I was struck by the fact that, none of the 4 major subchronic studies considered as the source of a POD for the RfD achieved an MTD; indeed except for the fact that EPA claims a LOAEL for the Shopp study, one could state that not a single subchronic study achieves a LOAEL. Furthermore all of the studies were one of a kind. Thus, you have one feeding study in rats one feeding study in mice, one drinking water (dw) study in rats and essentially only one dw study in mice (since the Barnes and Shopp studies involved the same mice and the same route of exposure). This lack of an MTD is despite the fact that highest doses exceeded 3 grams/kg in rats and approached 8 g/kg in mice. This kind of divergence in the database, as well as the very high doses that were found as NOAELs in well run, extremely thorough studies like that conducted by NTP, makes me question whether the database supports deriving an RfD for the trans-isomer of DCE.

Michael I. Luster

Yes, it is logical, clear and concise, presenting the data and analyses in a reasonable manner, with well-supported conclusions. One general suggestion:

Considerable amount of the literature review includes a review of studies involving the metabolism of cis- and trans-DCE which is important since metabolism is related to toxicity. While a lot of information on metabolism is accurately provided, it is a little hard to follow. A summary paragraph might be useful indicating the likely toxic metabolite (I presume the epoxide) and the suicide (inhibition) effect observed at higher doses which might explain some of the atypical dose-response curves seen. In the synthesis-discussion section, it might also be worth indicating whether inhalation vs. oral exposure would involve the same postulated metabolic processes (e.g., kinetics, biotransformation products).

General Charge Questions:

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

James V. Bruckner

I did not locate any additional published research papers that should be considered for deriving an RfD, RfC or cancer potency factor. Four supplementary references are included at the end of my review.

Robert A. Howd

Perspective on metabolite toxicity: Caldwell and Keshava. 2006. Key issues in the modes of action and effects of trichloroethylene metabolites for liver and kidney tumorigenesis. *Environ Health Perspect* 114(9):1457-63.

Ralph L. Kodell

I do not know of any additional studies that should be considered in the assessment of the noncancer and cancer health effects of cis- and trans-1,2-DCE.

Janice Longstreth

Additional references related to the drawbacks of choosing a corn oil gavage study for a liver end-point:

Ahmed, U., Redgrave, T.G. & Oates, P.S., 2009. Effect of dietary fat to produce non-alcoholic fatty liver in the rat. *Journal of Gastroenterology and Hepatology*, 24(8), 1463-1471.

Chetty, K.N. et al., 2006. Cholesterol-induced alteration in liver mineral concentrations in corn oil and olive oil fed rats. *Pathophysiology: The Official Journal of the International Society for Pathophysiology / ISP*, 13(1), 35-37.

Condie, L.W., 1985. Target organ toxicology of halocarbons commonly found contaminating drinking water. *The Science of the Total Environment*, 47, 433-442.

Huber, W.W. et al., 1997. Inhibition instead of enhancement of lipid peroxidation by pretreatment with the carcinogenic peroxisome proliferator nafenopin in rat liver exposed to a high single dose of corn oil. *Archives of Toxicology*, 71(9), 575-581.

Raymond, P. & Plaa, G.L., 1997. Effect of dosing vehicle on the hepatotoxicity of CCl₄ and nephrotoxicity of CHCl₃ in rats. *Journal of Toxicology and Environmental Health*, 51(5), 463-476.

Rivera, C.A. et al., 2006. Feeding a corn oil/sucrose-enriched diet enhances steatohepatitis in sedentary rats. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 290(2), G386-393.

Michael I. Luster

To my knowledge, all relevant studies were discussed and the data adequately synthesized. There might be, however, some additional support for the scientific rationale used to support the selection of the critical effect by discussing structurally or biologically similar acting chemicals. For example, CCl₄ also produces P450 enzyme inhibition at high doses and has been extensively studied for hepatotoxicity mechanism. Are there studies with this or other hepatotoxic compounds that can be used to support the basis for using elevated liver weights to establish the RfD?

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for cis-1,2-DCE

1. The McCauley et al. (1990, 1995) subchronic gavage study in rats was selected as the basis for the derivation of the RfD for cis-1,2-DCE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other study that should be selected as the principal study.

James V. Bruckner

The report by McCauley et al. (1995) must be used as the basis for derivation of the RfD for cis-1,2-DCE, as it is apparently the only subchronic oral study of this compound.

Robert A. Howd

This is the best available study, and should be used for the calculation.

Ralph L. Kodell

As stated in the document, the subchronic gavage study in rats by McCauley et al. (1990, 1995) is the only published oral, repeat-dose toxicity study of cis-1,2-DCE. According to EPA, some errors and inconsistencies were identified upon examination of the unpublished version (McCauley et al., 1990) and the published version (McCauley et al., 1995) of the study. But, EPA stated that the errors were principally related to the documentation of administered doses. These errors and inconsistencies suggested to EPA that there were issues with the quality of the report writing, but not with the study findings themselves, and they were not considered by EPA to compromise the reliability of the study findings. Therefore, the selection of this study as the principal study is scientifically justified. There is no other study to consider as the principal study.

Janice Longstreth

Since the McCauley et al. study is the only published study available, I would suggest that if an RfD is to be derived for cis-1,2-DCE then the McCauley et al. study must be used. However, there are so many things wrong with the study, I think that I would recommend not deriving an RfD for the cis 1,2-DCE (and perhaps let the RfD for the trans-1,2-DCE stand for 1,2-DCEs in general). First there are the discrepancies noted between the two publications. According to an EPA employee locator, a P. McCauley works for EPA in OH (513-569-7444, mccauley.paul@epa.gov). If this is the same P McCauley listed as first author on the publication, it should have been easy to check and resolve the discrepancies (and also determine why an author from the AF on the original report was left off of the published version). Second, corn oil gavage studies should be

viewed with suspicion given the many observations suggesting that corn oil by itself can enhance hepatic lipid peroxidation so may exacerbate the toxicity of compounds co-administered with it (K. N. Chetty et al., 2006; Ahmed et al., 2009; Rivera et al., 2006; Huber et al., 1997). Furthermore, several reports have commented on such interactions between corn oil and chloroalkenes (e.g., Raymond & Plaa, 1997).

Michael I. Luster

I think these studies are appropriate for development of the cis RfD. I have some minor concern regarding the statement that there were some errors and inconsistencies between the published and unpublished McCauley studies. The IRIS report only states that the inconsistencies consisted of documentation of administered dose and minor inconsistencies. In order for more transparency in the document, I suggest adding a short discussion detailing these differences. Regarding **Table 4-1 (relative liver and kidney weights)**: these changes are relatively small. Can you indicate historical ranges that might be expected and also include absolute weights?

(A) Oral Reference Dose (RfD) for cis-1,2-DCE

2. Increased relative liver weight in male rats (McCauley et al., 1990, 1995) was selected as the critical effect for the RfD for cis-1,2-DCE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoint that should be considered in the selection of the critical effect.

James V. Bruckner

Increased relative liver weight was selected as the critical effect, despite the absence of manifestations of more serious hepatic effects. It is noted in line 5 of pgr. 1 of page 79 that terminal body weights in male rats at the two highest doses were 10 – 11% lower than controls. This raises the question of whether absolute liver weights were significantly elevated? An increase in absolute liver weight would be more toxicologically significant than the increase in relative liver weight.

Robert A. Howd

A significant effect on kidney weight in male rats was observed at a lower dose than the liver effect, and standard risk assessment practice is to use the lowest LOAEL for the risk assessment. Benchmark modeling based on the kidney data would presumably result in a lower BMDL, and the resulting RfD should be considerably lower. So the question is does the dose-response pattern of toxic effects (kidney versus liver) justify considering the effects in the two organs to be quantitatively similar, so that modeling effects in the organ with the more consistent pattern of responses is adequately representative of both, and adequately health-protective for both? In my opinion, the answer is yes, and I support the use of the liver data for the extrapolation. I would have preferred the discussion to be more explicit about the quantitative effect of this choice, however – that a higher value was derived than if the default procedure were used.

Ralph L. Kodell

The selection of increased relative liver weight in male rats as the critical effect may be scientifically justified. There was a statistically significant dose-response relationship for average relative liver weight in both males and females. BMD modeling gave a lower candidate POD for males than for females, and thus increased relative liver weight in male rats was selected as the critical effect. Although the absence of elevated liver enzymes or histopathology make the changes in liver weight difficult to interpret, EPA cited shorter-term toxicity studies as supporting the liver as a potential target of cis-1,2-DCE toxicity.

