

External Peer Review

U. S. Environmental Protection Agency Toxicological Review for the Tetrahydrofuran Human Health Assessment

Tetrahydrofuran (THF) Final Report

Final Compilation of Reviewer Comments And Responses to Charge Questions

**Prepared for
Integrated Risk Information System (IRIS) Program
Office of Research and Development
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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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**CHARGE TO EXTERNAL REVIEWERS
FOR THE
TETRAHYDROFURAN INITIAL COMMENTS REPORT**

GENERAL CHARGE QUESTIONS

**Draft Charge to External Reviewers for the Toxicological Review of Tetrahydrofuran
August 8, 2007**

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of tetrahydrofuran (THF) that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is a database of EPA's scientific position on the human health effects that may result from exposure to various substances found in the environment. There is currently no assessment on the IRIS database for the health effects associated with THF exposure.

The draft health assessment includes chronic Reference Doses (RfD) and Reference Concentrations (RfC) and a carcinogenicity assessment. Below are a set of charge questions that address scientific issues in the assessment of THF. Please provide detailed explanations for responses to the charge questions.

(A) GENERAL CHARGE QUESTIONS

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and 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 THF.

CHEMICAL-SPECIFIC CHARGE QUESTIONS

(B) Oral Reference Dose (RfD) For Tetrahydrofuran

1. A chronic RfD for THF has been derived from the oral drinking water 2-generation reproductive toxicity study (BASF, 1996; Hellwig et al., 2002) in rats. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.
2. Decreased F2 male pup body weight was selected as the most appropriate critical effect. Please comment on whether the selection of this critical effect has been scientifically justified

and transparently and objectively described in the document. Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the point of departure (POD). All available models were fit to the individual male and female and combined incidence data (F1 and F2 pup body weight gain). Please comment on the appropriateness and scientific justification presented for individual and combined body weights to obtain a data set for BMD modeling. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.
4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document?
5. A two-generation reproductive toxicity study was used for the selection of the POD for the derivation of the RfD. Please comment on whether the rationale and justification for not applying a subchronic to chronic uncertainty factor has been scientifically justified and transparently described in the document.
6. Please comment on whether the rationale and justification for the selection of the database uncertainty factor has been scientifically justified and transparently described in the document.

(C) Inhalation Reference Concentration (RfC) For Tetrahydrofuran

1. A chronic RfC for THF has been derived from data from a 105 week chronic inhalation study (NTP, 1998) in mice and rats. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.
2. Liver toxicity and CNS effects were selected as the co-critical toxicological effects. Please comment on whether the selection of this critical effect has been scientifically justified and transparently and objectively described in the document. Specifically, please address whether the selection of liver effects and CNS toxicity as the co-critical effects instead of increased thymus weight has been adequately and transparently described. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.
3. The chronic RfC has been derived utilizing benchmark dose modeling to define the point of departure (based on liver cytomegaly). BMD modeling was conducted on liver weight and

cytomegaly data in both males and females. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively described? Please provide comments on whether the selection of a POD based on liver cytomegaly instead of liver weight is scientifically justified and transparently described. Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

4. No incidence data were presented for CNS effects. Thus, these data could not be evaluated by BMD modeling. However, a NOAEL LOAEL approach (based on the CNS data) for the derivation of the RfC has been presented for comparison purposes. Please provide comments as to whether the NOAEL LOAEL approach based on the POD for CNS effects is more appropriate for the derivation of the RfC. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure.
5. Please comment on whether the selection of the uncertainty factors applied to the POD for the derivation of the RfCs. For instance, are they scientifically justified and transparently and objectively described in the document.
6. Please comment on the transparency and scientific rationale and justification for the selection of the database uncertainty factor. Please comment on whether the application of the database uncertainty factor adequately represents the gap in inhalation reproductive and developmental toxicity and immunotoxicity data for THF. Please comment on whether the rationale for use of the oral data to inform this decision scientifically justifiable and transparently described in the document.
7. THF induces a spectrum of effects consistent with both Category 1 and Category 3 gases. Therefore, for the purposes of calculating human equivalent concentrations, respiratory tract effect levels were calculated using the default equations for Category 1 gases and extrarrespiratory tract effect levels were calculated using default equations for Category 3 gases. Please comment on the explanation for the dosimetry choice in the derivation of the RfC. Has the rationale been scientifically justified and transparently described?

(D) Carcinogenicity of Tetrahydrofuran

1. Under the EPA's 2005 *Guidelines for carcinogen risk assessment* (<http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283>), there is *suggestive evidence for the human carcinogenic potential* of THF. Please comment on the scientific justification for the cancer weight of the evidence characterization. A quantitative cancer assessment has been derived for THF. Do the data support estimation of a cancer slope factor for THF? Please comment on the scientific justification for deriving a quantitative cancer assessment considering the uncertainty in the data and the suggestive nature of the weight of the evidence of carcinogenic potential. Has the rationale and scientific justification for quantitation been transparently and objectively described?

2. The available data suggest that a plausible mode of action for THF-induced male rat kidney tumors may involve the accumulation of alpha-2u globulin. EPA concluded that the available data do not provide significant biological support to establish a mode of action for male rat kidney tumors and that these tumors are relevant to humans. Please comment on the transparency and scientific rationale and justification for the evaluation of these data and the conclusions regarding the possible mode(s) of action and human relevance for the male rat kidney tumors.
3. The available data suggest that increased proliferation and promotion in the liver may be a plausible mode of action for THF-induced female mouse liver tumors. EPA concluded that the data do not provide significant biological support to establish a mode of action for female mouse liver tumors and that these tumors are relevant to humans. Please comment on the transparency and scientific rationale and justification for the evaluation of these data and the conclusions regarding the possible mode(s) of action and human relevance for the female mouse liver tumors.
4. An inhalation unit risk has been derived utilizing benchmark dose modeling to define the point of departure of 10% extra risk followed by linear low-dose extrapolation below the point of departure (i.e., the default assumption). Please comment on the scientific justification and rationale supporting the estimation of an inhalation unit risk from the available data for THF. Specifically, please comment on whether the rationale for the quantitative analysis is objectively and transparently described, considering the uncertainty in the data and the suggestive nature of the weight of evidence. Please comment on the selection of linear low dose extrapolation. Has the justification of linear low dose extrapolation been objectively and transparently presented? Please identify and provide rationale for any alternative approaches for low dose extrapolation that the data for THF would support and if such approaches are preferred to EPA's approach.
5. THF induces a spectrum of effects consistent with both Category 1 and Category 3 gases. Therefore, for the purposes of calculating human equivalent concentrations, respiratory tract effect levels were calculated using the default equations for Category 1 gases and extraratory tract effect levels were calculated using default equations for Category 3 gases. Please comment on the explanation for the dosimetry choice in the derivation of the inhalation unit risk. Has the rationale been scientifically justified and transparently described?

External Peer Review

REVIEWER RESPONSES

(A) GENERAL CHARGE QUESTIONS

QUESTION A1

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

Response from John Christopher

Other than being repetitious regarding mode of action, the review is clear and very well presented. I had no difficulty understanding the authors' meaning. MOA is a crucial topic, but it doesn't need to be treated multiple times.

Response from George Corcoran

The Toxicological Review appears logical, clear and concise. EPA has accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard for tetrahydrofuran. This reviewer raises a number of serious concerns in the responses to questions under sections B) - D). The greatest of these relate to concern over selection of benchmark effects, the almost certain overestimation of cancer risk posed by this very weak possible human carcinogen, and an indefensible use of linear low-dose extrapolation to estimate the point of departure for a solvent that has a very strong weight of evidence showing that it is not genotoxic, but likely a promotional agent.

Response from David William Gaylor

The document is comprehensive and clearly written. The pertinent studies and results are well presented. Dosimetrics for extrapolations and uncertainty factors are clearly described and justified for the calculation of the oral RfD, inhalation RfC, and cancer inhalation unit risk.

Page 32, Table 4-2. Indicate how the Adjusted Rate is determined, as was done in the footnote for Table 5-5.

Page 33, Table 4-3. Same as above.

Response from Nancy Kerkvliet

This is a very thorough, well-written report that clearly presents the rationale and basis for the interpretation of the data available. The mode-of-action section is at times rambling and difficult to follow and would benefit from subheadings to better orient the reader and to orient presentation by topic rather than study. Editorial comments are listed here, mostly for readability of the document:

Page 24, first line – typo? Reference to 1 in 96 not clear.

Page 24, second paragraph – change ‘can induce’ to ‘might induce’

Page 25, relevance of ‘higher incidence of cytoplasmic homogeneity’ not clear

Page 41, line 3, delete the word ‘dramatic’

Page 49, section 4.4.1.3 last line: use of the term ‘plethora’ unclear

Page 52, paragraph starting “The systemic toxicity of GBL...”: accounting of thymic depletion in regard to low and high dose males is inconsistent/unclear.

