External Peer Review

Toxicological Review of 1,1,1-Trichloroethane

Compilation of Reviewer Comments and Responses to Charge Questions

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The ORISE IRIS Technical Assistance Team has neither altered nor edited these comments for grammatical or other errors.

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The U.S. Environmental Protection Agency (EPA) is conducting a peer review of the scientific basis for the human health assessment of 1,1,1-trichloroethane that will appear on the Agency's online database, the Integrated Risk Information System (IRIS).

Feedback on the Toxicological Review of 1,1,1-Trichloroethane was sought in three general areas: (1) general clarity and thoroughness of the documents, (2) issues concerning the derivation of reference values specific to 1,1,1-trichloroethane, and (3) characterization of the carcinogenic potential for 1,1,1-trichloroethane.

This report is a compilation of the final comments submitted following the peer review panel meeting for 1,1,1-trichloroethane where the peer reviewers met to discuss their initial comments on the draft Toxicological Review. The external peer review panel meeting was held April 20, 2007, in Washington, DC.

The final, written comments provided here are the individual opinions of the reviewers and not a consensus of the group.

GENERAL QUESTIONS

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

Jeffrey W. Fisher, Ph.D., Chair

RfD and RfC values for different periods of exposure (eg. less than 24 hrs) are not discussed. The classical definition (lifetime exposure) of RfC or RfD does not appear to fit for less than lifetime exposures. This can be confusing to readers. Is a new definition needed?

Scott E. Bowen, Ph.D

Yes, I found the Toxicological Review to be a logical, clear, and a somewhat concise (> 150 pages) report of the available database on 1,1,1-trichloroethane. The review provides detailed information on the toxicokinetics of 1,1,1-trichloroethane (primary target appears to be the central nervous system especially at higher concentrations) as well as a detailed review of the hazard identification information that is currently available for studies in humans and other species. The review also makes clear that there is not enough oral toxicity information available to support derivation of oral RfD values for acute, short-term, subchronic, or chronic exposure. Finally, the Toxicological Review clearly and logically concludes that the current database for 1,1,1-trichloroethane provides inadequate information to assess carcinogenic potential based on the weight-of-evidence categories in the EPA 2005 Guidelines for Carcinogen Risk Assessment.

Richard B. Mailman, Ph.D

The review was generally excellent, and showed a high level of scholarly thought. One addition I might suggest would be a brief review that provided context about the behavioral actions of 1,1,1-TCE. Specifically, although the review alludes to the similarity between 1,1,1-TCE and inhalational anesthetics, to the non-neurotoxicologist, the reasons for "non-monotonic" behavioral changes may not be clear. For this reason, I would recommend that a brief section expand on the minimal background currently present, such that readers will understand that similar patterns of effect can be seen with many agents that are either structurally or functionally similar.

Matthew D. Reed, Ph.D

The review is generally logical, clear and concise in presentation. EPA may consider referencing subchronic RfC/ RfD justification in the chronic situation. Both use essentially the same rationale. EPA has objectively and transparently represented and synthesized the scientific evidence of cancer and noncancer health effects.

D. Alan Warren, Ph.D

The toxicological review is logical and clear, but at 158 pages less references, not concise. By comparison, California's *Public Health Goal for 1,1,1-Trichloroethane in Drinking Water* document is only 33 pages with references. As EPA's review is not intended as an exhaustive literature review, those studies clearly not having any decisive relevance to RfD or RfC derivation or qualitative cancer evaluation could be excluded (or merely referenced), as they detract from rather than inform the issue at hand. This would reduce the length of the Hazard Identification section, allow for more detail to be provided on those studies of most import, and I dare say, perhaps eliminate the need for the synthesis section altogether which I found repetitious. Generally speaking, I believe the EPA's analysis to be objective and sufficiently transparent.

2. Are you aware of additional studies that should be considered in the assessment of the noncancer and cancer health effects of 1,1,1-trichloroethane?

Jeffrey W. Fisher, Ph.D., Chair

I am unaware of studies missing from this document.

Scott E. Bowen, Ph.D

The toxicological review may benefit with the addition of some or all of the following studies which were not included in the original document:

Loew, G. H., Rebagliati, M., Poulsen, M., 1984 Metabolism and relative carcinogenic

potency of chloroethanes: a quantum chemical structure-activity study. Cancer Biochem Biophys 7,109-32.
Lynge, E., Anttila, A., Hemminki, K. 1997 Organic solvents and cancer. Cancer Causes Control, 8, 406-19.
Stewart, P. A., Lee, J. S., Marano, D. E., Spirtas, R., Forbes, C. D., Blair, A. 1991. Retrospective cohort mortality study of workers at an aircraft maintenance facility. II. Exposures and their assessment. Br J Ind Med, 48, 531-7.
Dickerson, C. L., Biesemeier, J. A. (1982). Aspiration of methyl chloroform. Vet Hum Toxicol., 24(3), 167-8.
A. Muttray, B. Moll, M. Faas, L. Klimek, W. Mann, J. Konietzko, Acute effects of 1,1,1-trichloroethane on human olfactory functioning, American journal of rhinology 18 (2004) 113-7.
W.G. Troutman, Additional deaths associated with the intentional inhalation of typewriter correction fluid, Veterinary and human toxicology 30 (1988) 130-2.
H.G. Verschuuren, C.G. de Rooij, Health risk assessment of environmental exposure to 1,1,1-trichloroethane, Regul Toxicol Pharmacol 11 (1990) 90-9.
M.G. Yost, M.A. Rose, M.S. Morgan, An evaluation of Fourier transform infrared (FTIR) spectroscopy for detecting organic solvents in expired breath, Applied occupational and environmental hygiene 18 (2003) 160-9.

Richard B. Mailman, Ph.D

I was not aware of additional toxicological studies that should have been included from the literature, although other panelists noted a few including an unpublished study. I do feel that some data relevant to the current and predicted use and exposure levels would be a critical aspect of context for this document. This would have permitted a more precise answer about when one might have advised seeking other or better data to resolve uncertainties in RfDs or RfCs. I also would like to offer a general comment regarding the PBPK rather than have to refer to it several times throughout the charge questions. Although I am not a PBPK expert, I am experienced at modeling in general. I found the Yang report to be thorough, exhaustive, and accurate as it dealt with its particular goal. This said, I found the modeling *in toto* to be scientifically unsatisfying. I can illustrate this by examining the early figures (although this applies throughout). Clearly, the modeling used for Figures 2-4 (unlike Figure 1) is deficient in capturing important aspects of the data. Although I recognize the charge given to Dr. Yang (that he fulfilled admirably), an opportunity was missed in not developing models that fit these data more accurately, and hence providing additional toxicological insight.

Matthew D. Reed, Ph.D

Generally, the current phase out of trichloroethane for non-essential usage and the lack of definitive adverse events associated with previous long-term human exposure preclude the need of further testing of the oral and inhalation hazard associated with this compound.

D. Alan Warren, Ph.D

Although apparently not published in the peer-reviewed literature, the chronic inhalation study of 1,1,1-trichloroethane in Sprague-Dawley rats is relevant (Quast et al., 1978; Rampy et al., 1977). In this particular study, rats were exposed to 0, 875, or 1750 ppm for 6 hr/day, 5 days/wk, for 12 months and allowed to survive until 31 months. The study is similar to the inhalation bioassay of Quast et al. (1984, 1988) in that there was no evidence of a carcinogenic response, but differs with respect to the strain of rat utilized (i.e., Sprague-Dawley versus Fischer 344). Importantly, it serves as the only other chronic bioassay to that of Maltoni et al. (1986) to use the Sprague-Dawley rat, and thus is of value for comparative purposes despite different dosing routes and exposure lasting 1 year instead of two.