A few statistically significant changes in clinical chemistry and hematology parameters were found in the McCauley study, but these were not considered by the study authors or

by EPA to be biologically significant when compared to normal ranges for these parameters.

The only other potential candidate for the critical effect was an increase in relative kidney weight. For male rats, the means of all dose groups were statistically significantly higher than the control mean, and they increased in a dose-related fashion. Average relative kidney weight was elevated for females also, in a monotone, dose-related fashion; but no pair-wise mean comparisons of dose groups to controls were statistically significant at the 5% level by Tukey's multiple comparisons test. However, standard deviations were quite large in the two middle female dose groups, about 3 to 4 times the values for the control, low, and high dose groups. There are two concerns about using Tukey's test for these comparisons. First, the test makes an adjustment for all pair-wise comparisons among means, but the only comparisons of interest are comparisons of dose means to the control mean. Second, Tukey's test is based on an assumption of equal variances across dose groups, which appears to be violated. Although Dunnett's test would be a better choice because it is designed specifically for multiple comparisons of dose groups to controls, it too is based on having equal variances. A simple nonparametric test for dose-related trend in mean kidney weight in females is statistically significant at the 5% level ($p\text{-value} = 2/5! = 2/120 = 0.017$).

As noted above, errors and inconsistencies between the 1990 and 1995 reports were principally related to documentation of administered doses; however, EPA stated on page 62 that some errors in the values for a few of the relative organ weights were identified, which may have been due to either transcription errors or calculation errors. If there were such errors for relative kidney weights, it might help to explain the abnormally large variances in the two female dose groups. Histopathology findings for the kidney were negative, and indicators of renal dysfunction like BUN and creatinine levels were not elevated in any treatment groups; however, this does not make the case for kidney any weaker than that for liver, because there was a similar lack of indicators of liver toxicity. In the McCauley 14-day study, relative kidney weight was significantly elevated in female rats in the two highest dose groups. I believe that BMD modeling should be done for increased relative kidney weight in both males and females to produce candidate PODs on which to base the oral RfD. If the kidney is not considered further, then I think additional justification is needed for not considering it.

Janice Longstreth

I have some concerns about using an increase in liver weight, in the absence of histopathological or clinical chemical evidence supporting liver toxicity, as the critical effect. While it is clearly an effect, I question whether it is an adverse effect. I would feel more comfortable using the increase in kidney weights in males, which at least was accompanied by effects on serum phosphorous, BUN and creatinine levels (although these last two are going in the wrong direction and for some reason the decrease in creatinine was not mentioned in the Review).

Michael I. Luster

Yes - I believe that increased liver weight represents the critical effect. The assessment provides a convincing argument that the increased liver weight effect is chemical related (i.e., dose-response, reproducible observation among different studies). However, the effect is relatively small and not associated with any histopathology or serum liver enzyme changes (one usually equates changes in serum BUN with kidney effects – not liver). Thus, I feel the document should include a more convincing (scientifically-based) argument that increased weight is an adverse effect or precursor to an adverse effect, particularly in light of an earlier document prepared by the OPPT/EPA (CAS # 51798-33-5 a trifluoro, March 2009) which concluded modest changes in liver weights (10-15% above controls) not accompanied by histopathological or clinical chemistry changes do not represent an adverse effect. Despite that fact, I believe that increased liver weight can be a precursor of an adverse effect since it seems to be associated with increased P450 activity (increased metabolic activity can result in elevated liver weight probably detectable by EM but not histopathology or clinical chemistry). It might be helpful if the authors can identify any studies where persistent enzyme induction leads to an adverse effect (e.g., altering metabolism of other chemicals or drugs and causing an idiosyncratic reaction).

Regarding other critical effects, it might be worthwhile to look at the kidney as a potential critical target and use hypercalcemia as the critical effect. It appears that hypercalcemia occurs at relatively low doses. Ample data exists that elevated serum calcium can be a potential biomarker for altered renal function. With the reported changes in kidney weight, urinary protein (pg 50, Table 4-9) and elevated BUN (at least at higher doses), it might provide a better endpoint, but without modeling it is hard to tell.

(A) Oral Reference Dose (RfD) for cis-1,2-DCE

3. Benchmark dose (BMD) modeling methods were applied to liver weight data to derive the point of departure (POD) for the RfD. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., a 10% change in relative liver weight) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

James V. Bruckner

The use of BMD modeling to derive a POD is a reasonable approach to use with the dose-response data that are available. The modeling appears to have been appropriately conducted. Estimation of the BMDL for a 10% change in relative liver weight is a conservative approach to use with such a modest effect.

Robert A. Howd

Yes, the method appears to be reasonable for the available data sets, yielding a health-protective result.

Ralph L. Kodell

Yes, the BMD modeling has been appropriately conducted. The three available models for continuous endpoints in EPA's benchmark dose software, BMDS, were fitted and compared. The Hill model fit the best for both males and females based on AIC values and goodness-of-fit p-values. The fitted model was actually the Michaelis-Menten model, a special case of the Hill model, because the exponent on dose was estimated to be 1. Both a central estimate and a 95% lower confidence limit (BMDL) on the BMD corresponding to a BMR defined as a 10% change in mean relative liver weight compared to the control group were calculated, with the BMDL representing the candidate POD. The selection of a 10% change in mean relative liver weight as the BMR is scientifically justified, consistent with EPA's consideration of a 10% change in the mean body weight as a minimally biologically significant change.

For comparison purposes, EPA also defined the BMR as a change in the mean corresponding to a shift of one standard deviation from the control mean, and calculated the corresponding central estimates and BMDLs. BMDL values calculated on the basis of the standard deviation change were slightly less than half the values calculated on the basis of a 10% change. When transformed to a probability-based context, the standard-deviation approach gives a BMD that corresponds approximately to 10% excess risk for a normally distributed endpoint that is assumed to have a 1% background risk. I favor the one-standard-deviation change in the mean personally because the BMD can be

interpreted on an excess-risk basis like BMDs for quantal endpoints, but the 10% change in the mean used by EPA is scientifically justified and has ample historical precedence.

Janice Longstreth

Given that I am not a fan of using increased liver weight as the critical effect, I'm not going to comment on the application of BMD modeling to this effect. I would like to see the BMD modeling applied to the kidney weight, creatinine and BUN data, with a POD chosen which considers all of these data.

Michael I. Luster

The BMD is the most appropriate method to derive the RfD from liver weight data.

(A) Oral Reference Dose (RfD) for cis-1,2-DCE

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

James V. Bruckner

It is a standard EPA default policy to utilize an intraspecies uncertainty factor (UF) of 10. The full factor seems to be excessive for this modest endpoint, as there is little evidence of *cis*-1,2-DCE causing more serious hepatic effects in any species by any route of exposure, even when administered in extremely high dosages.

The 10X intraspecies factor of 10 is usually considered to consist of two factors of 3.3, one for potential inter-individual toxicokinetic (TK) and one for potential toxicodynamic (TD) differences (Renwick, AG, *Food Addit. Contam.* 15 (Suppl.): 17-25 (1998).

Adverse effects of DCE and other halocarbons, other than reversible central nervous system depression, are generally believed to be due to their metabolites. Therefore, inter-individual variance in xenobiotics metabolism, due either to genetic or environmental factors, can be an important contributor to one's susceptibility to liver changes (for *cis*-1,2-DCE) or immunosuppression. Cytochrome P4502E1 (CYP2E1) is primarily responsible for metabolic activation of halocarbons such as DCE in rats and humans. Thus, individuals with higher CYP2E1 activity should be more susceptible to the chemicals' toxic effects. This is true at relatively high exposure levels, but not at the very low exposures mandated by the EPA's current RfD derivations for *cis*- and *trans*-1,2-DCE. Kedderis, G.L. *Chem.-Biol. Interact.* 107: 109-121 (1997) used a PBPK model to predict that a 10-fold increase in CYP2E1 activity in humans inhaling 5 ppm DCE for 4 hours would result in only a 7% increase in DCE metabolism by the liver. Quantities of CYP2E1 and other constitutive CYP isozymes in all persons are far in excess of the amounts necessary to metabolize all low levels of DCE. Therefore, elevated CYP2E1 activity in some individuals is inconsequential to the total amount/dose of bioactive metabolite formed. For this reason, the 3.3-fold component of the 10-fold intraspecies factor should be omitted, leaving an intraspecies UF of 3.