Page 52, last paragraph, identify substance tested (assume it is GBL but not sure)

Page 53, after end of discussion of GHB there needs to be some sort of conclusion written that puts the studies cited regarding GBL and GHB into perspective for relevance to THF. Otherwise put them elsewhere as they distract from the train of thought regarding mode of action of THF.

Page 54, last paragraph, line 3: use of the term “additional” is confusing since the assays were mentioned in the prior paragraph. Change to: Results of.... the mode-of-action... are shown in Table 4-8. This paragraph could also use a concluding statement interpreting the relevance of the changes seen in labeling index.

Page 55 first paragraph: change ‘growth’ to ‘proliferation’

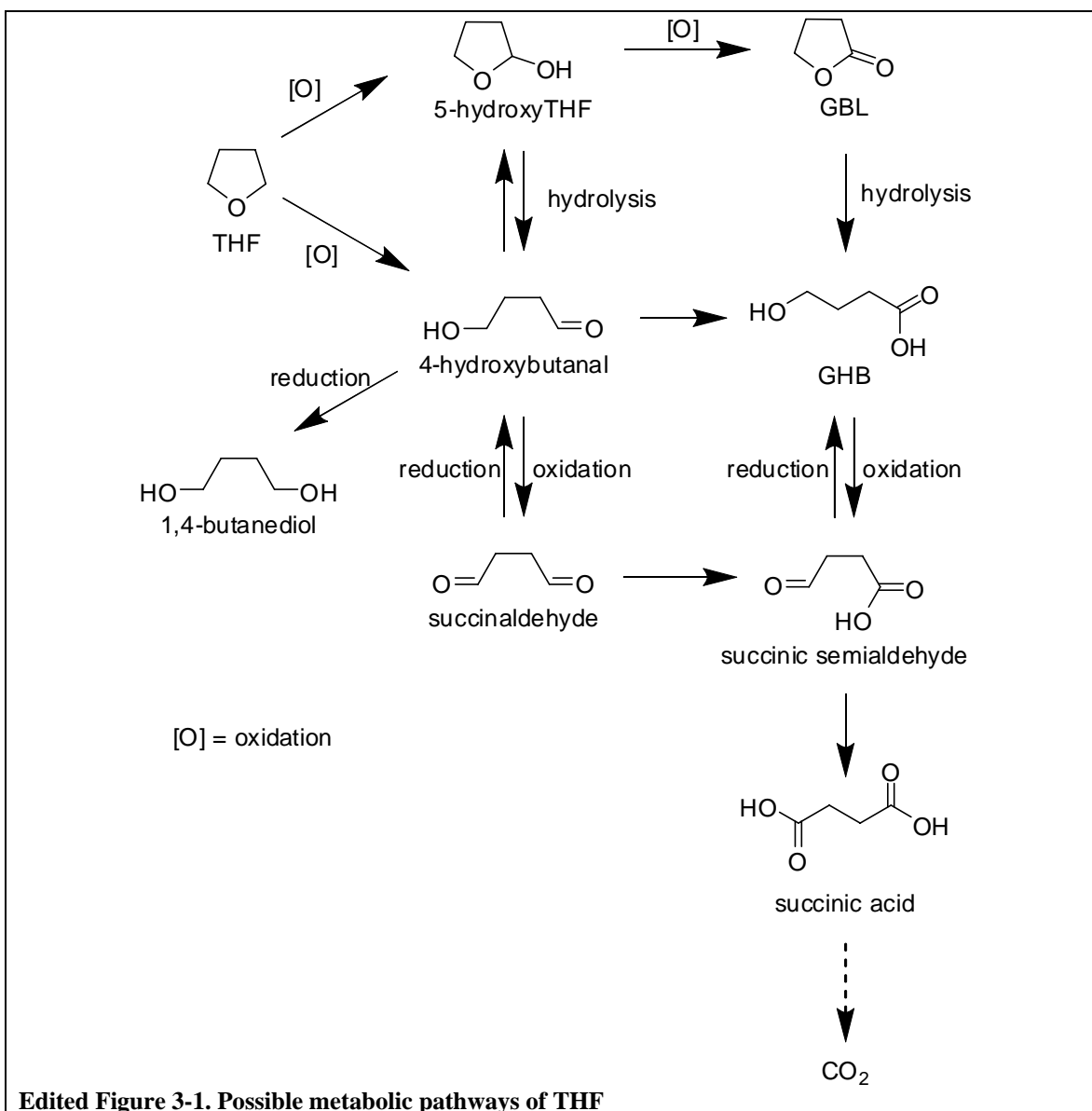
Page 57: The unqualified statement “THF exposure increased cell proliferation in female mouse liver” is misleading given the discussion of the limitations of the data on page 53. The paragraph needs a concluding statement, because the next paragraph jumps into the uterus without any transition.

Page 92, second line from bottom: change ‘with’ to ‘while’; use of term co-critical in same sentence is not clear here.

Response from Lisa Peterson

In general, the Toxicological Review is logical, clear and concise. There was one section (3.3 Metabolism) in which I noticed some errors. The pathways shown in Figure 3-1 are somewhat misrepresentative and, given that much of it based on structurally related molecules, I would

recommend some changes to this figure as indicated below. Given the absence of information regarding the metabolism of THF, I think a generic representation is more appropriate. For example, the current Figure 3.1 indicates that succinaldehyde and GBL result from P450 catalyzed oxidation. Since the conversion of THF to these two products would require an overall 4 electron oxidation and cytochrome P450 catalyzed reactions are 2 electron oxidation reactions, the initial product would be either 4-hydroxybutanal or 5-hydroxy-THF. It is likely that succinaldehyde and GBL are microsomal metabolites of THF and are formed from the intermediate via an additional two electron oxidation. In addition, the proposed conversion of THF to 4-hydroxybutanal is not a hydrolysis reaction as indicated in Figure 3-1 or in the text on page 15, but would result from oxidation of THF. This reaction could be catalyzed by enzymes other than cytochrome P450. The text should be corrected to better fit what is likely. Finally, the conversion of GHB to succinic semialdehyde is an oxidation reaction, not reduction as indicated on the first line of p. 14.



Response from Karl Rozman

This is a well-written, easy to read document with whose conclusions, however, I disagree. The authors make a compelling case for the parent compound being responsible for toxicity of THF. This compound is not mutagenic, does not bind to DNA and is not metabolized to reactive intermediates. The putative metabolites enter the citric acid cycle as indicated by exhalation of $^{14}\text{-CO}_2$ consisting of about 50% or more of the dose in both rats and mice after oral administration. This pathway is clearly a detoxification reaction. Consistent with this is the long half life (> 50 hours) of $^{14}\text{-C}$ radioactivity reflecting sequestration of the various citric acid cycle intermediates as part of the total carbon pool. In contrast the biological half life of THF is reported as 5-7 hours in rodents and possibly less than an hour in humans. Since it takes 3.32 half-lives to eliminate 90% of a compound, no accumulation of effect is expected from a chemical that does not cause irreversibility to accumulate in the organism. Irrespective of route of administration the steady state concentration of THF will be minimal (nearly zero but of course not quite that for a first order process) after subchronic and chronic administration. In fact each daily dose rate acts as a single dose. This is born out by identical NOAEL/LOAELS after subchronic and chronic administration.

Oral reference dose

In my view application of a total UF of 1000 ($10 \times 10 \times 10$) is not warranted for compounds that do not have a rate-limiting step originating in biotransformation. Intraspecies variability (human) in terms of metabolism might be as large as 10-fold in some instances. However, the document argues and it is indeed plausible that metabolism plays no role in the toxicity of THF. Absorption, distribution and elimination (if metabolism is not rate-limiting) does not have a 10-fold range among individuals or for that matter among species, particularly not when exhalation of the toxicant and its metabolite (CO_2) constitutes the major route of elimination. Therefore, in my opinion a UF_H of 3 and an UF_A of no more than 3 is justifiable according to this reasoning. I do not see a database deficiency to warrant a UF_{DB} of 10, rather a factor of 3 should suffice because the RD_f is based on a well-conducted study with very sensitive end points (pup weight, delayed eye opening). Thus a total UF of 27 (30) ($\sim 3 \times 3 \times 3$) would provide protection for the whole population regardless if it is derived from the NOAEL or the BMDL_{05} .

Inhalation reference concentration

The same arguments apply for derivation of the R_fC except that the studies used for it were perhaps even better documented. Therefore, a total UF of 27 (30) should suffice to protect the whole population from any adverse health effects of THF.