QUESTIONS RELATED TO THE DERIVATION OF ORAL REFERENCE DOSE (RfD) VALUES

1. The conclusion was reached that the available oral toxicity information was inadequate to support derivation of oral RfD values for acute and short-term exposure durations. Do you agree with this conclusion? Is the rationale for not developing an acute or short-term oral RfD transparent and objective? If you disagree, what study should be used to derive an oral RfD?

Jeffrey W. Fisher, Ph.D., Chair

I agree that there is a modest amount of oral data for setting acute or short term RfDs. I agree with this conclusion. The rationale for not providing acute or short term RfDs is too terse (page 120). I think a better summary of the weaknesses of each study should be given, leading to the conclusion that an oral RfD could not be developed. The use of inhalation data sets to perform route to route extrapolation (human PBPK modeling) was not conducted for CNS endpoints. Justification for not doing route to route extrapolation should be articulated. If inhalation exposures were used for neurobehavioral endpoints, such as the Mackay et al. study, and agreement could be reached on the oral uptake kinetics of methyl chloroform, the internal AUC calculations could be performed. If this is important, I think it is feasible to do these calculations (discussion below).

Scott E. Bowen, Ph.D

Yes, I agree with the conclusion that based on the available oral toxicity information of only one human exposure (accidental) and three rat studies (with conflicting reports-two report hepatotoxicity and the third did not) is currently insufficient to support derivation of oral RfD values for acute and short-term exposure durations.

Richard B. Mailman, Ph.D

I agree with the conclusion that the data are not adequate, and I think the rationale for reaching that conclusion was logical and transparent, although the rationale behind this could be somewhat expanded.

Matthew D. Reed, Ph.D

I agree with the general conclusion. The rationale is transparent and objective.

D. Alan Warren, Ph.D

I agree that the available oral toxicity data are inadequate to support derivation of acute and short-term oral RfDs based on the following: 1) human studies via the oral route are limited to a single case in which 1 ounce of 1,1,1-trichloroethane was accidentally ingested; 2) of three single gavage studies in rats, two reported mild hepatotoxicity based on marginal elevations of single enzyme markers, while a third failed to note any hepatotoxicity even at a higher dose; 3) the three short-term gavage studies in rats focused exclusively on liver and/or renal effects and suffered from high mortality rates, the use of a single dose level, or a lack of histopathological confirmation of urinary parameters; 4) of the oral reproductive/developmental studies, those focused on reproductive function and neurodevelopmental effects were negative, while the drinking water studies of cardiac development, although intriguing, were limited by the use of a single dose level, failure to regard litter as the experimental unit of analysis, and unsuccessful efforts at experimental replication; and 5) the 2-day gavage study of Spencer et al. (1990) examined only a single dose level and failed to demonstrate that the reported electrophysiological changes had functional consequences. The lack of suitable acute and short-term oral studies, coupled with the apparent inability of the Reitz et al. (1988) PBPK model to accurately simulate oral gavage data in rats (suggesting that it is not an appropriate tool for extrapolating acute inhalation exposures to the oral route), is an adequate explanation as to why acute and short-term RfDs were not derived.

2. The 90-day dietary study by the National Toxicology Program (NTP, 2000) was selected as the basis for the subchronic and chronic oral RfDs. Is the selection of NTP (2000) as the principal study scientifically justified? Is the rationale for selecting this transparent and objective? Are there any other studies that you believe would be justified scientifically as the bases for the subchronic and chronic RfDs?

Jeffrey W. Fisher, Ph.D., Chair

The 90 day NTP study is the best choice for determining subchronic and chronic oral RfDs. The NTP study has the advantage over academic studies of established QA/QC procedures, evaluation of many tissues or organs, and evaluation of tissues by boarded

pathologists. The Bruckner study had excess mortality.

Scott E. Bowen, Ph.D

I support the decision that the NTP 2000 study was the best for determining the subchronic and chronic oral RfDs. The rationale for selecting this study is transparent and objective. I also agree with the decision not to include the Bruckner et al. 2001 paper because of the use of oral bolus dosing and the high rates of mortality.

Richard B. Mailman, Ph.D

The rationale for using the 90-day NTP dietary study is transparent and objective in my opinion, and that the high mortality rate in the Bruckner et al. 2001 paper make that work less relevant.

Matthew D. Reed, Ph.D

The selection of the 2000 NTP study is justified. The rationale is transparent and objective. No further studies are necessary.

D. Alan Warren, Ph.D

I agree with EPA that the oral bolus dosing regimen employed in Bruckner et al. (2001) and the resulting high mortality rate make it less relevant for subchronic and chronic RfD derivation compared to the feeding study of NTP (2000). And while the Agency's rationale for selecting NTP (2000) as its principal study is transparent, it is nonetheless questionable, as the critical effect of reduced body weight gain could be considered adaptive rather than adverse. In addition, the majority of those studies cited by EPA in support of reduced body weight as a sensitive effect were conducted in rats (see section 5.1.3.1), not in female mice which serves as the basis for the subchronic and chronic RfDs. This is noteworthy given that rats in NTP (2000), especially female rats, were more resistant to reduced body weight gain compared to mice of either sex. Considering the paucity of subchronic/chronic oral dosing studies with 1,1,1-trichloroethane, it was appropriate for EPA to explore the possibility of extrapolating between the inhalation and oral routes. Granted, differences in relative hepatotoxicity by the oral and inhalation routes have been reported (i.e., the oral studies of NTP (2000) and Bruckner et al. (2001) failed to report even mild hepatotoxicity at less than lethal doses, whereas the inhalation study of McNutt et al. (1975) clearly did so). However, I can think of no reason (other than a difference in applied dose) why 1,1,1-trichloroethane via the oral route would be less effective at targeting the liver than when administered via inhalation. In fact, one might predict quite the opposite. Therefore, EPA's justification for not performing a route-to-route extrapolation (i.e., differences in relative hepatotoxicity by route of exposure) is not biologically based or rational. For this reason, the Agency should perhaps reconsider route-to-route extrapolation to establish subchronic and chronic RfDs, with a focus on those inhalation studies reporting mild hepatotoxicity or alternatively, the subchronic inhalation study of Rosengren et al. (1985) reporting astrogliosis in the

cerebral sensorimotor cortex.

3. A 10% decrease in mean terminal body weight of the mouse relative to the control mean served as the basis for the subchronic and chronic oral RfDs. Is the selection of decreased body weight gain as the critical effect scientifically justified? Has the rationale for selection of this critical effect been transparently and objectively described? Is a 10% decrease in mean terminal body weight the most scientifically justified response to use given the findings of NTP (2000) of a statistically significant decrease in mean terminal body weight (compared to the control mean) at a dose lower than the $BMDL_{10}$? Would presenting a BMD analysis of the 1% and 5% responses be helpful to the reader? If you disagree with the choice of body weight as the critical effect is there a preferable alternative?

Jeffrey W. Fisher, Ph.D., Chair

A 10% decline in body weight reflects a toxic effect usually seen with large doses of chemicals and may not be relevant at lower doses. The male body weight data were not dose dependent. Therefore, this endpoint is weak, but it is a toxic response. The rationale appears to be stated adequately. I do not know of an alternative endpoint.