The interspecies UF of 10 is definitely too high. Rodents consistently metabolize short-chain aliphatic hydrocarbons (halocarbons) to a greater extent than do humans. Although the mode(s) of action of *cis*-1,2-DCE is(are) unknown, it is generally accepted that oxidative metabolites (e.g., epoxides) are the most likely candidates for proximate toxicants. Therefore, the toxicokinetic component of 3.3 of the full 10X should be omitted, leaving an interspecies UF of 3 for potential toxicodynamic differences.

A UF of 10 due to lack of chronic data is probably too much, in light of the virtual absence of adverse effects on any organ in inhalation experiments with *cis*-1,2-DCE or experiments with mixed isomers. The DCE isomers will likely continue to inhibit their

own metabolic activation and thereby prevent adverse effects, no matter how long the exposures.

A UF of 3 to account for database deficiencies is reasonable.

It should be recognized that oral administration of DCE and other short-chain aliphatic hydrocarbons (halocarbons) by corn oil gavage produces more pronounced effects than when the chemicals are ingested in divided doses in food or water over the course of a day. Sanzgiri et al. *Toxicol. Appl. Pharmacol.* 134: 148-154 (1995), for example, gave the same doses of carbon tetrachloride (CCl₄) to rats by corn oil gavage and over 2 hours by gastric infusion. Blood CCl₄ levels and hepatic damage were significantly higher in the gavage group. La et al. *Toxicol. Appl. Pharmacol.* 140: 108-114 (1996) saw marked necrosis and ensuing proliferation of hepatocytes of mice given chloroform in corn by gavage, but no such effects in mice that consumed the same daily doses in their water. In such instances, halocarbons are rapidly/extensively absorbed from the GI tract and delivered to the liver via the portal blood in amounts high enough to exceed the capacity of cellular protection and repair mechanisms. It should also be recognized that administration of large quantities of corn oil promotes lipid accumulation and lipoperoxidative damage. Thus, the experimental design of the McCauley et al. study resulted in a more pronounced hepatic effect than would occur with real-life exposures. This argues against adoption of such large UFs to protect against such a modest effect.

Robert A. Howd

The UFs appear reasonable to me.

Ralph L. Kodell

A composite uncertainty factor of 3000 was applied to the selected BMDL to derive the RfD. This was composed of a UFH = 10, UFA = 10, UFL = 1, UFS = 10 and UFD = 3. The value of 10 for UFH and UFS is the default maximum value customarily applied for these uncertainty factors, and is justified in the absence of information to suggest a smaller value. Based on the panel discussion, I believe that the factor UFA should be reduced, provided that EPA can add some documentation that it is well known that cis-1,2-DCE is less toxic to humans than to rats. If so, there is no need for a full factor of 10 for uncertainty regarding both toxicokinetic and toxicodynamic differences, just a factor for toxicodynamic differences. UFA can be either 3.3 or 3, to give a composite uncertainty factor of 900 or 1000, instead of 3000. The value of 1 for UFL is probably justified based on precedent, although a “minimally biologically significant” change of 10% is still biologically significant. The value of 3 for UFD is also probably justified based on precedent, but I dislike reducing a POD quantitatively by a database deficiency factor. In my opinion, values up to 10 for the four uncertainty factors other than UFD can be justified based on analyses of historical data, and those factors have direct quantitative relevance to the uncertainty in the RfD. On the contrary, I do not see how database deficiency can be assigned a quantitative value in the form of a UFD between 1 and 10. I

would personally define UFL = 3 and UFD = 1, but the net effect is a total factor of 3 for these two uncertainties, so it wouldn't change anything in this case. In summary, I recommend reducing the composite uncertainty factor to either 900 or 1000, if it can be documented that a factor is not needed for interspecies toxicokinetic differences.

Janice Longstreth

I would quarrel with the selection of a factor of 3 for the database uncertainty. Having only a single corn oil gavage study and then picking a liver endpoint with no other confirmatory sub-chronic toxicity studies and no confirmatory data from within the study, I believe should be the largest uncertainty factor. I'm unaware of whether this has ever been done, but I believe that the choice of the liver endpoint with a single corn-oil gavage study would warrant a UF of 10, while I would be comfortable with a UF of 5 for the kidney data since the evidence for interactions of corn oil and kidney toxicity are much weaker and there are some internal data from the study supporting a potential effect on the kidney. This would suggest that they would be appropriate for a POD chosen from the kidney data.

Michael I. Luster

The 10X intraspecies UF is justified by known gender, age and genetic differences in P450 metabolism. Both the toxic metabolite (Epoxide) and the suicide/inhibition effect require P450 metabolites.

I agree with James Bruckner that the interspecies UF can be reduce to 3.0 by removing the 3.3X for the toxicokinetic factor.

No other changes recommended.

(B) Oral Reference Dose (RfD) for trans-1,2-DCE

1. The 90-day immunotoxicity study by Shopp et al. (1985) was selected as the basis for the RfD for trans-1,2-DCE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other study that should be selected as the principal study.

James V. Bruckner

The 90-day immunotoxicity study by Shopp et al. (1985) as the principal study has been scientifically justified in the document.

Robert A. Howd

This is just one part of a larger study (Barnes et al., 1985). The study, including this immunotoxicity part, was well-conducted and basically as good as it gets for this chemical.

Ralph L. Kodell

Two subchronic studies, Shopp et al. (1985) and NTP (2002), were considered principal studies for determining candidate PODs on which to base the oral RfD. The immunological response reported by Shopp et al. was regarded by EPA as biologically significant as was the increase in relative liver weights observed in female rats and in both male and female mice observed in the NTP study.

According to EPA, the most prominent effects in two other candidate studies, Barnes et al. (1985) and Hayes et al. (1987), were significantly elevated ALP levels in male mice and significant increases in absolute kidney weight at high doses in female rats, respectively, neither of which were considered as biologically meaningful as the critical effects observed in the two principal studies. (The previous oral RfD was based on increased serum ALP in male mice observed in the Barnes study.) The Barnes study also showed effects on absolute thymus weights. Although EPA states that, overall, the evidence does not support a conclusion that the thymus is a target of trans-1,2-DCE toxicity, the data on absolute thymus weight in Table 4-6 (p. 27) show a negative dose-response on the mean weights in female mice, with statistical significance at the highest dose by Duncan's multiple range test. Like Tukey's test discussed above, Duncan's test is designed to adjust for comparisons of all pairs of means and thus is a conservative way to adjust for multiple comparisons of each dose mean to the control mean (although not as conservative as Tukey's test). The dose-response in mean thymus weights is statistically significant at the 5% level with a nonparametric test ($p\text{-value} = 1/4! = 1/24 = 0.042$). I believe that BMD modeling should be done on decreased thymus weight in female mice to derive a potential POD.

Selection of Shopp et al. (1985) and NTP (2002) as principal studies is scientifically justified. However, I believe that Barnes et al. (1985) should also be considered a candidate co-principal study, unless a stronger rationale is given for excluding it. From the panel discussion, I gathered that the effect on thymus weights in the Barnes study may be indicative of an effect on the immune system like the critical effect in the Shopp study. Thus, it may well have relevance. Unfortunately, there does appear to be inconsistency in immune responses between genders, as only the males had a statistically significant response for the immunological response observed in the Shopp study, while only the females had a statistically significant response for the effect on thymus weights observed in the Barnes study.

Janice Longstreth

I do not believe that choice of the Shopp et al. (1985) study as the basis for the RfD is scientifically justified. The authors themselves did not find any compelling evidence suggesting that trans-1,2-DCE was responsible for any biologically significant adverse effects on the immune system and noted that the lack of response observed in nearly every assay, except the AFC assay in male mice, was evidence that trans-1,2-DCE did not specifically affect the immune system.

I would suggest that the NTP study be used as the principal study instead. The NTP was a very well run study; it was an oral study, not complicated by the use of corn oil as the vehicle, so there should be little concern with regard to an interaction between compound and vehicle. Therefore, the liver weight data could be used in the same fashion as it was considered in the document.

Michael I. Luster

Yes – the Shopp et al., 1985 publication seems appropriate to use as the principal study, but I have some suggestions/concerns detailed in response to question #2.