Cancer assessment

“A 2-year NTP (1998) inhalation cancer bioassay reported an increased incidence of renal tubule adenomas and carcinomas in male F 344/N rats (not significant)...” this is a contradictory statement. A not significant change is not a change even if it is slightly outside of historical controls. The $\alpha_{2\mu}$ -globulin response is from inconclusive to largely negative, therefore there is

no plausibility for this alleged response. In female mice the highest dose significantly increased hepatocellular adenomas and the combined rate of adenomas and carcinomas in this high background strain of mice. This leaves us with no effect in one species and a carcinogenic effect in one gender of another species. Cell proliferation assay and other mechanistic studies allow no other conclusion than that THC might be a very weak promoter. If the linear extrapolation from the POD is applied in this situation – and I will not belabor well-known arguments here – then the threshold approach will never be applied and you might consider replacing me with a more pliable toxicologist.

$$\begin{aligned}
 R_f D &= \text{BMDL}_{05} \div (\text{UF}_H \times \text{UF}_A \times \text{U}_{DB}) \\
 &= 309 \text{ mg/kg} - \text{day} \div (3 \times 3 \times 3) \\
 &\approx 309 \text{ mg/kg} - \text{day} \div 27 \\
 &11.4 \text{ mg/kg} - \text{day}
 \end{aligned}$$

$$\begin{aligned}
 R_f C &= \text{NOAEL} \div (\text{UF}_H \times \text{UF}_A \times \text{U}_{DB}) \\
 &= 316 \text{ mg/m}^3 \div (3 \times 3 \times 3) \\
 &\approx 11.7 \text{ mg/m}^3 \\
 &\text{with } 20 \text{ m}^3 \text{ air inhaled per day} \\
 &= 234 \text{ mg/day} \cong 3.3 \text{ mg/kg} - \text{day for } 70 \text{ kg humans}
 \end{aligned}$$

This would bring in line the oral and inhalation numbers. Clearly THF is less toxic orally since it is detoxified more efficiently due to first pass effect. As it is written the document implies, that THF is more toxic orally ($R_f D$ 0.3 mg/kg – day) than by inhalation ($R_f C = 3 \text{ mg/m}^3 \cong 0.9 \text{ mg/kg} - \text{day}$).

QUESTION A2

Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of THF.

Response from John Christopher

I know of no additional studies which the authors ought to include.

Response from George Corcoran

The only revision I wish to note along with Dr. Peterson is that there should not be an arrow leading from THF directly to 4-OH Butanal but rather that the arrow should go from GHB to 4-OH Butanal. I also agree with the other corrections described by Dr. Peterson. This report does a credible and effective job identifying gaps in existing studies and databases and points clearly to where future studies need to focus should THF undergo again undergo rigorous toxicological evaluation.

Response from David William Gaylor

No comment was provided to this question.

Response from Nancy Kerkvliet

The chronic toxicity of THF appears to be sufficiently low to not generate enough concern for further study.

Response from Lisa Peterson

I did not find any other published studies regarding the health effects of furan which were not incorporated in this assessment.

Response from Karl Rozman

I am not aware of better documented or more suitable studies for either noncancer or cancer health effects of THF.

CHEMICAL-SPECIFIC CHARGE QUESTIONS

(B) ORAL REFERENCE DOSE (RfD) FOR TETRAHYDROFURAN

QUESTION B1

A chronic RfD for THF has been derived from the oral drinking water 2-generation reproductive toxicity study (BASF, 1996; Hellwig et al., 2002) in rats. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Response from John Christopher

I agree with the selection of critical study. Justification for its selection is presented very well.

Response from George Corcoran

The selection of the BASF and Hellwig studies for estimation of the chronic oral RfD has been scientifically justified and transparently and objectively presented in the report. I do not concur with the conclusions of this report that studies demonstrate acceptably strong evidence that THF induces developmental toxicity. This difference in conclusions is detailed in responses to subsequent questions under this oral reference dose section. I am aware of no other studies that should be selected as the principal study.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

The use of the 2-generation drinking water studies for reproductive toxicity in rats (BASF, 1996; Hellwig et al. 2002) are most appropriate for deriving the chronic RfD. Drinking water (or food) represents the primary nonoccupational exposure pathways of concern. Using the relevant dosing regimen is important because of the rapid metabolism of THF. Bolus dosing with high doses of THF induced other toxicities not seen with drinking water exposure that are likely not relevant to the RfD.

I agree with Dr. Gaylor's recommendation that BMD.05 and BMDL.05 be dropped and that BMDL1SD be used as POD. I further believe that this represents an "abnormal" but not likely an "adverse" effect.

Response from Lisa Peterson

A chronic oral RfD for THF was developed using data from the oral drinking water 2-generation reproductive study. The justification for the selection of this particular study is clearly described in the document and is strongly supported by the available scientific data. The rationale for choosing this study over the other published studies is that it was conducted for a sufficiently long period of time, that the dose range was narrower than the other study and that it contained the most comprehensive toxicological data with histopathological data for liver, kidney, digestive and reproductive organs in males and females at all doses tested. Furthermore, since the reference dose is for oral exposure, the data best used to determine the oral RfD is with data obtained via oral exposure. The other oral chronic study, which was less complete, provides important supportive data. The decision not to use the oral toxicity data for the THF metabolites, GBL and GHB, is well-justified because there is substantial uncertainty whether these metabolites contribute to the toxicological effects of THF and it is difficult to draw conclusions regarding dose of parent compound based on the effects of the metabolites. Many factors can influence the extent of metabolism of the parent compound. In addition, there are data which suggest that the parent compound, not a metabolite, is responsible for the toxicological properties of THF.

Response from Karl Rozman

This is a well-conducted and – documented study. I would have picked this study for chronic R_fD myself. If anything, shortcomings of the experiment (incomplete histopathology) are exaggerated. THF does not cause much of pathology even at higher doses.

QUESTION B2

Decreased F2 male pup body weight was selected as the most appropriate critical effect. Please comment on whether the selection of this critical effect has been scientifically justified and transparently and objectively described in the document. Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Response from John Christopher

I agree with the authors' selection of decreased pup weight as the critical effect via the oral route. This effect is at most marginally adverse, as it seems possible that animals consumed less water at the higher exposure groups. It was most unfortunate that quantal data were not reported for the neurological and behavioral effects. Nevertheless, neurological and behavioral effects were reported at the same doses at which decreased pup weight occurred.

The authors should have stated that the critical effect via the oral route is caused by the parent chemical and not a metabolite. The justification for this lies in combining information from the oral and inhalation routes. The authors demonstrated clearly that THF is metabolized in the liver, making a first pass effect via the oral route a very definite possibility. Hepatotoxicity was seen in several studies via inhalation, but either minimally or not at all in oral studies. One may infer from this that the parent compound is the toxic principal. Identifying the ultimate toxin can prove to be very important if a physiologically-based toxicokinetic model is ever applied to these data to estimate a tissue dose.

Response from George Corcoran

The data presented in the primary studies (BASF, 1996; Hellwig et al., 2002) in rats are somewhat limited, both in scope and in extent of effect. Classifying decreased F2 pup weight as a developmental toxicity of THF appears tenuous due, in part, to its low overall magnitude, and due to the observed significant decrease in dam water intake. Thus, it is the opinion of this reviewer that, while these studies may remain the most appropriate to serve as principal studies because of the absence of more extensive high quality data sets, their interpretation as direct and secure evidence of THF developmental toxicity remains tenuous. The limited available data for oral exposure of animals to THF, including nominal effects on delayed eye opening and auditory canal opening, preclude their selection as more robust endpoints for this evaluation. This reviewer is aware of no other studies that would be more appropriate for basing the RfD calculation.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

Although the selection of decreased F2 male pup body weight is transparently and objectively described in this document, I am not convinced it is an appropriate critical effect. The effect is minimal and is only observable in a small window of time. The effect was also seen in the F1 pups and did not carry forward into changes in their growth or ability to reproduce. The data demonstrating the developmental effect of delayed eye opening that is mentioned in the document to support the F2 body weight as critical effect are also very weak, with a high degree of variation in each group. The data on auditory ear opening is not shown in the document so the strength of the data could not be assessed. Add in the confounder of decreased water consumption in the dams that could be due to palatability and pup data are even less impressive as representing a critical adverse effect of THF. However, what these data really point to is the low toxicity of THF in drinking water. There doesn't appear to be a better endpoint from these studies that could be used to calculate the RfD. I agree with the decision to not use inhalation data to calculate oral RfD.