Scott E. Bowen, Ph.D

I will agree that the 10% decrease in mean terminal body weight of exposed animals compared to control animals is scientifically justified but difficult to understand. One could also consider the renal lesions and decreases in liver weight observed in the male rats as one of concern (NTP, 2000). However, I do realize that these renal effects have not been reported in any other study suggesting that this effect is equivocal and may not be a valid marker. The rationale for the selection of this critical effect has been transparently and objectively described but may be improved even further by including more descriptions from the pharmacology/toxicology literature clarifying the use of body weight as a critical effect. Finally, I would support (but not necessarily recommend) presentation of the BMD analysis of the 1% and 5% responses.

Richard B. Mailman, Ph.D

In light of the relatively subtle effects of even high doses (and the lack of a clear dose-response effect), these choices seem to be the best available, if suboptimal. The rationale for the 10% body weight change as a threshold might, however, be useful to expand upon. Some might express the view that such a change is “adaptive” rather than “adverse.” My opinion is that this is a mechanistic, not toxicological, distinction. Specifically, if a compound caused an animal/person to loss weight from a healthy free-feeding weight from adaptive mechanisms, this might well be accompanied by other “adaptive” effects such as decreased motivation that would clearly be an adverse effect. Such an effect with this class of compound is likely to occur at doses/concentrations far

below those causing more typical toxicological changes. It is for this reason that I made the general recommendation (above) regarding mechanisms of anesthetics, as I believe this would provide a firmer for the offered conclusions.

Matthew D. Reed, Ph.D

Not particularly. The lack of associated daily observations, gross necropsy observations, or general histopathology associated with a decrease in BW make defending this selection difficult. An ~10 percent decrease in BW (although referenced as an acceptable adverse sign) without direct data reference for the reader (initial and time interval weight curves) makes assessing this effect difficult. However, given the overall lack of trichoroethane chronic exposure effects in the sum of studies and without redirecting the dogma of hazard assessment, the selection of BW to set RfD is reasonable. The rationale for the choice is as well defined as possible.Yes. Presentation of BMD analyses would be helpful. For the purposes of this document, there is not better parameter. For the purposes of this document, there is not better parameter.

D. Alan Warren, Ph.D

As alluded to earlier, the selection of reduced body weight gain as the critical effect is questionable, as it could be considered adaptive rather than adverse. Many studies indicate that repeated acute stress, such as might be caused by chemical exposure, induces a chronic change in weight independent of stress-induced hypophagia. Given 1,1,1-trichloroethane's propensity to partition to fat once absorbed, a leaner experimental animal would arguably experience a lower overall body burden, more rapid elimination, and a possible reduction in 1,1,1-trichloroethane-induced toxicity. Granted, if the subchronic and chronic RfDs are to be based on an oral study, there is little in the way of critical effects from which to choose. The question is thus whether defaulting to reduced body weight gain as a critical effect is preferred over the use of inhalation-to-oral extrapolation or awaiting a more suitable principal study for RfD derivation. Admittedly, my comfort level with reduced body weight gain as the critical effect could be increased if EPA provided some evidence that it is an indication of unequivocal impairment or that it somehow contributed to frank toxic effects. In the absence of this, my preference is to explore route-to route extrapolation as an option. My misgivings about the use of reduced body weight gain aside, the methodology by which subchronic and chronic RfDs were derived appears sound. According to EPA's Benchmark Dose Technical Support Document, a 10% change in body weight is the minimal level of change generally considered to be biologically significant. Considering that such data are measured on a continuum with no sharp demarcation between normal and "adverse" values, the biological basis for this statement would be a welcomed addition to EPA's review. The use of the $BMDL_{10}$ of 2155 mg/kg-day for RfD derivation is not problematic in my opinion, despite male mice exhibiting a 9% reduction in body weight gain at a fraction of this dose (i.e., 850 mg/kg-day or 5000 ppm). Not only was the dose-response in male mice essentially monotonic, but a statistically significant reduction in body weight gain among female mice (which served as the basis of the RfDs) was first observed at 10,000 ppm or 2820 mg/kg-day, a dose in excess of the $BMDL_{10}$. Thus, I see little to no benefit

from presenting benchmark dose analyses of the 1 and 5% responses.

4. Are the uncertainty factors applied to the point of departure for the derivation of the subchronic and chronic RfD values scientifically justified and transparently and objectively described?

Jeffrey W. Fisher, Ph.D., Chair

Subchronic and chronic UFs are ok.

Scott E. Bowen, Ph.D

Yes, I believe the composite uncertainty factor of 300 (10-fold UF for extrapolation from laboratory animal to humans, 10-fold UF for intraspecies variation, 3 for database deficiencies) is appropriate. These values are scientifically justified and transparently and objectively described especially with the lack of data establishing sensitive targets following acute oral exposure.

Richard B. Mailman, Ph.D

The composite uncertainty factor of 300 (multiple of 10-fold UF for extrapolation from laboratory animal to humans, 10-fold UF for intraspecies variation, 3 for database deficiencies) is appropriate and objectively described (albeit, see comments below).

Matthew D. Reed, Ph.D

Generally given the oral assumptions.

D. Alan Warren, Ph.D

Yes, the uncertainty factors (UFs) of 300 and 1000 are scientifically justified and transparently and objectively described. However, as discussed in EPA's review, there are experimental data that may stimulate conversation as to the need for any UF for subchronic to chronic extrapolation in the derivation of the chronic oral RfD.

5. A database uncertainty factor of 3 was applied in deriving the subchronic and chronic RfDs principally because the available oral studies did not specifically examine the potential for subtle neurotoxicity following repeated exposures. Has the rationale and justification for this uncertainty factor been transparently and objectively described? Is the application of this uncertainty factor scientifically justified? Please consider the appropriateness of this UF in light of the full database for 1,1,1-trichloroethane and, in particular, whether consideration of uncertainties

in the inhalation database with respect to neurotoxicity should be reflected in the database uncertainty factor for the oral reference values.

Jeffrey W. Fisher, Ph.D., Chair

The only hint of toxicity that could be used for RfD values was body weight decline. The oral administration studies were not focused on neurotoxicity, but the inhalation studies did focus on subtle changes in behavior. The PBPK route to route extrapolations from inhalation to oral probably should have been conducted. I disagree with the group that attempted to use the oral kinetic data for PBPK model development. If allowed, they should have altered the model code.

Scott E. Bowen, Ph.D

Yes, I believe the rationale and justification have been transparently and objectively described. Yes, I believe that the database uncertainty factor of 3 is scientifically justified and the rationale and justification appears to be appropriate and objectively supported.

Richard B. Mailman, Ph.D

I found this choice of UF conservative and acceptable, and clearly rationalized. My significant reservations about the Rosengren 1985 study (see pt. 10 below) make the use of that study to add in an additional UF problematic. Although this was a conservative approach, I do not think it necessarily is scientifically appropriate.

Matthew D. Reed, Ph.D

Yes, the rationale and justification are sound given the limitations in UF methodology. Yes, the rationale and justification are sound. Yes, the approach is reasonable given the limitation of UF methodology). Yes. The rationale and justification were reasonably justified. Again, the data limitations (limited effects in limited studies) for estimating RfD are what they are. There is no advantage of extrapolating the inhalation UF to the oral reference dose.

D. Alan Warren, Ph.D

Yes, I believe the database UF of 3 is justified and the rationale and justification for its application has been adequately described. However, I find it interesting that EPA embraces the inhalation study of Rosengren et al. to justify the database UF for oral RfD derivation, but considers the study inadequate to establish inhalation RfCs. With that said, like EPA, I feel as though the inhalation database for 1,1,1-trichloroethane is relevant to the oral route (and visa versa), especially considering that the chemical's toxicokinetic profile suggests many of its toxicities should be the same regardless of the pathway by which it is absorbed.