(B) Oral Reference Dose (RfD) for trans-1,2-DCE

2. Immune suppression, as indicated by the decrease of sheep red blood cell (sRBC)-specific IgM antibody-forming cells (AFCs) in the spleen in male mice, was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoint that should be considered in the selection of the critical effect.

James V. Bruckner

Consideration of decreased immune function as a critical effect was well justified by the authors. It was chosen rather than relative liver weight increase, because BMD modeling resulted in a lower BMDL10. Selection of relative liver weight increase, instead, would have allowed consideration/expression of the relative potency of *cis*- and *trans*-1,2-DCE.

Robert A. Howd

The toxic effect data for trans-1,2-DCE is quite limited. I am not impressed by the robustness and clear toxicological significance of any of the endpoints. This makes it difficult to estimate a safe dose. Choice of the immune response to sheep red blood cells, expressed as antibody-forming cells per million spleen cells in male mouse, seems potentially under-protective, considering the several significant effects reported by Barnes et al. at 17 mg/kg-day. The lack of effect in females also makes this endpoint difficult to understand in terms of mechanism of effect and physiological significance. However, none of the other endpoints exhibit a clear dose-response, and may not represent true compound-related effects. It appears to me that application of the benchmark modeling approach to this SRBC endpoint results in a reasonable estimated minimal effect level. In addition, this derivation seems at least as defensible as the endpoint OEHHA used in our recent public health goal (the increased liver weight in male mice at 175 mg/kg-day only) in Barnes et al., 1985. Therefore I support the choice of endpoint used in this risk assessment.

The use of effects on liver weight from NTP (2002) would, it seems to me, be quite under-protective (with a BMDL10 of 867.3 mg/kg-day or NOAEL of 190 mg/kg-day).

Ralph L. Kodell

The selection of decreased number of AFCs against sRBCs in the spleen of male mice (Shopp et al, 1985) as a critical effect for the RfD is scientifically justified. It is indicative of suppression in humoral immune status and was observed at relatively low doses. The data chosen by EPA for POD determination was the response measured on day 4 of the 90-day study, which I understand from the panel discussion is when the response is expected to peak.

As indicated above, an increase in relative liver weights in female rats and in both male and female mice (NTP, 2002) was also considered as a critical effect for deriving candidate PODs for setting the RfD. This effect was also considered biologically meaningful by EPA and is scientifically justified. As noted above, effects from two other subchronic studies were considered in the selection of the critical effect, but were not considered by EPA to be as biologically relevant as the effects selected. Still, I think that the decrease in absolute thymus weights (Barnes et al., 1985) should be considered as a potential critical effect for developing a candidate POD, even though the gender response is opposite that of the immunological response in the Shopp study. Ultimately, the selection of the lowest candidate POD (immune response, so far) is scientifically justified on the basis of being conservatively health protective.

Janice Longstreth

In general, I am not in favor of selecting as a critical effect one that is not supported by the authors' conclusions. It is my feeling that, in addition to the data such authors publish, their conclusions are the result of their other experiences with the assays they've used. Thus, to choose an endpoint in contravention of the authors conclusions, particularly when those authors can be considered leaders in this field, should require information from other sources, e.g., other studies with the same compound, which support the contrary conclusion. At the very least, failing such information, it would be worth talking to the authors about their conclusion to see if they would be comfortable with the EPA's interpretation of the data given their experiences over the intervening time period (and so indicating this change via citation of a personal communication to that effect that could go into the public record). The lack of consistency in the findings of the study vis à vis the other assay systems is telling and given this particular laboratory's long experience with such assays, I would not challenge their position without some fairly strong supporting evidence from another trans-1,2-DCE study of such an effect (which I did not see in the discussion of this choice).

Note added after discussion at the Peer Review Meeting: In the discussion with Dr. Luster about the use of this study, he indicated that there are data indicating that depression of the anti-sRBC response is associated with greater susceptibility to infections. While I was unable to find these data, I believe that they would lend valuable support to the decision to consider the Shopp data as evidence of a LOAEL. I would still recommend contacting Shopp and/or Munson and talking to them about the data to see if they would be comfortable considering the data as representative of a LOAEL rather than a NOAEL.

Michael I. Luster

I believe the use of decreased AFC responses is justified for use as the critical effect. The major weaknesses (although these would not negate using these data to develop an RfD) include: there are no other published studies to confirm or repute the effect on AFCs on DCE; no positive controls were used; and the effects were only apparent in male and not

female mice. These concerns might increase the UF for data quality. The strength of the observation is that the AFC response shows a relatively good dose-response curve and the study seems to have been conducted appropriately (control values with normal historical range). One issue that adds support to the observation, and is not discussed in the report, are that thymus weights were found to be decreased in the Barnes et al. study, at least at the higher dose levels. Decreased thymus weights can be a good indication for immunotoxicity and when accompanied by decreased AFC response, in the absence of general toxicity, provides an excellent predictor of immunotoxicity (see Luster et al., 1992 reference).

It is not surprising that neither HI titers nor LPS response were affected. Since HI titers involve measuring 1:2 dilutions, a significant effect would require at least a 50% decrease in serum antibody levels and the AFC response was only down 26%. With respect to the LPS response, this measures a non-specific activation of certain B cells and is a fairly insensitive assay and is seldom being used today in immunology testing.

Regarding the biological significance of a decreased AFC response, similar to the increased liver weights, the report does not provide a sufficiently detailed scientific explanation to support their argument (i.e., that this represents an adverse effect). The report simply references two other publications (i.e., an old EPA guidance document and Luster et al., 1992) that indicate it is adverse. I think there is general agreement at present that the AFC is a good predictor of immune function and loss of immune function is an adverse effect (i.e., increases susceptibility to infection). The issue is whether a 25% decrease in the AFC is adverse, although I would not recommend basing adverse effects on the caveat of how much (is a 25% loss in the liver adverse?) Nonetheless, the document could discuss the data presented in Table 5 of Luster et al. (1993) which shows animal studies where decreases of even less than 20% in the AFC are likely to increase susceptibility to infection when the animal is challenged with an infectious agent. Although equivalent quantitative data in humans are not available, it might be useful to examine a review by *Luster, M.I., Blanciforti, L.M., Germolec, D.R., Parks, C., Kashon, M. and Luebke, R. Associating changes in the immune system with clinical diseases for interpretation in risk assessment. In Current Protocols in Toxicology eds. D.A. Lawrence et al., Wiley and Sons, New York pp18.1-18.20, 2004.* This review summarizes effects of moderate losses in immune function on infectious diseases including: immune suppression from chronic stress and relationship to latent virus infections and vaccine responses; immunosuppressive therapy and respiratory infections; and pesticide exposure and HSV incidence. Unfortunately, precise quantitative relationships are not available in humans and no studies with DCE were available but, none-the-less, such information should help provide support for the use of modest AFC decreases as a critical effect.

I prepared a short paragraph that helps support the use of the AFC response as a critical effect that EPA may find helpful.

Decreased ability to respond to infectious agents (in toxicology studies SRBCs are used as a surrogate for a typical foreign material or infectious agent) represents an adverse

effect. Measurement of the antibody response to a T-dependent antigen, such as SRBCs, represents a sensitive and reproducible biomarker of immune function (WHO, 1996). A functional immune system is required by the host to properly defend against infections, as most recently evidenced by the numerous fatalities from infectious diseases in individuals with AIDS or increased deaths from influenza in individuals with compromised immune systems. The exact quantitative association between loss in immune function (or the AFC response) and development of infectious disease is difficult to ascertain. Luster et al., (1993) conducted mouse studies in which groups of mice were administered increasing doses of an immunosuppressive drug (cyclophosphamide) and increasing amounts of infectious agents (e.g., Listeria). Although no longer used, survival was monitored as the indicator to resist Listeria infection. The data were modeled and the results indicated that decreases of even less than 20% in the AFC response decreases the ability of the host to survive infection. Conducting similar studies in humans to determine if similar quantitative relationships would apply of course is not possible. However, there have been a number of studies where groups of individuals with moderately compromised immune system were studied for both infectious disease incidence and immune function. For example, studies in immunosuppressed patients following hematopoietic stem cell transplantation showed a 1.7- fold higher rate of infections with only 2-fold decreases in certain types of CD4 T cells (Storek et al., 2000). Similarly, transplant patients, even when on very low levels of immunosuppressive therapy, show a 1.5 fold increased risk of in immune function and associated increased levels of antibodies to latent viruses, such as CMV, EBV and HSV, an indication of viral reactivation (rev by Kiecolt-Glaser JK and/or Glaser R (I do not have their recent reviews but should be easy to find)).