Response from Lisa Peterson

Decreased F2 male pup body weight was selected as the most appropriate critical effect. This selection was justified based on a clear consistent dose response and biological significance. They were also occurred with no visible effect on the mother indicating that they were connected to THF exposure, rather than toxic effects on the mother. Endpoints such as delayed eye opening by pups are more difficult to quantitatively measure and, therefore, are difficult to model. In addition, this effect was more sensitive to THF exposure: the effects on pup kidney weight paralleled those on body weight but the changes with increasing dose were larger with pup body weight, making this measurement a more sensitive tool. In addition, the effects on pup weight gain were observed at THF doses at which there were no CNS or liver effects in the adults. Therefore, this toxic effect (pup weight gain) is a more sensitive measure of THF's adverse effects. The justification of this choice is clearly described in the document and strongly supported by the available scientific data.

Response from Karl Rozman

The answer is yes to both questions. Other end points did not allow for dose-response modeling,

QUESTION B3

The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the point of departure (POD). All available models were fit to the individual male and female and combined incidence data (F1 and F2 pup body weight gain). Please comment on the appropriateness and scientific justification presented for individual and combined body weights to obtain a data set for BMD modeling. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Response from John Christopher

I agree that benchmark dose modeling is the best method for describing the dose-response relationship. However, I agree with Dr. Gaylor, another reviewer, that the point of departure should be based the lower limit on one standard deviation below the mean for body weight in the control groups, because pup weight is a continuous variable. The point of departure should be 671 mg/kg-day, the value shown in Table 5-3 as the $BMDL_{1SD}$ for decreased pup weight for F₁ males and females. Among the various values for $BMDL_{1SD}$ for decreased pup weight for both sexes, this value has the lowest value for the Akaike Information Criterion. Using the standard deviation instead of a fixed response as the benchmark yields a higher point of departure by more than a factor of 2: 671 vs. 309 mg/kg-day.

Note in Table 5-3 that “ $BMD_{0.5}$ ” and “ $BMDL_{0.5}$ ” are misprints which should read “ $BMD_{0.05}$ ” and “ $BMDL_{0.05}$ ”. Appendix B ought to present greater detail on the definition and use of the Akaike Information Criterion.

Response from George Corcoran

The use of combined F1 and F2 pup body weight gain can be advocated as appropriate and scientifically justified to obtain a data set for BMD modeling. However, this statement should be considered in light of responses to questions B1 and B2. The decreases in pup body weight gain are weak effects, at the very best. Their linkage to THF development is not robust. Notwithstanding these caveats, BMD selection and BMD modeling to determine the POD appear reasonable.

There is a more appropriate approach to selecting the level of abnormal response (here adverse effect) for the purpose of BMD and POD calculations. I support a proposal that will be elaborated in detail in the responses of Dr. Gaylor in which data variance is considered rather than arbitrarily selecting a 5% decline in body weight as the adverse effect onset. This approach utilizes 1 standard deviation below the BMD to establish the Point of Departure. I strongly

support this science-based approach over the arbitrary selection of a 5% decline in body weight gain as the onset of a THF adverse effect of F2 pups.

The use of combined F1 and F2 pup body weight gain can be advocated as appropriate and scientifically justified to obtain a data set for BMD modeling. However, these data sets are not robust enough for this purpose (see responses to questions B1 and B2).

Use of the BMD / POD approach is highly rational and appropriate for this toxicology review. It is superior to other possible approaches including use of NOAEL and LOAEL values.

Response from David William Gaylor

Page 96, Table 5-3. Change decimal points in column headings to BMD_{.05} and BMDL_{.05}

Pages 95-97, Section 5.1.2.1.

Pup body weight gain in F₂ males, as presented in Table 5-3, appears to be an appropriate sensitive effect for calculating the point of departure (POD) in order to derive an oral RfD. Selection of a 5% reduction in body weight gain is an arbitrary choice and has no defined biological significance for the derivation of the POD. It is **highly recommended that BMD_{.05} and BMDL_{.05} be dropped**. In order to establish the biological impact of a continuous variable, a change in the mean response due to exposure needs to be evaluated relative to the variation among animals as measured by the standard deviation. For continuous data, in the absence of a specified value associated with an adverse biological effect, an extreme percentile, e.g., estimated 1st and/or 99th percentile, of the values in the controls may be used to define abnormal (not necessarily adverse) values. For example, with normally distributed data, values outside the range defined by the control mean \pm 2.33 standard deviations would be considered abnormal. From the dose response model, the dose associated with a specified excess risk, e.g., 5% or 10%, of animals with abnormal values of the selected continuous endpoint can be estimated (Gaylor and Slikker, 1990; Crump, 1995). Allen *et al.* (1994) show for reproductive and developmental effects that the lower 95% confidence limit for an excess risk of 5% is often similar to the NOAEL. For normally distributed data, an excess risk of 5% of animals with abnormal levels (outside the 1st or 99th percentile) occurs at the dose where the mean value changes from baseline (controls) by an amount equal to (0.77 x standard deviation). Similarly, an excess risk of 10% of animals with abnormal levels occurs at the dose where the mean value changes from baseline by an amount equal to (1.1 x standard deviation). BMDL_{1SD} presented in the document, representing a shift in the mean equal to (1.0 x standard deviation), is an appropriate choice for the POD. This value provides an estimated excess risk of 10% if the abnormal cut-offs are set at the estimated 1.5th and 98.5th percentiles of the control animal levels. Hence, this approach provides a measure of the biological effect expressed as an excess risk of animals with abnormally low body weight gains in pups. Rather than using BMDL_{.05}, it is **highly recommended that BMDL_{1SD} = 601 mg/kg-day**, for decreased pup weight gain in F₂ males based on the restricted power model presented in Table 5-3, be used for the POD.

Pages 99-101, Section 5.1.3. Using the BMDL_{1SD} = 601 mg/kg-day discussed above for the POD and dividing by the overall uncertainty factor of 1000 gives an oral RfD = 601 / 1000 = 0.6 mg/kg-day. It is **highly recommended that the oral RfD be set at 0.6 mg/kg-day**.

References

Allen, BC, Kavlock, RJ, Kimmel, CA, and Faustman, EM. Dose-response assessment for developmental toxicology. II. Comparison of generic benchmark dose estimates with no observed adverse effect levels. *Fundamental Applied Toxicology* 23: 487-495 (1994).

Crump, KS. Calculation of benchmark doses from continuous data. *Risk Analysis* 15: 79-89 (1995).

Gaylor, DW and Slikker, W, Jr. Risk assessment for neurotoxic effects. *NeuroToxicology* 11: 211-218 (1990).

Response from Nancy Kerkvliet

Although BMD modeling is not my area of expertise, the description of the process appears to be fully justified. The only troubling point is that the pup body weight data is the only data set that was amenable to BMD modeling so it had to be used in order to do the process at all.

Response from Lisa Peterson

The chronic RfD was derived utilizing benchmark dose (BMD) modeling. This approach seems appropriate since this modeling was performed with a chronic dosing study with 25 animals of each sex per dose. In addition, they used a critical effect endpoint that was dependent on the dose. Since males and females seem to respond differently to THF, it was appropriate that they modeled these groups separately. I would recommend changing the point of departure (POD) from BMDL_{0.5} to BMDL_{1SD} since the latter is based on an adverse effect of true significance.

Response from Karl Rozman

Without dose response, BMD modeling could not have been conducted to identify the POD. When there are enough and closely spaced doses selected, there is not much difference between the BMD and NOAEL - based approaches. When there are only a few high doses, BMD may be more suitable to establish a POD.

QUESTION B4

Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document?

Response from John Christopher

I agree with the authors' selection of uncertainty factors: $UF_H = 10$, $UF_A = 10$, and $UF_{DB} = 10$ for a total $UF = 1,000$. No information is available of differential sensitivity of human populations to THF, so the default value of 10 for UF_H is appropriate. Information is essentially missing on toxicokinetic and toxicodynamic differences between animals and humans in their responses to THF via the oral route. Hence, the default of 10 for UF_A is appropriate. This data base lacks a chronic study via the oral route and no study on teratology is presented. Hence, the default of 10 should be used for UF_{DB} .

Response from George Corcoran

The uncertainty factors applied to the POD for the derivation of the chronic oral RfD appear scientifically justified and transparently and objectively described. The data set is incomplete and not robust, justifying the factor of 10 for database uncertainty.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

The selection of uncertainty factors is transparent and objectively described. However, I question the need to use such a large overall uncertainty factor when the oral toxicity of THF was so low that it was difficult to find an endpoint of concern.