6. As an alternative to the subchronic and chronic oral RfDs derived using data from the NTP (2000) dietary study, consideration was given to use of physiologically-based pharmacokinetic (PBPK) modeling to extrapolate findings from a two-year inhalation bioassay (Quast et al. 1984, 1988) to the oral route (i.e., route extrapolation). Is the decision not to use route extrapolation to derive oral RfD values (as discussed in Section 5.1.1. of the Toxicological Review) transparently and objectively described?

Jeffrey W. Fisher, Ph.D., Chair

The PBPK modeling (route-to-route) could inform you on the UF selection, even if you did not extrapolate both route (inhalation to oral) and response (neurotoxicity from inhalation to oral ingestion). Gavage doses give transient high peak concentrations, which may skew the interpretation somewhat. In the Hilton Head route to route PBPK modeling meeting sponsored by the US EPA (many years ago), I presented (and is published) a route-to-route comparison for trichloroethylene. The authors of the PBPK modeling effort should have used other approaches for describing the unpublished Bruckner kinetic data. The Bruckner data show a fast phase and a slower phase for oral uptake kinetics. This complex kinetic behavior can be better described by using two compartments for oral absorption. A one compartment approach was used by the authors. This approach did not work very well. Also, placement of error bars on the data points would be very useful for understanding how well model predictions compared with observation.

Scott E. Bowen, Ph.D

Yes, I believe the rationale and justification for not using route-to-route extrapolation have been transparently and objectively described. The apparent differences in comparative hepatotoxicity following oral (mild hepatotoxicity) and inhalation (pronounced hepatotoxicity) exposures of 1,1,1-trichloroethane preclude route-to-route extrapolation at this time.

Richard B. Mailman, Ph.D

The basis for this decision was transparent, objectively described, and one with which I agree completely, although comments about the PBPK modeling were made above.

Matthew D. Reed, Ph.D

Yes. The rationale is sound in this case.

D. Alan Warren, Ph.D

Yes, EPA's decision to forego route-to-route extrapolation for oral RfD derivation is

adequately described (see section 5.1.5.2), but is not biologically based or rational. The decision was based largely upon reported differences in relative hepatotoxicity by the oral and inhalation routes (i.e., the oral studies of NTP (2000) and Bruckner et al. (2001) failed to report even mild hepatotoxicity at less than lethal doses, whereas the inhalation study of McNutt et al. (1975) clearly did so). However, as I have stated before, I can think of no reason why 1,1,1-trichloroethane via the oral route would be less effective at targeting the liver than when administered via inhalation (e.g., hepatic first-pass elimination is essentially a non-issue with 1,1,1-trichloroethane). In fact, one might predict quite the opposite. For this reason, the Agency should perhaps reconsider inhalation-to-oral extrapolation, whether it be based on the hepatotoxicity NOAEL of Quast et al. or the LOAEL of McNutt et al. (or a combination of the two), or a study examining another critical effect altogether.

QUESTIONS RELATED TO THE DERIVATION OF INHALATION REFERENCE CONCENTRATION (RfC) VALUES

1. The acute inhalation study by Mackay et al. (1987) involving the examination of neurobehavioral effects in humans was selected as the basis for the acute inhalation RfC. Is the selection of Mackay et al. (1987) as the principal study scientifically justified? Is the rationale for selecting this study transparent and objective? Are there any other studies that you believe would be justified scientifically as the basis for the acute RfC?

Jeffrey W. Fisher, Ph.D., Chair

The use of human data is the best approach for derivation of an acute RfC. I agree that the Mackay et al. is a good data set for the acute RfC. I do not know of other studies. Usually an UF of 3 is used (not 10), when using PBPK to predict kinetics (3) and for dynamics, an UF of 3. You mention an UF of 10 was used for susceptible populations. Perhaps, this should be 3 since it is presented as a pharmacodynamic issue.

Scott E. Bowen, Ph.D

Yes, the choice of the study by Mackay et al. (1987) as the basis for the acute inhalation RfC is appropriate and scientifically justified. This was a well done study which examined several concentrations of 1,1,1-trichloroethane on a battery of psychomotor tests. Mackay et al. (1987) demonstrated neurobehavioral effects at one of the lowest 1,1,1-trichloroethane concentrations (175 ppm or 950 mg/m^3) of all the human studies in the current database. I believe the rationale and justification for not using other studies has been transparently and objectively described.

Richard B. Mailman, Ph.D

The reasoning here was transparent and objectively described, and I believe selection of Mackay et al. (1987) was scientifically justified. Neurobehavioral effects appear to be a sensitive endpoint for inhaled 1,1,1-TCE, and often can pick up changes at lower concentrations in humans than in laboratory animals. The Mackay 1987 experiment was a good study of normal human volunteers with appropriate endpoints. Although (as noted in the IRIS review) there were some minor deficiencies, this is one case where there is highly relevant and sensitive human data to be applied to such questions.

Matthew D. Reed, Ph.D

The selection of this study is scientifically justified The rationale is transparent and objective. No.

D. Alan Warren, Ph.D

Yes, the selection of Mackay et al. (1987) is scientifically justified. Neurological effects appear to be the most sensitive endpoint for inhaled 1,1,1-trichloroethane, and have typically been observed at lower concentrations in humans than experimental animals. Mackay et al. is an extremely well-designed and well-controlled study of human volunteers that employed a battery of psychomotor tests to identify what is thought to be the lowest 1,1,1-trichloroethane concentration to result in documented neurological effects (i.e., 175 ppm). As pointed out in EPA's review, the study does lack pair-wise comparisons that preclude one from determining the exposure levels at which statistically significant changes in specific psychomotor tests occurred. However, it does not suffer from the fatal flaws that often accompany psychomotor evaluations of solvent-exposed human subjects. EPA's rationale (section 5.2.1.1) for selecting Mackay et al. to establish points of departure is sound and other acute studies, human or animal, are less than optimal to serve this purpose, comparatively speaking.

2. PBPK modeling was used to extrapolate from the LOAEL (950 mg/m^3 exposure for one hour) to 4-, 8-, and 24-hour exposure durations. Is this duration extrapolation scientifically supported? Was duration extrapolation correctly performed? Please provide any other comments concerning EPA's conduct of this extrapolation. Is the PBPK approach transparently and objectively described?

Jeffrey Fisher, Ph.D, Chair

Yes, I have read how they did the bootstrapping. The description is adequate. How the short term RfC values (less than 24 hrs) fit into this document is unclear. Is this part of the AEGL process? The discussion about C x T not doing a great job with predicting outcomes should be extended to how the AEGL process uses a modified form of Haber's law to describe C x T. However, if PBPK models are available, the use of PBPK

modeling is preferred over the modified Haber's law approach (ten Berge).

Scott E. Bowen, Ph.D

Yes, I believe the duration extrapolation is scientifically supported. The Reitz et al. (1988) PBPK model was appropriately used with human pharmacokinetic data sets from a study by Mackay et al. (1987) in which concentrations in blood at time of testing were available. This model acceptably predicted the experimental data sets. The description of the PBPK approach is transparently and objectively described.

Richard B. Mailman, Ph.D

The PBPK approach is transparent and objectively described (albeit see general comments above).

Matthew D. Reed, Ph.D

Yes. The extrapolation is scientifically supported. Yes. The committee's collective opinion is that this was the case. This is not the reviewer's direct area of expertise, but the authors and contractors present a sound rationale. Yes. The approach is transparently and objectively described.