WHO (1996) Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. A report of the International Programme on Chemical Safety (*Environmental Health Criteria*; 180, World Health Organization, Geneva.

You can find all the other references and a summary of these in the Luster et al. chapter that was sent earlier.

(B) Oral Reference Dose (RfD) for trans-1,2-DCE

3. BMD modeling was applied to data for suppression of AFCs in the spleen in male mice in the Shopp et al. (1985) study to derive the POD for the RfD. Has the BMD modeling been appropriately conducted? Is the BMR selected for use in deriving the POD (i.e., a change in response of 1 standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

James V. Bruckner

The decision to use a change of 1 standard deviation from the control mean is reasonable and scientifically justifiable. The BMD modeling appears to have been appropriately conducted according to standard EPA methodology.

Robert A. Howd

The modeling looks good, and overall this seems an appropriate approach, yielding a non-arbitrary estimate of a “threshold” effect dose that falls within a range that I can be comfortable with.

Ralph L. Kodell

Yes, the BMD modeling has been appropriately conducted. All three models for continuous data in BMDS were fitted to the immune response data (decrease in IgM AFCs per 106 spleen cells) in male mice. A second-degree polynomial model provided the best fit based on AIC and goodness-of-fit p-value. Although the Hill model gave lower BMD and BMDL values than the selected second-degree polynomial model (Appendix B), it was not used because it was over-parameterized (over-fitted), as indicated by no degrees of freedom to assess goodness of fit. Because of a lack of information as to the biological significance of particular changes in AFC levels in rodents, and what these changes would correspond to in humans, EPA defined the BMR as a change in the mean response equal to one standard deviation from the control mean. EPA noted that in this case, a BMR of one standard deviation corresponds to a 20% decrease in AFCs per 106 spleen cells. As noted above, the standard deviation approach gives a BMD that corresponds approximately to 10% excess risk for a normally distributed endpoint that is assumed to have a 1% background risk, and thus the BMD can be interpreted on an excess-risk basis like BMDs for quantal endpoints. The BMR is scientifically justified.

EPA also attempted to fit the three continuous-endpoint models to the relative liver weight data. Only the male mouse relative liver weight data could be modeled adequately, with the Hill model exhibiting the best fit based on AIC value and goodness-of-fit p-value. The BMR was defined both as a 10% change in the mean and as a one-

standard deviation change in the mean, the latter approach giving BMD and BMDL estimates a little less than half the former approach. For the female mice and rats, because BMD modeling could not be done, a NOAEL was appropriately identified in each case as a candidate POD.

The candidate POD based on altered immune status in male mice was lower than the candidate PODs based on increased relative liver weight. Thus, the immune response was considered more sensitive and was chosen as the critical effect, with its estimated BMDL representing the POD for derivation of the oral RfD.

Janice Longstreth

Given that I cannot support concluding that 175mg/kg is a LOAEL in this study, I would suggest that if the Shopp et al. study is to be used then a traditional approach with 175 mg/kg as the NOAEL would be more defensible than the BMD modeling approach.

Michael I. Luster

Yes - the BMD is the most appropriate model.

(B) Oral Reference Dose (RfD) for trans-1,2-DCE

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

James V. Bruckner

My comments about UFs utilized in calculation of the RfD for *cis*-1,2-DCE apply here to *trans*-1,2-DCE.

Robert A. Howd

Considering the large uncertainty on the toxicity of this compound, using the maximum UF of 3,000 is a good idea, in my opinion.

Ralph L. Kodell

A composite uncertainty factor of 3000 was applied to the selected BMDL to derive the RfD. This was composed of a UFH = 10, UFA = 10, UFL = 1, UFS = 10 and UFD = 3. The value of 10 for UFA, UFH and UFS is the default maximum value customarily applied for these uncertainty factors, and is justified in the absence of information to suggest a smaller value. The value of 1 for UFL may be justified, although a dose corresponding to a mean change of one standard deviation can be interpreted to correspond approximately to an excess risk of 10% above an assumed 1% background risk. This may be more than a minimal biologically significant change, and thus may warrant a larger UFL. The value of 3 for UFD is also probably justified based on precedent, but as stated above, I dislike reducing a POD quantitatively by a database deficiency factor. I would personally define UFL = 3 and UFD = 1 and arrive at the same composite uncertainty factor of 3000. Thus, I agree with the composite factor.

Janice Longstreth

I believe that the UFs used could remain the same if the traditional approach for this study was to be used, except that a UF of 10 for extrapolating from a LOAEL to a NOAEL would not be necessary.

Michael I. Luster

The 10X intraspecies UF is justified by known gender, age and genetic differences in P450 metabolism. Both the toxic metabolite (Epoxide) and the suicide/inhibition effect requires P450 metabolites.

I agree with James Bruckner that the interspecies UF can be reduce to 3.0 by removing the 3.3X for the toxicokinetic factor.

No other changes recommended.

(C) Inhalation Reference Concentration (RfC) for cis-1,2-DCE

1. An RfC was not derived due to the lack of available studies to characterize the health effects associated with cis-1,2-DCE administered via the inhalation route. Are there available data that might support development of an RfC for cis-1,2-DCE?

James V. Bruckner

I do not know of other appropriate inhalation studies of *cis*-1,2-DCE to utilize as the basis for calculation of an RfC.

Robert A. Howd

Yes. Since the effects of *cis*-1,2-DCE used for the RfD are systemic, in my opinion all the oral data are relevant for the estimation of an effect level for the inhalation route.

Ralph L. Kodell

Not to my knowledge.

Janice Longstreth

I could not find evidence of any additional studies.

Michael I. Luster

I am not aware of any other available data that will help support development of an RfC.

(D) Inhalation Reference Concentration (RfC) for trans-1,2-DCE

1. An RfC was not derived for trans-1,2-DCE. Has the scientific justification for not deriving an RfC been clearly described in the document? Are there available data that might support development of an RfC for trans-1,2-DCE?

James V. Bruckner

Although *trans*-1,2-DCE was reported to elicit alveolar distension and pulmonary capillary hyperemia, I am not sure these modest effects are consistent with the qualifier for a category 2 gas, namely that it be reactive in respiratory tissue. In addition, DCE is lipophilic and has quite limited water solubility. Therefore, its ability to penetrate the mucus layer in the upper respiratory tract should be limited.

EPA's method for calculating HECs for inhaled halocarbons such as DCE is unsuitable. It takes only animal/human blood:air partition coefficients (PCs) into consideration. Rodents (e.g., mice and rats) absorb substantially larger amounts of halocarbons than humans due to: (1) higher blood:air PCs; (2) higher alveolar ventilation rates; (3) greater cardiac output (i.e., pulmonary blood flow rates); and (4) elevated halocarbon metabolic rates (Brown et al., 1997). The EPA sorely needs to update their HEC methodology. Volkel et al. (1998), and Pahler et al. (1999), for example, subjected rats and humans to equivalent inhalation exposures to perchloroethylene (PERC). The rats absorbed ~ 7X more PERC than the humans and formed substantially larger amounts of potentially toxic/carcinogenic metabolites and adducts. Physiologically-based pharmacokinetic (PBPK) modeling by Bruckner et al. (2004) demonstrated significantly greater systemically uptake of inhaled trichloroethylene by rats than by humans.

A full intraspecies UF of 10 and a subchronic to chronic UF of 10 seem excessive for an endpoint modest as fatty liver. UFs of 3 each should be adequate. Total UFs of 900 (3 X 3 X 3 X 10 X 3) or 1,500 (3 X 3 X 5 X 10 X 3) are more reasonable. Adoption of these or similar UFs would allow derivation of an RfC for *trans*-1,2-DCE. Although the inhalation database is limited, I believe it is better to have a value for this relatively non-toxic halocarbon than to provide no guidance at all.