I believe that the change in body weight gain in the F2 pups should not be used to determine Rfd without some adjustment in the UFs applied to reflect that the effect being used to determine the Rfd is abnormal for the experimental data base rather than inherently adverse. I agree with Dr. Rozman's comments that the water solubility of THF makes it unlikely that THF would be absorbed and distributed much differently in rodents compared to man. This reduces the need for a UF of 10 for animal to human extrapolation. I also think that the total amount of toxicity data available from oral and inhalation routes of exposure to THF is more than adequate to estimate chronic toxicity and reduces the need for a UF of 10 for database insufficiency.

Response from Lisa Peterson

The selection of uncertainty factors applied to the POD for the derivation of the RfD were scientifically justified and clearly described in the document.

Response from Karl Rozman

I disagree with the selection of the uncertainty factors as can be read on page 16 under Oral reference dose.

QUESTION B5

A two-generation reproductive toxicity study was used for the selection of the POD for the derivation of the RfD. Please comment on whether the rationale and justification for not applying a subchronic to chronic uncertainty factor has been scientifically justified and transparently described in the document.

Response from John Christopher

I agree with USEPA that no UF for extrapolating from subchronic to chronic exposures is required. The best evidence for this is the identification of the same LOAELs and NOAELs for toxic effects via inhalation, a route by which THF is more toxic. Exposure in the 2-generation reproduction study was as long as could be done for this type of protocol.

Response from George Corcoran

The rationale and justification for not applying a sub-chronic to chronic uncertainty factor has been scientifically justified and transparently described. Results from sub-chronic and chronic studies are quite similar, arguing against use of any uncertainty factor for this aspect of the evaluation.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

The rationale for not applying a subchronic to chronic uncertainty factor for the derivation of the RfD based on the 2-generation reproductive study is scientifically justified and transparently described.

Response from Lisa Peterson

The rationale and justification for not applying a subchronic to chronic uncertainty factor was scientifically justified and clearly described in the document.

Response from Karl Rozman

No need indeed, since subchronic and chronic NOAEL/LOAELs are the same.

QUESTION B6

Please comment on whether the rationale and justification for the selection of the database uncertainty factor has been scientifically justified and transparently described in the document.

Response from John Christopher

See 4 above.

Using the point of departure recommended in 3 above and the UFs summarized in 4, 5, and 6 above, I recommend an RfD value of 0.67 or 7 E-1 mg/kg-day.

Response from George Corcoran

The rationale and justification for the selection of the database uncertainty factor have been scientifically justified and transparently described. The dataset is incomplete (especially in the area of histopathology) and not robust. This justifies using a factor of 10 for database uncertainty.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

The selection of uncertainty factors is transparent and objectively described. However, I question the need to use such a large overall uncertainty factor when the oral toxicity of THF was so low that it was difficult to find an endpoint of concern. The UF of 10 for database insufficiency may need better justification. A discussion of this point at the meeting is anticipated.

I remain unchanged in my opinion that the change in body weight gain in the F2 pups should not be used to determine Rfd without some adjustment in the UFs applied to reflect that the effect being used to determine the Rfd is abnormal for the experimental data base rather than inherently adverse.

I agree with Dr. Rozman's comments that the water solubility of THF makes it unlikely that THF would be absorbed and distributed much differently in rodents compared to man. This reduces the need for a UF of 10 for animal to human extrapolation. I also think that the total amount of toxicity data available from oral and inhalation routes of exposure to THF is more than adequate to estimate chronic toxicity and reduces the need for a UF of 10 for database insufficiency.

Response from Lisa Peterson

The rationale and justification for the selection of the database uncertainty factor was scientifically justified and transparently described in the document.

Response from Karl Rozman

No, it is not justifiable, the study was well-documented with a very sensitive end point, which significantly correlated between pup body weight gain and maternal THF intake after accounting for other confounding factors.

**(C) INHALATION REFERENCE CONCENTRATION (RfC)
FOR TETRAHYDROFURAN**

QUESTION C1

A chronic RfC for THF has been derived from data from a 105 week chronic inhalation study (NTP, 1998) in mice and rats. Please comment on whether the selection of this study as the principal study has been scientifically justified and transparently and objectively described in the document. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Response from John Christopher

I agree with the selection of critical study. Justification for its selection is presented very well.

Response from George Corcoran

Selection of the NTP 105 week chronic inhalation study in mice and rats has been scientifically justified and transparently and objectively described in the document. Other studies that could serve this purpose are not apparent. This study provides a robust database for determining RfC and POD.

Response from David William Gaylor

Pages 101-113, Section 5.2. This section is comprehensive, clearly written, considers several options and supports the derivation of the inhalation RfC = 3 mg/m³.

Response from Nancy Kerkvliet

The selection of the chronic 2-year inhalation study by NTP (1998) to derive the RfC is described transparently and objectively in the document.

Response from Lisa Peterson

The selection of the 105 week chronic inhalation study as the principal study scientifically justified and transparently and objectively described. This study was performed with an adequate number of animals and includes toxicological data on all organs and systems. There are no human studies that provide sufficient information regarding the duration and/or concentration of THF exposure. In addition, when humans are exposed to THF, they are also exposed to other potentially hazardous materials. There are no other studies in rodents that are as extensive as the chosen study.

Response from Karl Rozman

Good selection, well justified.

QUESTION C2

Liver toxicity and CNS effects were selected as the co-critical toxicological effects. Please comment on whether the selection of this critical effect has been scientifically justified and transparently and objectively described in the document. Specifically, please address whether the selection of liver effects and CNS toxicity as the co-critical effects instead of increased thymus weight has been adequately and transparently described. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Response from John Christopher

I agree with the authors that hepatotoxicity and CNS effects are co-critical effects. It was most unfortunate that useable data on incidence of CNS effects was not presented, preventing the possibility benchmark modeling of the CNS effect. The effect in liver is minimally adverse, but appropriate to select nonetheless. Centrilobular hepatocytomegaly in male mice, unaccompanied by cell death or apoptotic changes, was dose-related in the chronic inhalation study. THF does induce its own metabolism, so the cytomegaly could have been related to enzyme induction. Even though the hepatocytomegaly was minimally adverse, I prefer this endpoint to increased liver weight. The former is a recognized early change in the pathobiology of centrilobular liver damage caused by many toxins, while the latter is a relatively non-specific effect. Changes in thymus weight cannot be used as a critical effect, when they are not accompanied by either histopathological changes or measured alterations in immune competence. The additional information from Luster *et al.* on the ambiguous predictive power of changes in thymus weight for immunotoxicity was helpful.

Page 104 is ambiguous on whether this hepatotoxic effect occurs in males or females. The authors should clarify their language.

Response from George Corcoran

The CNS effects are an excellent critical toxicological effect. This reviewer differs with selection of liver effects as a co-critical effect. Observations of hepatomegaly, without well characterized and convincingly established events such as sustained increases in cell proliferation or notably decreased apoptosis, is a questionable critical toxicologic effect. Results of the 105 week inhalation study do not convincingly establish either sustained cell proliferation or sustained inhibition of apoptosis as supportable modes of hepatomegaly. Cytomegaly is consistently reported. However, this readily reversible effect is not considered a serious toxic effect by many in the field. Considering the observed liver effects together with the absence of other key effects, these results collectively argue strongly against the use of liver changes as a critical effect. This report would be benefited substantially by limiting itself to the use of CNS effects as the critical toxicological effect for inhaled THF. Such changes would not materially change the analysis following THF inhalation exposure.

The selection of CNS effects as a critical toxicological effects over thymus or any other effects has been scientifically justified and transparently and objectively described. Neither thymus or liver effects should be considered critical effects because of the incomplete data sets and the lack of hisptoathology to establish that changes in thymus and liver were indeed adverse effects.

This reviewer can offer rationale for no other endpoints that should be considered in selection of the critical THF effect after inhalation exposure.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

Liver toxicity and CNS effects are appropriate co-critical endpoints that are scientifically justified in a transparent and objective manner in this document.

Thymus weight was decreased (not increased) in animals exposed to THF, and the effect was dose-dependent with significance at 600 ppm. Spleen weight was affected only at the highest concentration of 5000 ppm. Although the selective accumulation of THF in the thymus and spleen was noted, the lack of cytotoxic effects of THF in mode of action studies alleviate concern to some extent. The accumulation of THF in thymus and spleen may reflect lymphatic distribution and/or the lower metabolic capacity of these tissues. Decreased thymic weight is not an adverse effect per se, and can occur nonspecifically. Although I would usually discount the standard “stress” explanation in the absence of corticosteroid levels, (since I presume all animals were treated the same), it is possible that irritant effects of THF induced a stress response that could be related to dose. However, since the effect was only seen in male mice, and since spleen weights were not affected at the lower doses, I agree with the decision to not use the thymus weight loss as the critical effect. The lack of consistency between studies also supports this decision as Kawata and Ito (1984) reported no histopathology in spleen or thymus following THF exposure, and Horiguchi et al (1984) reported increased spleen weight following THF exposure. Inconsistent effects on WBC counts also lessen concern that THF is immunotoxic at the concentrations tested.