D. Alan Warren, Ph.D

First, let me admit to having only slightly more than a rudimentary knowledge of PBPK modeling. Even so, the duration extrapolation performed by Yang (2006) and discussed in EPA's review was readily transparent. It is scientifically supported, based on 1) experimental evidence that the 1,1,1-trichloroethane concentration in blood at any given time is likely to be an appropriate dose metric (i.e., correlates with brain concentrations and is predictive of the degree of neurobehavioral/neurological deficit); and 2) the capability of the Reitz et al. PBPK model to accurately simulate venous blood concentrations experimentally determined by Mackay et al. (see section IV.2.4 of Yang (2006)). The method of duration extrapolation seems logical, especially as the target internal dose was empirically determined and not a modeled value.

3. The study results of Mackay et al. (1987) were used to derive the short-term RfC, with PBPK modeling used to extrapolate to steady state conditions. Is the Mackay et al. (1987) study the most appropriate as the basis for the short-term RfC? If so, is this extrapolation scientifically justified? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and supported? Are there any other studies that you believe would be justified scientifically as the basis for the short-term RfC?

Jeffrey Fisher, Ph.D, Chair

I agree with the use of the Mackay et al. data and PBPK modeling to derive a short-term RfC. This time extrapolation approach using human information is preferred over animal data for CNS effects. I could not find the information where a 336 hr simulation was conducted to derive the concentration of 526 mg/m^3 in the Yang 2006 technical report. The value seems ok, I just could not find it, except as part of a graph on page 73.

Scott E. Bowen, Ph.D

Yes, the choice of the study by Mackay et al. (1987) as the basis for the derivation of the short-term RfC is appropriate. The arguments for the extrapolation appear to be sound and the model assumption, parameter values, and selection of dose metrics are clearly presented and supported. I am not aware of any other study that would be a better basis for the short-term RfC.

Richard B. Mailman, Ph.D

The IRIS Review clearly presents and provides support for its methodology. The method of duration extrapolation was appropriate, especially as the target internal dose was empirically determined and not a modeled value.

Matthew D. Reed, Ph.D

Yes. This study is appropriate and the extrapolation is scientifically justified. Yes. The assumptions, parameter values, and selections of dose metrics are clearly presented and supported. No. There are no other studies that are needed.

D. Alan Warren, Ph.D

EPA readily admits that *"toxicity data in animals provide limited information related to the potential for effects to occur at lower concentrations with repeated versus acute exposure."* However, the few available studies, with the exception of that of Geller et al. (1982) in baboons, suggest repeated exposure does not result in a threshold reduction for neurobehavioral effects compared to acute exposure [given the results of Geller et al., PBPK modeled estimates of 1,1,1-trichloroethane blood concentrations after a 4-hour exposure and continuous exposure for 7 days might be informative]. Even so, one must not fully discount the possibility (based on the study of Rosengren et al., for example) that repetitive exposure to 1,1,1-trichloroethane could have neurotoxic consequences that are not sensitive to traditional tests of neurobehavioral function. Nonetheless, given the human LOAEL from Mackay et al. (1987) is less than any identified from short-term studies (e.g., the unpublished study of Albee et al. (1990b) in rats), the use of Mackay et al. for short-term RfC derivation is most appropriate. The method of analysis (see section 5.2.2.2), similar to that used for acute RfC derivation, is scientifically supported based on 1) similarities in the disposition of 1,1,1-trichloroethane after single and repeated exposures; 2) experimental evidence that the 1,1,1-trichloroethane concentration in blood

at any given time is likely to be an appropriate dose metric (i.e., correlates with brain concentrations and is predictive of the degree of neurobehavioral/neurological deficit); and 3) the capability of the Reitz et al. PBPK model to accurately simulate venous blood concentrations experimentally determined by Mackay et al. (see section IV.2.4 of Yang (2006)). The method of duration extrapolation seems logical, especially as the target internal dose was empirically determined and not a modeled value. EPA has clearly presented and provided sufficient support for its methodology. Its rationale for selecting Mackay et al. to establish a point of departure is sound and short-term studies in animal models are less than optimal to serve this purpose, comparatively speaking.

4. The Quast et al. (1984, 1988) 2-year inhalation bioassay and the McNutt et al. (1975) 14-week inhalation study were jointly used as the basis for the subchronic and chronic RfCs. Is the selection of these as co-principal studies (see Sections 5.2.3.1. and 5.2.4.1.) appropriate? Is the rationale for selecting these studies transparent and objective? Are there any other studies that you believe would be justified scientifically as the basis for the subchronic and chronic RfCs?

Jeffrey Fisher, Ph.D, Chair

These data sets for subchronic and chronic appear adequate to me (Quast and McNutt). I am ok with the selection of these studies. I am not qualified to comment on the changes in GFAP in the brain and an endpoint (Rosengren et al. 1985).

Scott E. Bowen, Ph.D

Yes, I agree with the decision to combine the Quast et al. (1984, 1988) 2-year inhalation bioassay and the McNutt et al. (1975) 14-week inhalation study to obtain the subchronic and chronic RfCs. Many of the other subchronic studies report ambiguous evidence for 1,1,1-trichloroethane's detrimental effects. The rationale presented for the selection of these studies is transparent and objective. I am not aware of any other studies that would be justified scientifically as the basis for the subchronic and chronic RfCs.

Richard B. Mailman, Ph.D

The decisions to use the Quast (1984, 1988) studies and the McNutt et al. (1975) together is appropriate and transparent, and I am not aware of any other relevant studies that could be a basis for determining subchronic and chronic RfCs. As noted later, I feel the decision to not use Rosengren 1985 was clearly scientifically justified.

Matthew D. Reed, Ph.D

Yes. These studies are appropriate. Yes. These studies are appropriate. Yes. The rationale are transparent and objective. No. No other studies are required.

D. Alan Warren, Ph.D

In my opinion, EPA has probably interpreted the Quast et al. (1984, 1988) and McNutt et al. (1975) studies correctly (e.g., accurately made the distinction between adaptive and adverse) and in so doing, justified their joint use for subchronic and chronic inhalation RfC derivation. While the NOAELs from Quast et al. (i.e., 1460 mg/m^3) and McNutt et al. (i.e., 1360 mg/m^3) are quite similar, use of the former for RfC derivation obviates the need for an UF to account for extrapolation across exposure durations. Likewise, use of the Quast et al. NOAEL rather than the McNutt et al. LOAEL (i.e., 5460 mg/m^3) eliminates the UF for NOAEL to LOAEL extrapolation. While EPA has provided a clear rationale for the selection of the two hepatotoxicity studies, its attempt to dismiss Rosengren et al. from consideration as the principal study is less than convincing. Granted, there are uncertainties surrounding the study, but its LOAEL of 210 ppm (and NOAEL of 70 ppm) suggests that what is quite possibly the most sensitive effect of 1,1,1-trichloroethane warrants further consideration (or at least further explanation as to why it was not selected as the principal study). Although EPA's review makes no mention of them, there are several studies, many nearly identical in design to that of Rosengren et al. (1985), which report GFAP increases following toluene, styrene, xylene and dichloromethane exposures [note that while GFAP increases may be statistically significant, they tend to be very small; also, GFAP is decreased by solvent exposure in some cases; see several relevant citations below]. In addition, studies show that S-100 and GFA proteins are frequently expressed differentially in various brain regions following chemical insult, something EPA views as suspect. Lastly, GFAP has been validated as an indicator of neurotoxicity under conditions where traditional histopathology has failed to reveal the damage [note that such validation has been accomplished by former EPA scientist, J.P. O'Callaghan, and others]. Admittedly however, the database UF of 3, driven largely by questions surrounding 1,1,1-trichloroethane's neurotoxicity that stem from Rosengren et al., makes EPA's choice of the hepatotoxicity studies more palatable.