I have serious reservations about considering the use of the Freundt et al. (1977) study as the basis for deriving the RfC for *trans*-1,2-DCE, after reviewing the study report by Kelly (1998). The investigation by DuPont was clearly superior to that by Freundt et al. (1977). The former is much more robust in that it was: more recent; conducted in compliance with GLP requirements; and considerably more comprehensive and detailed in terms of study design, numbers and sexes of rats/group, numbers of dosage levels, tissues and toxicity parameters evaluated, statistical analyses and presentation of data, etc. The report by Freundt et al. is quite old (> 30 years) and does not establish the purity of the test chemical. It is difficult to tell whether the frequency and/or magnitude of the fatty changes are statistically significant. Fat accumulation in Kupffer cells seems more pronounced at 8 than at 16 weeks. In light of these

considerations, there is greater confidence in the investigation and findings of Kelly (1998) of no adverse effects following inhalation of up to 3,000 ppm *trans*-1,2-DCE for 90 days. The absence of adverse hepatic effects in a number of subchronic oral studies of very high doses of the chemical supports the findings of Kelly (1998). It should be recognized that short-chain aliphatic hydrocarbons [e.g., carbon tetrachloride (Sanzgiri et al., 1995) and 1,1-dichloroethylene (Bruckner et al., 2010)] are substantially more hepatotoxic in rats by ingestion than by inhalation. The results of Kelly (1998) should be used, in conjunction with reasonable uncertainty factors (see above) to derive an RfC for *trans*-1,2-DCE.

Robert A. Howd

No, the rationale for not using data from other routes for consideration of toxic effects levels by the inhalation route has not been justified in this document. (I realize this is an EPA policy decision which also, by policy, is not re-explained in every document.) Yes, there are available data that support the development of an RfC for *trans*-1,2-DCE. I disagree with the (apparent) decision not to use oral data in a PBPK model for *trans*-1,2-DCE, and also with the decision not to derive an RfC because the combined UF using the one inhalation study would be 10,000. We would truncate the UF at 3,000 for estimation of a health-protective level.

Ralph L. Kodell

The database may be inadequate to permit derivation of an RfC. The justification provided by EPA is that, if one attempts to derive an RfC, a total uncertainty factor of 10,000 results. If only a study in abstract form (Kelly et al., 1999) and a study with a single exposure concentration (Freundt et al. 1977) provide inhalation data on *trans*-1,2-DCE, I believe that is sufficient justification for not setting an RfC, regardless of the size of the total uncertainty factor that might result from going through the steps of the process. I believe that it is sufficient to state that the database is too deficient to justify derivation of an RfC, and that it is not necessary to attempt to derive one from the available inadequate data. However, based on the panel discussion, the (unpublished) study of Kelly et al. (1999) upon which the abstract is based, appears to be a well conducted study with data that ought to be evaluated by EPA as a possible basis for setting an RfC. Specifically, I believe that there are several shallow dose-responses on organ weights in Tables 29 (males) and 30 (females) that could be considered. The responses appear more consistent in females than males (e.g., absolute and relative liver weights). Although the dose-responses are shallow, and effects may reach only around 10% at the highest dose (and not reach statistical significance), still they might be used for BMD modeling to derive BMDLs for potential PODs. If one or more BMDLs corresponding to a BMR of 10% can be estimated, then I think they should be considered as possible PODs for setting an RfC for *trans*-1,2-DCE. If a BMDL corresponding to a BMR of 10% cannot be estimated (or is estimated to be greater than 4000 ppm), then I would recommend that 4000 ppm be considered a NOAEL and considered for a POD. Thus, my recommendation is that EPA looks at the Kelly et al. (1999) study and

evaluates the organ weights for possible RfC derivation. If the data are not suitable, then I recommend just stating that the database is inadequate for setting an RfC and leave it at that.

Janice Longstreth

Same comment as for (C)

Michael I. Luster

DuPont Study-Kelly et al 1998,
Trans DCE Inhalation:

Design: SD Rats 9- day inhalation at 0, 200, (400 or 1000) and 4000.
6hrs/day, 5days/wk, 90 days

It looks like a well conducted and fairly complete subchronic study. One concern is that all that animals (including controls) were reported to have liver inflammation. If the animals had viral hepatitis (or something similar), it would impact the results, particularly for a chemical that requires liver enzyme metabolism.

I do not agree with the author's conclusion that no effects in the Kelly study were observed. A toxic effect may be leukopenia (decreased WBC, lymphocytes). These levels were decreased by 25% at the high dose compared to controls and in both sexes and dose responsive. I believe this would constitute an adverse effect. (The authors suggest these changes were due to stress and are biologically insignificant, but no scientific support for their argument is provided.

There were some other less pronounced effects:

Osmolarity of urine (could indicate diabetes or kidney function). Not statistically significant at any dose but looks like a very significant dose-response. Also, some liver effects (for example, increased SDH) but not consistent with other liver enzymes that were decreased. Surprised not too see some increase in liver weights as with oral exposures.

Also- liver inflammation was seen in all animals independent of dose. Was this viral hepatitis and would this compromise the study?

RECOMMENDATION – move forward for trans and cis DCE RfDs but consider reviewing the Kelly study for the potential to establish an RfC for trans DCE.

(E) Carcinogenicity of cis- and trans-1,2-DCE

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgr-d.htm), the Agency concluded that there is inadequate information to assess the carcinogenic potential of cis- and trans-1,2-DCE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

James V. Bruckner

There are no cancer bioassays to provide data on which to base cancer risk assessments.

Robert A. Howd

Yes, the cancer characterization is appropriate. Although a minor metabolite, dichloroacetic acid, is a well-recognized carcinogen in rodents, and is listed as a Known Carcinogen by the State of California, there are no positive cancer studies on either cis- or trans-1,2-DCE. It is not clear to me whether the metabolite would be formed in a high enough proportion to yield a significant increased tumor rate in a 1,2-DCE cancer bioassay.

Ralph L. Kodell

There are no chronic animal studies on either cis- or trans-1,2-DCE and there is only extremely limited acute exposure information on humans. Thus, the Agency's conclusion that there is inadequate information to assess the carcinogenic potential of cis- and trans-1,2-DCE is scientifically justified.

Janice Longstreth

Yes, given the lack of any cancer bioassay data, concluding that there is inadequate information is scientifically justified.

Michael I. Luster

Yes – the cancer weight of evidence is scientifically justified.

V. SPECIFIC OBSERVATIONS

James V. Bruckner

p. 5, pgr. 1: Many studies of the oral absorption of short-chain aliphatic hydrocarbons (halocarbons) very similar to DCE have been conducted. These studies typically show that systemic GI absorption in fasted rodents exceeds 90%. It is generally assumed in risk estimation that absorption is complete.

p. 10, pgr. 2, lines 7 & 8: This last sentence in the paragraph is not pertinent to this document.

p. 19, pgr. 2, line 4: The words “oral” and “gavage” are usually considered to be redundant.

p. 20, pgr. 1: Were there changes in absolute liver and kidney weights? Body weights were slightly lower at the two highest dosage levels.

p. 28, pgr. 2, line 1: Serum LDH, AST and ALP activities are considered as indices of hepatocellular injury rather than function.

p. 36, pgrs. 1 & 2: Were the durations of the observation periods of Hayes et al. (1987) and Freundt et al. (1977) different? It is possible the lower LD50 in the latter study was due to a longer monitoring period, in which there was time for histopathological changes to occur. Nevertheless, numerous subchronic oral studies involving administration of oral doses considerably higher than 1,280 mg/kg showed little/no evidence of any adverse effects in rats.

p. 38, pgr. 2 & 3: Did Freundt et al. (1977) establish the purity of their *trans*-1,2-DCE? Contaminants may have been responsible for their findings of adverse effects, as other researchers appear to have consistently found few if any such effects.

p. 40, pgr. 1: An explanation, of the biological significance of the finding of reduced immobility in the behavior despair swimming test, should be given.

p. 42, pgr. 3, lines 7 – 10: Why bother to explain that “the expression of AFCs on a per spleen basis is affected by changes in the relative size of the spleen”, when there was no effect on spleen weight?

p. 44, pgr. 3, line 4: Were liver GSH levels measured?

p. 45, pgr. 2, lines 5 & 8: Was liver GSH content measured?

p. 46, pgr. 3: It is surprising that opacity was observed in the washed eye but not in the unwashed eye.

p. 48, pgr. 2, line 2: Did Jenkins et al. give DCE by (oral) gavage?

p. 64, pgr. 5, lines 7 – 9: It should be noted here that McMillan (1986) administered very high doses of *trans*-1,2-DCE.

p. 70, pgr. 3: The source of the data in this paragraph should be identified.

ADDITIONAL REFERENCES

Brown, RP; Delp, ML; Lindstedt, SL; et al. (1997). Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind Health* 13: 407-484.