It is unfortunate that the thymi were not weighed or examined in the pups from the developmental study or the oral repro-tox study since this might have provided insight into the direct thymic toxicity of THF.

Response from Lisa Peterson

The selection of liver toxicity and CNS effects as the co-critical toxicological effects rather than thymus weight is supported by the known scientific data. This is clearly discussed in the document. Given the incomplete information regarding the potential for THF to be an immune toxicant, the decision to use the more complete information for the known liver and CNS effects of this compound is reasonable.

Response from Karl Rozman

It is fine as is, although I attribute less toxicological significance to a marginal liver effect than to a CNS effect. I agree with the argument that the functional significance of the thymus weight is unknown and probably there is none.

QUESTION C3

The chronic RfC has been derived utilizing benchmark dose modeling to define the point of departure (based on liver cytomegaly). BMD modeling was conducted on liver weight and cytomegaly data in both males and females. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified and transparently and objectively described? Please provide comments on whether the selection of a POD based on liver cytomegaly instead of liver weight is scientifically justified and transparently described. Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Response from John Christopher

The benchmark dose modeling is presented well, although the ambiguity mentioned above makes unclear why male mice were modeled instead of females or both sexes. Lack of modeling fit makes clear why effects on centilobular hepatocytes were used in preference to changed liver weights. I agree that selecting a level of 2 mg/m³ based on changes in liver weight would have placed the RfC in an inappropriate range where no adverse effect occurred in the mice. Therefore, the effect on liver weight cannot be used. As mentioned above for the RfD, the authors should expand their explanation in Appendix B of the Akaike Information Criterion, because it is employed in selecting the modeled point of departure.

Response from George Corcoran

Deriving the RfC using BMD to define the point of departure is an appropriate and preferred approach when the dataset is adequately robust and when the appropriate response is selected. The latter standard is not adequately met with selection of cytomegaly as the benchmark response. Concerns about the use of cytomegaly alone for this purpose are outlined in comments to Question C2.

While the report scientifically justifies and objectively describes selection of cytomegaly as the benchmark response to THF, this reviewer differs with this selection. The use of increased liver weight may be a more acceptable approach, but there are many who believe that a simple increase in liver weight is not a form of toxicity unless it is accompanied by sustained proliferation and / or decreased apoptosis.

This reviewer believes that POD should be assessed using CNS effects of THF, and not liver effects. It is also important to supplement the existing report with a more complete basis and explanation of the use of the Akaike Information Criterion. Appendix B provides some information on the use of this approach. However, the goal of providing adequate background, rationale and transparency is not achieved by this section. If the Appendix approach is continued, the report requires a considerable section in the body of the text addressing this deficiency.

Response from David William Gaylor

I agree with the approach, analysis, discussion, and conclusions as presented in the document.

Response from Nancy Kerkvliet

BMD modeling was objectively and transparently described in the document. The selection of centrolobular cytomegaly rather than liver weight is scientifically justified and transparently described.

Response from Lisa Peterson

BMD modeling on liver weight and cytomegaly data in both males and females appears to be appropriately conducted. The explanation for the approach and the choices made are clearly presented and supported by the scientific data.

Response from Karl Rozman

If you chose the BMD approach you have to find the best model fit and cytomegaly can be related to hepatomegaly. Therefore, I do not see an issue here.

QUESTION C4

No incidence data were presented for CNS effects. Thus, these data could not be evaluated by BMD modeling. However, a NOAEL LOAEL approach (based on the CNS data) for the derivation of the RfC has been presented for comparison purposes. Please provide comments as to whether the NOAEL LOAEL approach based on the POD for CNS effects is more appropriate for the derivation of the RfC. Please provide comments with regards to whether BMD modeling is the best approach for determining the point of departure.

Response from John Christopher

The authors demonstrate quite clearly that a LOAEL/NOAEL approach based on CNS effects reaches the same RfC as benchmark modeling of the effects in liver. I prefer the result based on benchmark modeling, because the data on which it is based are described in detail in the NTP report. This is not the case for the CNS effects.

Response from George Corcoran

Many scientists advocate BMD modeling as the best approach for determining the point of departure when an adequate and robust database of effects is available. This is not the case for the dataset describing CNS adverse effects. In this instance, the more historic approach of using NOAEL and LOAEL doses for the derivation of the RfC is not only justified, but it is the superior approach in this setting, including determination of the point of departure.

Response from David William Gaylor

I agree with the approach, analysis, discussion, and conclusions as presented in the document.

Response from Nancy Kerkvliet

I think both approaches are appropriate. Confidence in the RfC is increased by the fact that both approaches achieve the same the value.

Response from Lisa Peterson

Both approaches give the same result supporting the decision to use BMD modeling which is considered by many scientists to be the best approach for determining the POD.

Response from Karl Rozman

As far as I remember the NOAEL and the $BMCL_{10}$ were similar and therefore for the numerical value it doesn't matter. However, I consider a CNS effect of greater toxicological significance than hepatic cytomegaly. Therefore, the use of a NOAEL/LOAEL approach might be better.

QUESTION C5

Please comment on whether the selection of the uncertainty factors applied to the POD for the derivation of the RfCs. For instance, are they scientifically justified and transparently and objectively described in the document.

Response from John Christopher

I agree with the authors' selection of the uncertainty for extrapolating from humans to sensitive humans, $UF_H = 10$. No information is available of differential sensitivity of human populations to THF, so the default value of 10 for UF_H is appropriate. I disagree with the authors' selection of $UF_A = 3$. The authors justified reducing UF_A to 3, stating that toxicokinetic differences between rodents and humans were accounted for. In fact, the authors accomplished this with a default value of 1 for the ratio of human to animal blood-air partition coefficients of THF. If UFs are placeholders for lack of information, then surely one cannot be reduced with a statement of another lack of information. This UF should not be reduced until it can be replaced with a data-driven UF based on a physiologically-based toxicokinetic model. Therefore, I recommend a total UF of 100 for extrapolating from animal to sensitive human of animal.

Response from George Corcoran

Scientific justification and rationale are provided for the selection and use of uncertainty factors for POD and RfCs determinations. However, these UFs in combination may be overly conservative. The report should provide additional discussion on attempts to reduce the overall uncertainty factor, and provide a more compelling argument as to why this is not possible.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

Overall, I agree with the rationale for the uncertainty factors applied, which was scientifically based and transparently described.

Response from Lisa Peterson

The selection of the uncertainty factors applied to the POD for the derivation of the RfCs seem appropriate. The discussion in the document is clear and supported by the known scientific information.

Response from Karl Rozman

I disagreed, as shown on page 16 under Oral reference dose.

QUESTION C6

Please comment on the transparency and scientific rationale and justification for the selection of the database uncertainty factor. Please comment on whether the application of the database uncertainty factor adequately represents the gap in inhalation reproductive and developmental toxicity and immunotoxicity data for THF. Please comment on whether the rationale for use of the oral data to inform this decision scientifically justifiable and transparently described in the document.

Response from John Christopher

I agree with the authors' selection of 3 for UF_{DB} . Their explanation is transparent. The inhalation data base is more complete than the oral, principally because it contains the NTP lifetime study in rats and mice. Teratology studies are still lacking, however, so a factor greater than 1 is called for.

Response from George Corcoran

Scientific rationale and justification are provided for the calculation of uncertainty factors. Again, the aggregated uncertainty factor may be overly protective.

The application of a database uncertainty factor of 3 is a reasonable approach to account for the gap between reproductive and developmental toxicity data.

The use of oral data to inform this decision is scientifically justifiable, transparent, and a reasonable approach given these circumstances.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

I think the database uncertainty factor of 3 is acceptable. I am not overly concerned about the lack of functional immunotoxicity data since there is no evidence to suspect that lymphocyte responses would be selectively sensitive to THF versus other types of cells. Cytotoxicity would have been a concern if it had been demonstrated in mode-of-action studies. The rapid metabolism of THF also lessens concern about the immune system being a target for chronic low level exposures to THF. Secondary effects that might occur as a result of inflammatory mediators produced following high exposure levels would not be relevant to low dose concerns. I think using the oral data to inform this decision is valid.

Response from Lisa Peterson

The scientific rationale and justification for the selection of the data base uncertainty factor is clear and well explained.