1. Rosengren and Haglid. Long term neurotoxicity of styrene. A quantitative study of glial fibrillary acidic protein (GFA) and S-100. *British Journal of Industrial Medicine* 46:316-320, 1989.
2. Rosengren et al. Irreversible effects of dichloromethane on the brain after long term exposure: a quantitative study of DNA and the glial cell marker proteins S-100 and GFA. *British Journal of Industrial Medicine* 43:291-299, 1986.
3. Rosengren et al. Irreversible effects of xylene on the brain after long term exposure: a quantitative study of DNA and the glial cell marker proteins S-100 and GFA. *Neurotoxicology* 7(3):121-135, 1986.
4. Gotohda et al. Effect of toluene inhalation on astrocytes and neurotrophic factor in rat brain. *Forensic Sci. Int.* 113(1-3):233-8, 2000.
5. Little et al. Decreases in brain glial fibrillary acidic protein (GFAP) are associated with increased serum corticosterone following inhalation exposure to toluene. *Neurotoxicology* 19(4-5):739-47, 1998.
6. Wang et al. Perchloroethylene-induced reduction in glial and neuronal cell marker proteins in rat brain. *Pharmacol. Toxicol.* 72(4-5):273-8, 1993.

5. The minimal histopathological findings in the liver observed in the Quast et al. (1984, 1988) rat study were judged to reflect an adaptive physiological response and not an adverse effect. Is this judgment scientifically appropriate and objectively supported?

Jeffrey Fisher, Ph.D, Chair

I think this is ok. Not adverse.

Scott E. Bowen, Ph.D

After reading the review and the articles cited, the histopathological findings reported do appear to be fairly minor as compared to the other types of hepatic reactions that can occur (e.g., lesions, tumors, fatty changes and necrosis). As such, the judgment appears to be appropriate and objectively supported.

Richard B. Mailman, Ph.D

The conclusions about the Quast studies were made in a scientifically appropriate fashion, and were objectively supported.

Matthew D. Reed, Ph.D

Yes. The histopathological finding is appropriate in this case.

D. Alan Warren, Ph.D

EPA has probably interpreted the Quast et al. (1984, 1988) and McNutt et al. (1975) studies correctly (e.g., accurately made the distinction between adaptive and adverse) and in so doing, justified their joint use for subchronic and chronic inhalation RfC derivation. With that said, the EPA review contains little or no discussion of what, in its opinion, constitutes adaptive and adverse. It would be extremely valuable if the criteria for each classification were disclosed and the demarcation between normal (adaptive) and adverse clarified. Also helpful would be a mechanistic explanation, that could be ever so brief, as to why certain cell populations in the liver undergo morphological change in their adaptive response to 1,1,1-trichloroethane. These additions to the text would enable the reader to better understand EPA's justification for classifying effects as NOAELS or LOAELs.

6. PBPK modeling was used to extrapolate the point of departure from Quast et al. (1984, 1988) to humans. Is the PBPK modeling for interspecies extrapolation scientifically justified and transparently and objectively described? Are the model

assumptions, parameter values, and selection of dose metrics clearly presented and supported?

Jeffrey Fisher, Ph.D, Chair

I evaluated the information using steady state assumptions. I think you will have different results if you use blood instead of liver AUC as the dosimetric and if you use peak concentration instead of AUC. Is the liver AUC ok without validation in humans? How confident are you in a calculated liver AUC in humans based on a partition coefficient value (Gargas)? Did anyone in the EPA run the model or double check what is in the Yang 2006 technical report? …Just a QA question. Page 146 (Yang 2006) provides a table for rat and human. Calculating an AUC for a rat exposure of 6 h/day, 5 d/week and translating this to a continuous exposure in human causes the big difference between the species. At steady state the blood levels should be depicted by the blood:air PC values of 5.8 (rat) and 2.53 (human), thus for a given air concentration of methyl chloroform at steady state, the rat circulating blood level of methyl chloroform would be greater than the human by about 2x. The Agency should evaluate the modeling portion of this document with reference to the agency guidance document for using PBPK models in regulatory decisions (document provided).

Scott E. Bowen, Ph.D

Yes, I believe the PBPK model for interspecies extrapolation (rat-to-human) is scientifically justified. The description of the PBPK approach is transparently and objectively described.

Richard B. Mailman, Ph.D

The overall strategy was clearly presented, although aspects of the PBPK modeling suggest to me that the models in some cases are not totally adequate in describing the actual kinetics (see General Comments).

Matthew D. Reed, Ph.D

Yes. The PBPK model is scientifically justified and objectively described and the assumptions, parameter values and selection of dose metrics are clearly presented and supported. More data nearer the derivation would be appreciated and make the reader's assessment easier.

D. Alan Warren, Ph.D

Yes, rat-to-human extrapolation using PBPK modeling is scientifically justified in this case and adequately described. Inclusion of Table 22 in the text, along with its footnotes, is extremely valuable in making the process transparent. The choice of TWA AUC-liver as the dose metric is logical. So too is its calculation at 6 months given steady

state had been achieved and toxicity was independent of exposure duration.

7. Are the uncertainty factors applied to the point of departure for the derivation of the acute, short-term, subchronic, and chronic RfC values scientifically justified and transparently and objectively described?

Jeffrey Fisher, Ph.D, Chair

UF ok. Comment on the acute UF is provided in comment 1. I agree with the logic given for lowering the subchronic and chronic RfC values to 5 mg/m^3 (p. 149).

Scott E. Bowen, Ph.D

Yes, I believe the composite uncertainty factor of 100 (10-fold UF for extrapolation from LOAEL to NOAEL and 10-fold UF for intraspecies variation) is appropriate. I believe these values to be scientifically justified and transparently and objectively described.

Richard B. Mailman, Ph.D

I believe these values to be scientifically justified and transparently and objectively described. The composite UF (10-fold for extrapolation from LOAEL to NOAEL and 10-fold for intraspecies variation) is appropriate.

Matthew D. Reed, Ph.D

Yes. The UF derivations of the RfC values are scientifically justified and transparently and objectively describied.

D. Alan Warren, Ph.D

Yes, the UFs used in the derivation of all four RfCs are scientifically justified and adequately described.

8. Database uncertainty factors were not applied in deriving the acute and short-term RfCs. A database uncertainty factor of 3 was used in deriving subchronic and chronic RfCs. Has the rationale and justification for the application of the database uncertainty factor been transparently and objectively described? Is the application of this uncertainty factor scientifically justified, particularly with respect to the existing literature (both human and animal) on 1,1,1-trichloroethane neurotoxicity?

Jeffrey Fisher, Ph.D, Chair

I am ok with database uncertainty discussions.

Scott E. Bowen, Ph.D

Yes, I believe the rationale and justification for the application of the database uncertainty factor been transparently and objectively described. The uncertainty factor of 3 is appropriate for derivation of the subchronic and chronic RfCs.

Richard B. Mailman, Ph.D

I felt the strategies described were appropriate, and objectively described. My sentiments about Rosengren 1985 are described later, and use of this study for UF's leaves me uncomfortable.

Matthew D. Reed, Ph.D

The rationale and justification for the database UF has been transparently and objectively described. Yes.