Bruckner JV; White, CA; Muralidhara, S; Hines, C; Dallas, CE (2010). Influence of exposure route and oral dosage regimen on 1,1-dichloroethylene toxicokinetics and target organ toxicity. *J. Pharmacol Exp Therap* (in press).

Bruckner, JV; Keys, DA; Fisher, JW (2004). The acute exposure guideline level (AEGL) program: Applications of physiologically based pharmacokinetic modeling. *J Toxicol Environ Health, Part A*, 67: 621-634.

Kedderis, GL (1997). Extrapolation of in vitro enzyme induction data to humans in vivo. *Chem-Biol Interact* 107: 109-121.

La, DK; Schoonhoven, R; Ito, N; Swenberg, JA (1996). The effects of exposure route on DNA adduction formation and cellular proliferation by 1,2,3-trichloropropane. *Toxicol Appl Pharmacol* 140: 108-114.

Pahler, A; Parker, J; Dekant, W (1999). Dose-dependent protein adduct formation in kidney, liver, and blood of rats and human blood after perchloroethylene inhalation. *Toxicol Sci* 48: 5-13.

Renwick, AG (1998). Toxicokinetics in infants and children in relation to the ADI and TDI. *Food Addit Contam* 15 (Suppl): 17-35.

Sanzgiri, UY; Kim, HJ; Muralidhara, S; Dallas, CE; Bruckner JV (1995). Effect of route and pattern of exposure on the pharmacokinetics and acute hepatotoxicity of carbon tetrachloride. *Toxicol Appl Pharmacol* 134: 148-154.

Volkel, W; Friedwald, M; Lederer, E; et al. (1998). Biotransformation of perchloroethylene: Dose-dependent excretion of trichloroacetic acid, dichloroacetic acid, and N-acetyl-S-(trichlorovinyl)-L-cysteine in rats and humans after inhalation. *Toxicol Appl Pharmacol* 153: 20-27.

Robert A. Howd

p. viii, definition of ID₅₀: "concentration to achieve 50% decrease in immobility" is appropriate for the behavioral assay described p. 40, but certainly not for the cell culture assay described p. 50. Shouldn't both definitions be given, or different terms used for the two measures?

p. 4, Table 2-1. Henry's Law coefficients look strange - measured or calculated at 24°C? That's an unusual temperature, and the vapor pressure and water solubility listed above (from which Henry's Law coefficients are calculated) are both at 25°. Also, the mixture at 25° has a Henry's law coefficient that's identical to the cis number at 24°C (i.e., actually lower), when it should be between the two values.

p. 6, third paragraph: "In an experiment using isolated perfused liver from female Wistar rats and exposing the perfusate to cis- or trans-1,2-DCE in the gas phase, Bonse et al. (1975) found that, at a given concentration in the gas phase, trans-1,2-DCE attained less than one-half the concentration of cis-1,2-DCE in liver, which was attributed in part to inhibition of CYP450 by the trans-isomer." I don't follow this explanation; multiple experiments show trans taken up 1/2 as much as cis. This is understandable, since its water solubility is less and its vapor pressure is more. It seems to me that inhibition of a metabolizing enzyme (by trans) would lead to higher equilibrium levels, not lower.

p. 7, third paragraph, discussing dermal uptake. I think it should say that the higher lipophilicity of the trans isomer may increase its dermal absorption, not just "affect" its absorption. It's better to be specific, to minimize potential confusion because of the other data on higher tissue uptake and equilibration for the cis isomer. Absorption through the stratum corneum is significantly different than the other processes, and this deserves a clear statement.

p. 8, Section 3.3. "...epoxidation of the ethylene double-bond-forming dichlorinated epoxides, which can undergo a nonenzymatic rearrangement..." Here is a typo that alters the meaning; the hyphen between bond and forming should be replaced with a comma and a space.

p. 14, first para under Section 3.5, last line. It appears that KM should be K_M. Also could be changed to K_M in List of Acronyms

pp. 19, 20, 24-26, 28, 31, 32, 46. Relative organ weights are mentioned in study descriptions, without stating what the comparison is. Are these relative to body weight or brain weight? The mention of the author's comment on organ-to-body weight data near the end of the middle paragraph on p. 19 is not adequate, since the very next paragraph also refers to both percent of body weight and organ to brain ratios. In later discussions of the relative weight endpoints (such as pp. 87-88), it's not crucial, as long as one can check back to the original study descriptions.

pp. 25, 26. It is stated here that Table 4-6 shows relative liver weights, but Table 4-6 (p. 27) actually shows absolute liver (and lung) weights. The text says there was an 11% decrease in relative lung weights in females at the highest dose, while the Table shows an 11% decrease in absolute lung weights. Both may be true, but the disparity is confusing. It would also be good to show body weights in Table 4-6.

p. 48, description of Freundt and Macholz study. The experimental methods should be stated (evaluation of hexobarbital sleeping time, zoxazolamine paralysis time, and formation of 4-aminoantipyrine from aminopyrine). It's confusing to report results, i.e., that oxidative metabolism was inhibited, without reporting the test method.

p. 50. The study of Mochida et al. reports "ID₅₀" levels at approximately the water solubility limits of cis and trans 1,2-DCE. In addition to questions about the relevance of such high concentrations, some clarification of the meaning of ID₅₀ might be appropriate. The context of the research would indicate that this might mean the concentration causing half-maximal inhibition of growth or cell division. The listed definition of ID₅₀ (p. viii), "concentration to achieve 50% decrease in immobility," is not applicable here.

p. 50, last paragraph. Discussion of Tse et al. (1990) refers to effects of trans-1,2-DCE in cell culture at 2%, v/v. It should be noted that the water solubility of this chemical is ~0.5%. While the fetal calf serum and other supplements in the cell culture medium may increase solubility of chemicals, compared to solubility in pure water, the relevance of any effect at this unphysiologically high concentration, if achievable, should certainly be questioned.

p. 77, Section 4.8.3.1. In inferring that variations in CYP2E1 may cause sensitivity to toxic effects of cis or trans 1,2-DCE, some mention should be made of why this would be so. Presumably this would involve increased formation of a particular toxic metabolite.

Section 4.8.3.2. p. 78. Also, in the discussion of GST, the statement is made that a low-activity allele of GSTZ may result in increased susceptibility to toxic effects of DCA; it may be appropriate to mention the presumed cause of this (higher concentrations or more prolonged exposure to DCA). Also, a recent article by Caldwell and Keshava (Key issues in the modes of action and effects of trichloroethylene metabolites for liver and kidney tumorigenesis. *Environ Health Perspect.* 2006 Sep;114(9):1457-63) provides some relevant perspectives on toxicity of DCA that might be useful to cite.

Section 5.1.1.1, second para, p. 79. Increases in kidney weight caused by cis-1,2-DCE were statistically significant only in males, but were quantitatively similar in females. Given this similarity, and the fact that the magnitude of the changes is similar to that in liver, it seems strange that the kidney effects should be discounted. The LOAEL is lower for the kidney effect (32 mg/kg-day than for the liver effect (97 mg/kg-day) and the BMDL for the kidney data should be lower (particularly because of the less consistent dose-response pattern).

On the other hand, the argument that the liver weight effects are more consistent (resulting in a better model fit) appears reasonable, and the calculated BMDL for males, based on liver effects, appears low enough to be representative of the effects on both liver and kidney. If the extrapolation to a health-protective level were based on the kidney LOAEL (no benchmark calculation), the combined UF would be over 3,000, and thus either be truncated at 3,000 or the data judged inadequate to derive an RfD.

Section 5.1.2.1, p. 84. Discussion of NTP (2002), near the end of the major paragraph, says a 915% increase in liver weight in male mice. This should be 9-15%.

Paragraph below Table 5-3, p. 87. Discussion of the 10% change in relative liver weight for the BMR for cis-1,2-DCE as representative of a “minimally biologically significant” change (equivalent to a LOAEL) might also mention that the use of the lower confidence limit on the dose to calculate the BMDL was justified as reasonably equivalent to a NOAEL by U.S. EPA 2000b. This provides a distinction between the 10% change as the BMR, which equals the BMD, and the BMDL as the value used for the extrapolation. This is used differently for trans-DCE as described below.

Section 5.1.2.3, 3rd bullet, p. 89. Here the term BMR is used to describe the BMDL_{1SD} for trans-1,2-DCE. It is used as the point of departure and essentially treated as a NOAEL (with a UF of 1 for LOAEL to NOAEL), but the same wording as above is used to describe it as “a minimally significant biological change.” It does not seem appropriate to use this wording to describe a LOAEL equivalent for one chemical and the NOAEL equivalent for the other.