Response from Karl Rozman

Immunotoxicity (thymic atrophy) was observed only in the inhalation studies with high tissue distribution of THF into spleen and thymus. THF as a small water-soluble compound will distribute into total body water initially during inhalation. After oral gavage the portal blood will carry the whole dose to the liver, a fraction of which will be extracted and much of it metabolized. A smaller fraction will escape extraction and enter into the systemic circulation. Thus, tissue parent THF will be possibly lower and for a much shorter period of time after oral than after inhalation exposure. Thus, there is a natural pharmacokinetic protection for the thymus after oral administration as compared to inhalation unless it is the metabolite (GBL) that causes this effect for which there seems to be some evidence. However, there is no evidence for massive production of GBL in liver followed by re-compartmentalization into the thymus, in fact this is highly unlikely. A factor of 3 may not be even needed as an UF_{DB} for deriving the $R_f C_f$.

QUESTION C7

THF induces a spectrum of effects consistent with both Category 1 and Category 3 gases. Therefore, for the purposes of calculating human equivalent concentrations, respiratory tract effect levels were calculated using the default equations for Category 1 gases and extrarespiratory tract effect levels were calculated using default equations for Category 3 gases. Please comment on the explanation for the dosimetry choice in the derivation of the RfC. Has the rationale been scientifically justified and transparently described?

Response from John Christopher

I agree with the authors' designation of THF as a Category 1 gas when calculating human equivalent concentrations for systemic toxic effects. Category 1 gases are those which reach the deep lung for gaseous exchange with blood.

Response from George Corcoran

The dosimetry choices in the derivation of the RfC appear rationale, scientifically justified, and transparently described. The report would benefit from an expanded discussion of the criteria and justifications of derivation of gas characterization categories used by EPA, as well as a brief treatment that contrasts the EPA approach with other categorical methodologies.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

The rationale was transparent and appeared sound.

Response from Lisa Peterson

The rationale for the decision to use the default equations for Category 1 gases for the respiratory tract effects and those for Category 3 gases for the extra-respiratory effects is logical and clearly presented. The available experimental evidence supports this decision. This section could be improved by a better explanation of the differences between category 1, 2 and 3 gases.

Response from Karl Rozman

I am not familiar with EPA's categorization of gases. I used one possible categorization in one of my chapters based on thermodynamics, basically very reactive gases (1) inert gases (3) and in between (2) using Gibb's free energy as criterion. I didn't find anything wrong with the calculations and explanations, as conducted by authors of this document.

(D) CARCINOGENICITY OF TETRAHYDROFURAN

QUESTION D1

Under the EPA's 2005 Guidelines for carcinogen risk assessment (<http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283>), there is suggestive evidence for the human carcinogenic potential of THF. Please comment on the scientific justification for the cancer weight of the evidence characterization. A quantitative cancer assessment has been derived for THF. Do the data support estimation of a cancer slope factor for THF? Please comment on the scientific justification for deriving a quantitative cancer assessment considering the uncertainty in the data and the suggestive nature of the weight of the evidence of carcinogenic potential. Has the rationale and scientific justification for quantitation been transparently and objectively described?

Response from John Christopher

I agree with the authors that positive results in two species constitute adequate evidence that THF has carcinogenic potential for humans. Of the two carcinogenic responses seen in the NTP inhalation bioassay, only hepatocellular adenoma and carcinoma in female mice support quantitative estimation of cancer potency. The dose-response is weak in male rat kidney. I address the advisability of estimating cancer potency, slope factor, and/or unit risk in 4 below.

Response from George Corcoran

There is little question that there is suggestive evidence of THF carcinogenicity based on the EPA 2005 Guidelines for Carcinogen Risk Assessment. The weight of evidence is for increased appearance of female mouse liver tumors is clear and straightforward. The same can not be said of male rat renal tumors. This reviewer considers the weight evidence for this tumor to be marginal to inadequate, despite use of 50 animals per group. This does not provide adequate power to detect relatively rare renal tumors, as is the conclusion of this report.

The quantitative cancer assessment derived for THF has at least 2 very substantial flaws that work to weaken the primary conclusions of this report. The first of these concerns is that the use of a quantitative cancer assessment will almost certainly overestimate, by many magnitudes, the risk actually posed by this very weak possible human carcinogen. This constitutes a problem of the largest magnitude and must be adequately addressed by revision of the report. The second very large conceptual and practical drawback to the manner in which this quantitative cancer assessment was performed is the use of low-dose linear extrapolation. This issue is addressed in greater detail in the response to Question D4.

The report provides a credible cancer weight of the evidence characterization. The report appears scientifically justified and transparently described. The conclusions of the report must be more greatly defended against the shortcomings pointed out by this reviewer.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

Since cancer risk assessment is not my area of expertise, I found it difficult to comment on the questions posed. I agreed with the overall weight-of-evidence summary and the approaches used and decisions made appeared to be logical and transparent. If anything, I was disappointed to see how little progress we have made as a toxicology community in science-based risk assessments.

I disagree with the default to a linear low-dose extrapolation model for the carcinogenic response to THF in the liver of female mice or kidney of male rats. There is ample evidence that THF is not mutagenic and therefore not a direct carcinogen. Furthermore the metabolism of THF is rapid and there will not be a body burden accumulating in humans from low dose exposures. The nonreactive nature of THF and its documented low toxicity also precludes the likelihood that damage would accumulate following multiple exposure to low doses of THF. Add these facts to the very weak tumor response that was observed only after exposure to the highest dose of THF and it further argues for the use of a nonlinear threshold dose-response model. The application of a nonthreshold model would be defaulting to a model that is clearly inappropriate and not based on sound scientific principles. Even though the mode of action of THF is unclear, the ability of THF to induce an unsustained (and therefore not documented by histopathology) proliferative response is a plausible mechanism of action in line with other known tumor promoters.

Response from Lisa Peterson

Given the observation that THF induces liver tumors in female B6C3F1 mice and the fact that humans can be exposed to THF, it is reasonable to suggest that THF may cause cancer in humans. Based on EPA guidelines, the data support estimation of a cancer slope factor for THF because there isn't sufficient information to indicate a threshold for THF exposure that would not cause cancer. The derivation of a quantitative cancer assessment considering the uncertainty in the data and the suggestive nature of the weight of the evidence of carcinogenic potential may provide a measure of the magnitude of the carcinogenic concern. For example, if this RfC is higher than those calculated for the toxic effects of THF, it would indicate that the potential for cancer risk is likely to be lower than other toxic effects of THF. The rationale and scientific justification for quantitation has been transparently and objectively described.

Response from Karl Rozman

I do not dispute the fact that statistically increased incidence of adenomas and carcinomas in female mice is "suggestive evidence for the human carcinogenic potential" because animal data are relevant for evaluation of human risk. However, an increase in high background cancer incidence in one gender of one species at a dose exceeding the maximum tolerated dose (narcosis observed at 1,800 ppm) is indicative of an exceptionally low potency compound. I am the

opinion that a linear extrapolation from the POD represents a vast exaggeration of risk which does not yield any benefit for public health. Hundred thousands of organic chemists have used THF in large quantities during the past 100 years, for the most part without the protection of a hood, not to speak about the millions of workers who have been exposed to THF during said time period. There is no indication that THF caused or contributed to the development of cancer in this huge population. The linear cancer extrapolation suggest a magnitude of risk for THF – induced cancer that is simply not supported by facts and to suggest otherwise is a disservice to workers who are still currently exposed to THF. I am convinced that the R_fC, which is much lower (orders of magnitude lower) than past exposures of workers is adequate to protect the public from any cancer risk that might result at very high exposure concentrations of THF.

QUESTION D2

The available data suggest that a plausible mode of action for THF-induced male rat kidney tumors may involve the accumulation of alpha-2u globulin. EPA concluded that the available data do not provide significant biological support to establish a mode of action for male rat kidney tumors and that these tumors are relevant to humans. Please comment on the transparency and scientific rationale and justification for the evaluation of these data and the conclusions regarding the possible mode(s) of action and human relevance for the male rat kidney tumors.

Response from John Christopher

The authors make an excellent presentation on all aspects of mode of action which might bear on renal carcinogenicity. Renal neoplasms ascribed to the pathological sequence beginning with deposition and accumulation of α_2 urinary globulin are thought to be a phenomenon singular to male rats. If this mode of action could be ascribed to THF, then one would not extrapolate this carcinogenic effect to humans. The available evidence seems to show that THF can trigger accumulation of α_2 urinary globulin, but its potency is very low and the more advanced stages of the disease process leading to cancer are not evident. Dr. Hard makes an interesting case that these renal tumors could be secondary to cellular proliferation to chronic nephropathy, the incidence and severity of which were unrelated to RHF. Although it is interesting that renal tumors were found in the areas of the most severe nephropathy, no information is available from the literature to support this mode of action for THF. I agree with EPA that no mode of action for causation of these neoplasms can be identified with reasonable certainty.