D. Alan Warren, Ph.D

I believe the UFs used in the derivation of all four RfCs are appropriate and that the rationale for them has been clearly described. One has only to examine Figures 2a and 2b and Table 20 to realize that the 1,1,1-trichloroethane database contains many more acute and short-term inhalation studies than it does subchronic and chronic ones. In addition, and in apparent agreement with EPA, I feel as though the study of Rosengren et al. (1985) is alone sufficient to generate some degree of uncertainty over the risk for neurotoxicity posed by repetitive exposures over a prolonged period (e.g., the LOAEL from Rosengren et al. (1985) is 210 ppm (continuous exposure for 12 weeks) while that from McNutt et al. is 1000 ppm (continuous exposure for 14 weeks)).

9. Because the value of the subchronic and chronic RfC exceeded the values of the acute and short-term RfCs, the subchronic and chronic RfC was set at 5 mg/m^3 so as not to exceed the limiting reference value derived for short-term exposure. Is this decision scientifically justified and transparently and objectively described? Please comment on whether you believe there might be more appropriate explanations than those discussed in Sections 5.2.3.3 and 5.2.4.3 for why the acute and short-term inhalation RfC values were smaller than the subchronic and chronic RfC values.

Jeffrey Fisher, Ph.D, Chair

I think what you did was great. I am glad to see this careful explanation. This helps with

the transparency issue. On the AEGL NAS committee we call this evaluation of the calculated values a 'reality check' and look for consistency across risk numbers. When different endpoints and methods are used, one can end up with corrections that are needed to have a consistent set of risk numbers.

Scott E. Bowen, Ph.D

The decision to set the subchronic and chronic RfC at 5 mg/m^3 is scientifically justified and transparently and objectively described. I don't have any additional comments beyond explanations than those discussed in Sections 5.2.3.3 and 5.2.4.3 for why the acute and short-term inhalation RfC values were smaller than the subchronic and chronic RfC values.

Richard B. Mailman, Ph.D

The decision to reduce the subchronic and chronic RfCs, EPA's decision to do so is transparently and objectively described, and the unexpected discrepancy between acute and short-term RfCs being smaller than their subchronic and chronic counterparts is addressed adequately. Reducing the subchronic and chronic RfCs to that of the short-term RfC may be of little practical importance in this case (3 to 1 ppm), but one wonders if there is not an alternate approach for hazard and dose-response evaluation.

Matthew D. Reed, Ph.D

Yes. The decision is scientifically justified and transparently and objectively described. There is no more appropriate explanation. The explanation is thoroughly described.

D. Alan Warren, Ph.D

One needs no formal training in toxicology to understand that as exposure duration increases, the allowable exposure concentration should decrease. Of course, this logic is predicated on the assumption that risk is a function of exposure duration, which it generally is. While it may not be universally true that risk increases as exposure duration increases, I'm hard pressed to think of a circumstance where risk declines the longer one is exposed. In this respect, it is difficult to argue against reducing the subchronic and chronic RfCs to that of the short-term RfC. However, doing so is not apt to be of any practical importance considering the reduction is from 3 to 1 ppm (aren't these essentially "background" concentrations in many parts of the country). Of most concern to me is that reducing the RfCs merely because they "don't make sense" when examined relative to one another belittles EPA's process of hazard and dose-response evaluation. This is particular disturbing given the obvious time, financial resources, and effort expended at applying state-of-the-science methods to RfD and RfC derivation in the present case. Personal opinions as to principal study and critical effect aside, the process of RfD and RfC derivation is sufficiently rigorous such that it should not require reductions simply to maintain a continuum from high/acute to low/chronic. Doing so might be logical, but cannot be scientifically justified if good science is so readily dismissed. While I may not

agree with the decision to reduce the subchronic and chronic RfCs, EPA's decision to do so is transparently and objectively described. Its explanation for why acute and short-term RfCs were smaller than their subchronic and chronic counterparts is adequate, though perhaps it should be pointed out that it was not a function of uncertainty (as all four UFs are equivalent (i.e., 100)).

10. Rosengren et al. (1985) reported increased glial fibrillary acidic protein (GFAP), a marker for formation of astroglial fibrils in response to brain injury, in the sensorimotor cerebral cortex of 1,1,1-trichloroethane-exposed gerbils. The EPA did not consider these findings to be sufficiently reliable or of sufficient toxicological significance to use as the basis for the subchronic RfC. Is this decision scientifically justified, particularly in light of observed neurobehavioral effects associated with acute exposure to 1,1,1-trichloroethane? Is this decision transparently and objectively described? The Rosengren et al. study was used to inform the value of the database uncertainty factor used in deriving the subchronic and chronic RfDs and RfCs. Was consideration of this study appropriate in the context of the database uncertainty factor?

Jeffrey Fisher, Ph.D, Chair

The use of this marker protein (Rosengren study) as a surrogate for dose response analysis (toxicity) appears to be inappropriate. More information is needed to link the protein changes in the brain with pathology of the brain and alterations in behavior, combined with a hypothesis about mode of action.

Scott E. Bowen, Ph.D

After reading the report, I will agree that there are enough inconsistencies (i.e., stimulation can also produce increases in GFAP, lack of dose-dependent effects, and the importance of consistent dissections of brain due to different levels of GFAP throughout the brain) to support the conclusion that the findings of Rosengren et al. (1985) are not sufficiently reliable to be used as the basis for the subchronic RfC. I believe that EPA's decision is transparently and objectively described. The Rosengren et al. (1985) study does raise potential concern for the effects of 1,1,1-trichloroethane on the CNS, so consideration of this study was appropriate in the context of the database uncertainty factor.

Richard B. Mailman, Ph.D

I felt the positions taken in the document as regards the Rosengren study to be well-formulated in terms of the UF. The review concludes that this study was unreliable, and I agree wholeheartedly although I have specific reservations above and beyond those cited in the EPA review. Some of my specific concerns may be useful to consider adding after appropriate examination. The IRIS review notes that a significant decrease in brain

weight echoing the text and Table 1 of Rosengren. I do not believe the data supports Rosengren's conclusion. Specifically, my analysis (extracting those data as well as I could from a reprint) shows that the high concentration of 1,1,1-TCE did not cause a significant decrease in brain weight (this from an upaired t-test without correcting for multiple comparisons). It is unclear why Rosengren here (and elsewhere through this paper) used a non-parametric analysis method for parametric data, and how they detected differences they report (the histogram data could not be reanalyzed). In addition to this clear issue, I also have concerns about the extremely small SEMs reported in this work, e.g., as related to the GFAP studies (although this point is a "feeling" from my experience rather than a criticism supported by hard data). My reasoning is as follows. In bioanalytical chemistry a method often is considered acceptable if it has a Coefficient of Variation (CV) ≤ 10%, According to my estimations made from the Figures, one could estimate a total "Coefficient of Variation" of these data of 15-25% (e.g., Figures 1 and 2). Since this estimate includes all sources of biological and toxicant-induced variability on top of the variance from the analytical endpoint (in this case, a relatively imprecise technique), it seems unexpectedly precise. To underscore, the Rosengren 1985 assays have to account for: 1) normal inter-animal variability; 2) inter-animal differences in response to 1,1,1-TCE); 3) the fact that only of a small portion of the extractable cellular GFAP is sampled (another source of variability); and 4) an assay method that is indirect and itself would be expected to have a relatively large CV (certainly greater than 10%). Thus, although I agree with the IRIS review conclusion that this study unreliable, I do not feel it should be used in any of the review's calculations (e.g., in determination of an uncertainty factor in the RfC).

Matthew D. Reed, Ph.D

Yes. This conclusion is justified. Yes. This decision is transparently and objectively described. Yes. Consideration was appropriate in deriving the uncertainly factor.