REM ONLY: Section 5.1.2.4, p. 91, discusses arriving at the same RfD for trans-DCE as in the previous EPA risk assessment, by another means. Previously EPA used the Barnes study, with the 17 mg/kg-day NOAEL, and a UF of 1000. Now they use Shopp et al. and a BMR approach with a UF of 3000, which acknowledges the data base deficiencies.

Section 5.2.2.3, RfC Derivation, p. 94. There is no RfC for trans because the UF would be 10,000, as a policy decision. Lack of consideration of other routes of exposure in deriving RfCs for systemic effects limits the ability to derive informative values and contributes in this case to the high UF. We would generally use both oral and inhalation studies, and convert them all to systemic doses for derivation of appropriate health-protective values. Also, we would more likely truncate the UF to 3,000 to develop a value, concentrating on the guidance statement in U.S. EPA (2002), page 5-5, “the Technical Panel recommends limiting the total UF applied to a chronic reference value for any particular chemical to 3000.” We think that the accompanying guidance statement, “Even when there is uncertainty in four areas, the database should be carefully evaluated to determine whether the derivation of a reference value is appropriate,” would likely be answered in the affirmative in this case, if all the data were considered. We in fact did truncate the UF to 3,000 for the derivation of our public health goal for cis-1,2-DCE.

Section 6.1, first para, p. 97. Here it states that boiling points of these two chemicals are between 50 and 60°C. However, Table 2-1, p. 4, gives a boiling point of 48.7°C for the trans isomer.

Appendix B. No comments; calculations look fine.

References

U.S. EPA (2002). A review of the reference dose and reference concentration processes. Final Report. Prepared by the Risk Assessment Forum for U.S. EPA, Washington, D.C. EPA/630/P-02/002F, December 2002.

Ralph L. Kodell

Page 20, Table 4-1.

Are the values means \pm SDs or means \pm SEs? I think they're SEs. Tables of means throughout the report need to be consistent with respect to the accompanying measure of variation. Some tables indicate SDs, others SEs and still others give no indication.

Page 21.

I think the study by Shopp et al. (1985) should be mentioned in section 4.2.1.2.2 along with the other subchronic studies, with a note that discussion of the study will be deferred to section 4.4.3.2 because it is a targeted immunology study and not a general toxicity study.

Page 27, Table 4-6.

This is a table of absolute organ weights. To be consistent with the effects listed as major effects in the summary table, Table 4-12, Table 4-6 needs to include relative liver weights for males, and relative lung and thymus weights for females. The footnote should state that Duncan's multiple range test was used to determine statistical significance.

Page 43, Table 4-8.

To be consistent with the summary table, Table 4-12, the exposures in Table 4-8 need to be expressed in mg/kg-day. The footnote should state that Duncan's multiple range test was used to determine statistical significance.

Page 60, Table 4-12.

Most of the NOAEL values don't make sense. For all except the Shopp study, I believe they are incorrect. Also, where NOAELs exist, I think the NOAEL for each sex ought to be listed instead of just the NOAEL for one sex.

For the NTP rat study, there needs to be an entry for Females indicating reduced RBC count at 1580 and higher.

For the Shopp study, if the significant result for sRBC-responsive cells in Females is to be disregarded because of its per-spleen basis, then it ought not to be listed in Table 4-12 because that gives a false impression that it will be considered for deriving a POD.

Page 85, Table 5-2.

To be consistent with the summary table, Table 4-12, the exposures in Table 5-2 need to be expressed in mg/kg-day.

Page B-5 – B-7.

BMD results for the one-standard-deviation change are included along with the results for the 10% change on page B-4, so separate modeling isn't needed.

Page B-11 – B-13.

BMD results for the one-standard-deviation change are included along with the results for the 10% change on page B-10, so separate modeling isn't needed.

Janice Longstreth

General Comments

There are still numerous acronyms that should be added to the Acronym list on page viii. These include Koc, Kow, Kp, Km, Kd, Kde, NADPH, nmol, NTP, SKF-5, Hz, V, ppm, Ca, Cv1, Qi, Pi, SPF, G-6-Pase, Fe(III)ADP, dw, dL, BMDL, BMDx, BMDL_{ISD}, UF_A, and UF_H, but I also suggest the document be carefully reviewed for others including those used in Tables and Figures. Also, RAM is explained as rate of amount metabolized on pg 16 and as rate of metabolism in acronym list – which is it?

For future documents, suggest that you provide a line numbered document to facilitate request to “Provide specific observations, corrections, or comments on the document, mentioning page, paragraph, and/or **line number** [emphasis added].”

Section 2 Chemical and Physical Information

I realize that it is not the intent to make these documents comprehensive, but given EPA's ownership of vast quantities of exposure information, I would like to see that some of those data at least be mentioned or pointed to in this section. Examples might include:

- Information on how these materials are produced, as this can be important to knowing possible contaminants. Also, for the cis- and trans- isomers, how are they made/separated, what is the typical purity and what are the contaminants? Should also provide information as to production volume of these chemicals, the releases registered under TRI, and current information from groundwater surveys.
- Half-lives in air are provided, but no mention is made of the degradation reaction involved in the loss.

- Which isomer (or what is the ratio of isomers) is produced from anaerobic degradation of trichloro- and tetrachloroethylene?

Suggestions for the other Sections:

Section 3. Toxicokinetics

- Section 3.1.2, page 6. It is noted that results obtained in a given rat strain should not be extrapolated to another strain, but for many of the references discussed under 3.1.2 strain is not indicated. Examples include Eger et al. (2001), Gargas et al. (1989, 1988). Please indicate strain or “strain not specified” for all species and all studies.
- Also, Section 3.1.2. Bonse (1975), specify which CYP450 is inhibited by trans isomer.
- Section 3.1.3. In the interim report on dermal exposure, did EPA calculate the cited dermal permeability coefficient and, if so, how has it undergone review? If not, suggest primary source be cited rather than the EPA document. As another question, the version of the interim report I have says do not cite or quote; has that policy been changed? Might this not violate IRIS’s strong preference for using peer reviewed information?
- Also, Section 3.1.3. Indicate whether there is any validation of the Potts and Guy (1992) proposed formula for deriving Kp values.
- Section 3.2. Please provide additional details about the perfusate used in Bonse et al. 1975, since this can be important to understanding this experiment.
- Section 3.4. I’m not sure I understand why the Pleil and Lindstrom study was not considered a study that assessed the elimination of cis-1,2-DCE by the inhalation route and thus why the first sentence in this section is not in conflict with the third sentence in this section.
- Section 3.5. Given the fact that the work on PBTK cannot be used for this report, it is not clear to me why three pages are devoted to characterizing what has been done in this area. Why not just refer to the studies and then give the bottom line provided in the last paragraph of this section?
- Section 4.2.2.2.2, pg 30, last sentence. I found no discussion of statistical analysis of the histopathological changes in the liver in the Freundt et al. 1977 study so I am at a loss to explain where this information came from.
- Section 4.5.5, pg 59. In the discussion of the Cronin study, in the next to the last sentence: “The author was able to obtain a description...” please indicate where DCE fell (presumably in the less neurotoxic ones).

Michael I. Luster

Minor editorial comments and suggestions:

- Table 3-1, p 8: Indicate in header that this represents in vitro (not in vivo) studies.

- TCE is a 'high profile' chemical. Although different toxic profile, I wonder whether it would be worth providing a brief comment indicating that toxicokinetics, toxicity, similarities or differences, if any, between DCE and TCE (short paragraph).
- Table 4-1, p 20: I assume these weights are relative to BW. Might be helpful to include actual body weight.
- Since some of the studies used cis/trans mixture, it might be helpful to briefly discuss similarities/differences in target organ and relative potency between the two.
- The Kelly 1999 reference seems like a potentially good study but only the abstract was available. Did EPA try to obtain the original study results?
- Pg 67. Can you define the significance, if known, of an erythroid mass in the liver?
- Not my expertise, but I think the increased liver weights from DCE were probably due to increased metabolic activity from P-450 induction. However, the increased liver weight induced by many chemicals which manifest very minimal liver pathology is due to blockage of hepatic triglycerides. Since the metabolite DCA effects lipid metabolism, any evidence found for mild steatosis?