D. Alan Warren, Ph.D

EPA has not adequately justified the dismissal of Rosengren et al. from consideration as its principal study. Granted, there are uncertainties surrounding the study, but its LOAEL of 210 ppm (and NOAEL of 70 ppm) suggests that what is quite possibly the most sensitive effect of 1,1,1-trichloroethane warrants further consideration. Although EPA's review makes no mention of them, there are several studies, many nearly identical in design to that of Rosengren et al. (1985), which report GFAP increases following toluene, styrene, xylene, and dichloromethane exposures. In addition, studies show that S-100 and GFA proteins are frequently expressed differentially in various brain regions following chemical insult, something EPA views as suspect. Furthermore, GFAP has been validated as an indicator of neurotoxicity under conditions where traditional histopathology has failed to reveal the damage [note that such validation has been accomplished by former EPA scientist, JP O'Callaghan, and others). Regarding GFAP, Dr. O'Callaghan stated – *"Elevations in GFAP are widely accepted as indicators of brain damage associated with neurological diseases such as Alzheimers and multiple sclerosis. More recently, enhanced expression of GFAP has been validated as an indicator of*

neurotoxicity by using a wide variety of prototype chemical neurotoxicants. These include agents that damage many regions of the brain and many different cell types within a brain region, as would be expected to occur under "real-world" conditions. Moreover, increases in GFAP reveal subtle damage to neurons, such as loss of nerve endings, under conditions where traditional neuropathological stains fail to reveal the damage…Thus, GFAP assessments fulfill the desired requirements for an indicator of toxicity." Therefore, failure to consider Rosengren et al. as the basis for the subchronic RfC does not appear scientifically justified. Another quote is relevant to the issue at hand, this one recently made in response to one of several EPA comments on California's public health goal (PHG) technical support document for 1,1,1-trichloroethane [in which the Rosengren et al. study was judged best suited for calculation of the PHG] – "*We share U.S. EPA's concern and we are also not aware of attempts to replicate the findings of Rosengren et al. Nevertheless, it is not unusual to find a critical study on a chemical for which a replication study has not been attempted or is lacking in some other fashion. Identifying the "best available" research often involves using a study with identified weaknesses."* While EPA is correct that a replicate study with 1,1,1-trichloroethane has not been conducted, Rosengren et al. have consistently shown GFAP elevations across multiple solvents with similar chemico-physical properties. While I am not necessarily advocating for the Quast et al. and McNutt et al. studies to be shelved in favor of Rosengren et al., neither am I convinced that EPA's subchronic RfC is based on the "best available" study. My opinion on the suitability of Rosengren et al. for subchronic RfC derivation is not influenced by reports of neurobehavioral effects following acute exposure. Such neurobehavioral effects are transient (reversible) and occur by a mechanism distinct from that marked by an increase in GFAP. As stated earlier, I feel as though the study of Rosengren et al. is alone sufficient to generate some degree of uncertainty over the risk for neurotoxicity posed by repetitive exposures over a prolonged period (e.g., the LOAEL from Rosengren et al. (1985) is 210 ppm (continuous exposure for 12 weeks) while that from McNutt et al. is 1000 ppm (continuous exposure for 14 weeks)). Given that many of 1,1,1-trichloroethane's toxicities are likely to be independent of exposure route, the uncertainty generated by Rosengren et al. is arguably as applicable to the oral route as to inhalation [this is supported by California's use of the study to set a PHG for 1,1,1-trichloroethane in drinking water].

QUESTIONS RELATED TO THE CANCER ASSESSMENT

1. Do the available data support the conclusion that the database for 1,1,1-trichloroethane provides inadequate information to assess carcinogenic potential based on the weight-of-evidence categories in the EPA 2005 *Guidelines for Carcinogen Risk Assessment*? Please describe the basis for your view.

<u>Jeffrey Fisher, Ph.D, Chair</u>

The potency of methyl chloroform (as a carcinogen) can not be calculated. I agree. More discussion is needed in the document about the Maltoni study, which suggests some

positive sporadic findings. This evaluation is consistent with the new cancer guidelines because the document evaluates the data (both human and animal) and makes the determination that there are no positive cancer studies. Appendix A-4 in 'Guidelines for Carcinogen Risk Assessment' discusses the situation where animal and human studies are negative for a chemical.

Scott E. Bowen, Ph.D

As discussed in the document, I support the conclusion that the available database for assessment of the carcinogenic potential of 1,1,1-trichloroethane is inadequate. This decision is based on the lack of evidence and the necessary conditions for 1,1,1-trichloroethane exposure to become a carcinogenic hazard to humans. There is a lack of fundamental information on dose response and levels of exposure necessary for carcinogenic effects. It is also unclear whether the risks are different for the various routes of exposure. Finally, there is inadequate information regarding the nature and extent of the risk from 1,1,1-trichloroethane exposure.

Richard B. Mailman, Ph.D

Although I agree that the particular descriptor (*inadequate information to assess carcinogenic potential*) is scientifically justified, I felt that the sum total of the available data might have led to a conclusion that was a bit more definitive. As an example, the genotoxicity data certainly is suggestive of a very low risk of carcinogenicity. This is not my area of expertise, but my feeling is that there are many examples of non-carcinogenic compounds that give positive genotoxicity results, but not vice versa. If so, this might certainly justify a conclusion when put in concert with the available data (however inadequate those data may be). It would seem that the carcinogenic potential of 1,1,1-trichloroethane was likely to be very low as regards potential exposure levels (albeit see general comment regarding the need for a summary of production and exposure data). This said, I am not a specialist in carcinogenesis risk assessment, and my basis for this opinion may not be sound.

Matthew D. Reed, Ph.D

This is true. The rationale generated by the authors sufficiently describes the deficiencies in the data. The literature and data generated thus far are not sufficient to prove carcinogenic potential.

D. Alan Warren, Ph.D

Yes, the descriptor selected for use (i.e., *inadequate information to assess carcinogenic potential*) is appropriate based on available data. The narrative in section 4.7 is clearly written and comprehensive, with the exception of its failure to reference a chronic inhalation study of 1,1,1-trichloroethane in Sprague-Dawley rats (Quast et al., 1978; Rampy et al., 1977). In this particular study, rats were exposed to 0, 875, or 1750 ppm for 6 hr/day, 5 days/wk, for 12 months and allowed to survive until 31 months. The study

is similar to the inhalation bioassay of Quast et al. (1984, 1988) in that there was no evidence of a carcinogenic response, but differs with respect to the strain of rat utilized (i.e., Sprague-Dawley versus Fischer 344). Importantly, it serves as the only other chronic bioassay to that of Maltoni et al. (1986) to use the Sprague-Dawley rat, and thus is of value for comparative purposes despite different dosing routes and exposure lasting 1 year instead of two. While cancer bioassays of 1,1,1-trichloroethane have been conducted in rats and mice by the oral and inhalation routes, virtually all have shortcomings. That of NCI (1977) suffers from high early mortality, that of Maltoni et al. (1986) from inadequacies in methodology and in data collection and reporting, and that of Quast et al. (1984, 1988) from an apparent failure to rule out tumors across the full range of tolerable doses. The epidemiological studies also have limitations, in that most are confounded with exposures to solvents other than 1,1,1-trichloroethane and/or report large effect estimates bracketed by extremely wide confidence intervals (reflective of a small number of cancer cases), implying that excess cancer rates have a relatively high probability of not being real but rather chance findings. The above, coupled with the absence of *in vitro* studies suggestive of carcinogenic potential, including most of those for genotoxicity, constitute a body of work inadequate for carcinogenic classification to an appropriate degree of scientific certainty. Nonetheless, EPA should provide information on the specific inadequacies of the Maltoni et al. (1986) study instead of alluding to them in a generic fashion.