Response from George Corcoran

The conclusions drawn by EPA that the available data do not provide significant biological support to establish a mode of action for male rat kidney tumors and that these tumors are relevant to humans appear scientifically justified and transparently described. Plausible arguments can be made that THF is a weak alpha-2u globulin inducer, resulting in observation of only some of what are considered to be the 5 hallmark changes induced by potent alpha-2u globulin inducers typified by trimethylpentane. It is not possible to say that this mechanism is not relevant to humans. Thus, the position taken by EPA in this report is fully justified.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

No response was provided to this question.

Response from Lisa Peterson

While the available data suggest that a plausible mode of action for THF-induced male rat kidney tumors may involve the accumulation of alpha-2u globulin, these data are not conclusive. Therefore, the conclusion by the EPA that the available data do not provide significant biological support to establish a mode of action for male rat kidney tumors is appropriate. Since the data are not conclusive, it is appropriate to consider that humans may also be at risk of kidney carcinogenesis when chronically exposed to THF.

Response from Karl Rozman

I concur with EPA's conclusion but I do not accept the claim that THF causes kidney cancer in male rats.

QUESTION D3

The available data suggest that increased proliferation and promotion in the liver may be a plausible mode of action for THF-induced female mouse liver tumors. EPA concluded that the data do not provide significant biological support to establish a mode of action for female mouse liver tumors and that these tumors are relevant to humans. Please comment on the transparency and scientific rationale and justification for the evaluation of these data and the conclusions regarding the possible mode(s) of action and human relevance for the female mouse liver tumors.

Response from John Christopher

Once again, the authors make an excellent presentation on all aspects of mode of action which might bear on hepatocarcinogenicity. Some evidence suggests that proliferation of centrilobular hepatocytes is caused by THF in a dose-related manner. However, other portions of well known processes leading to murine liver cancer, such as necrosis and regeneration of hepatocytes, was not observed. As was the case with kidney, THF seems capable of causing early events but its potency is very low. Hepatocellular proliferation was observed in response to THF, but the effect was not of the type usually associated with a carcinogenic response (hyperplastic nodules, eosinophilic foci, *etc.*). It seems possible that THF could be a promoter of liver cancer in mice, but the evidence for this is speculative. I agree with EPA that no mode of action for causation of these neoplasms can be identified with reasonable certainty.

Response from George Corcoran

This reviewer agrees with the position taken by EPA in this report regarding the roles of proliferation and promotion in mouse liver tumors. The conclusions drawn by EPA that the available data do not provide significant biological support to establish a mode of action for THF-induced female mouse liver tumors and that these tumors are relevant to humans appear scientifically justified and transparently described.

Response from David William Gaylor

THF is not considered to be genotoxic. This allows for the possibility of a nonlinear carcinogenic dose response and calculation of a Reference Concentration (RfC) based on the $BMCL_{10}$ divided by appropriate uncertainty factors, typically 10 for animal to human extrapolation and 10 for variability of sensitivity within the human population. It has been suggested that liver tumors in female mice may be the result of cell regeneration produced by high dose cytotoxicity that would be greatly diminished or even zero at lower concentrations. If so, an $RfC = BMCL_{10} / 100$ could be considered. Since the tumorigenic mode of action has not been fully established, the EPA has chosen to estimate potential cancer risks based on linear extrapolation to zero from the $BMCL_{10}$. For example, the cancer risk is estimated to be less than 10^{-5} at the $BMCL_{10} / 10,000$. However, an intermediate value between the RfC and linear extrapolation could be considered. Since THF is nongenotoxic, but the mode of action is not

firmly established, an additional uncertainty factor of 10 akin to an inadequate database might be employed producing an $RfC = BMCL_{10}/1000$.

Response from Nancy Kerkvliet

No response was provided to this question.

Response from Lisa Peterson

While the available data suggest that increased proliferation and promotion in the liver may provide a probable mode of action for THF-induced female mouse livers, there are insufficient data to exclude other possibilities. I agree with the EPA's conclusions that the data do not provide significant biological support to establish a mode of action.

Response from Karl Rozman

I agree with EPA's conclusion regarding the mode of action statement. The most plausible mechanism of action for very low potency chemicals to cause cancer is chronic irritation, which is not discussed. The mode of action of liver cancer in female mice is irrelevant for humans for lack of sufficiently high exposures and because liver cancer in humans – unlike in B6C3F1 mice – is a very low incidence disease.

QUESTION D4

An inhalation unit risk has been derived utilizing benchmark dose modeling to define the point of departure of 10% extra risk followed by linear low-dose extrapolation below the point of departure (i.e., the default assumption). Please comment on the scientific justification and rationale supporting the estimation of an inhalation unit risk from the available data for THF. Specifically, please comment on whether the rationale for the quantitative analysis is objectively and transparently described, considering the uncertainty in the data and the suggestive nature of the weight of evidence. Please comment on the selection of linear low dose extrapolation. Has the justification of linear low dose extrapolation been objectively and transparently presented? Please identify and provide rationale for any alternative approaches for low dose extrapolation that the data for THF would support and if such approaches are preferred to EPA's approach.

Response from John Christopher

The authors developed an inhalation risk factor using benchmark dose and linear extrapolation, following the default process from EPA's 2005 guidelines for assessing carcinogenic risk: (1) The carcinogenic hazard is correctly identified. (2) The chemical is not genotoxic. (3) No mode of action for the carcinogenic effect could be identified. (4) Benchmark dose modeling was followed with linear extrapolation with data from a large, well conducted chronic study. Although EPA's guidelines were followed to the letter, I believe these guidelines are misapplied in the case of THF.

Linear extrapolation downward from a point of departure and use of a unit risk for estimating carcinogenic effect are both based on the premise that any dose of a putative carcinogen however small is associated with an increased risk of developing cancer. This premise relies, in turn, on the gravity and irreversibility of the lesion. The default of extrapolating downward through zero is a conservative, health-protective one, intended to protect public health against potent toxins. This default procedure of linear extrapolation is intended for use when information is lacking.

But we have quite a bit of information on THF, and I believe that this chemical does not cause irreversible damage with every unit increase in dose. THF has been assayed at very high doses and exposure levels in several species. THF is not a potent toxicant in any organ. RfD and RfC values are estimated to lie near E+0 mg/kg-day and E+0 mg/m³, respectively. The ultimate toxin for THF is apparently the parent molecule. The panel's expert on metabolism recommended that the toxic effects of THF should not be assigned to a reactive intermediate. THF is neither genotoxic nor bioaccumulative. THF does not induce proliferative lesions which are identifiably pre-neoplastic. **The weight of the evidence presented by the authors strongly suggests that all the biological effects identified for THF are those which are commonly thought to exhibit thresholds.**

At the end of the day, the authors must ask themselves if they believe the end result of their carcinogenic risk assessment. When the peer panel did this at the end of our meeting in Washington, we were all struggling with how to reconcile the toxicity with the policy. I do not believe that THF causes irreversible damage necessitating use of a unit risk factor. I recommend

that EPA use benchmark dose modeling and uncertainty factors for estimating the maximum non-carcinogenic dose, *i.e.* the RfC model with mouse liver tumors as the critical effect.

Response from George Corcoran

The allegiance to the use of linear low-dose extrapolation when mode of action is not sufficiently established by the weight of evidence is troubling in the case of THF. It is understood that this approach is current EPA policy. However, the adherence to such policy in this case not only renders useless but is directly counter to a large and robust database showing that THF is not genotoxic. For this reason, it is this reviewers opinion that the use of linear low-dose extrapolation is not only unacceptably conservative, it is not rational, and it provides an effective basis to attack the excellent work done throughout the report.

For the approach taken by the EPA, the rationale for the quantitative analysis is objectively and transparently described, and may be viewed as generally credible considering the uncertainty in the data and the suggestive nature of the weight of evidence. It remains the opinion of this reviewer that the quantitative analysis overestimates cancer risk by many fold based on the factors elaborated here and in earlier sections.

The use of linear low-dose extrapolation is a major weakness of the analysis, and of the final report. The justification for linear low-dose extrapolation has not been adequately presented and defended, other than to indicate that it falls under EPA guidelines or practices for these circumstances.

The rationale for the estimation of an inhalation unit risk is objectively and transparently described, considering the uncertainty in the data and the suggestive nature of the weight of evidence. Linear dose extrapolation is supported by many experts but is challenged by others, including this reviewer.

Response from David William Gaylor

Pages 113-119, Section 5.3.

Page 114, Table 5-5. Female B6C3F1 mice, Hepatocellular adenomas, Logistic regression, decimal point missing in p-value for 200 ppm.

Page 116-117, Section 5.3.3. The $BMCL_{10}$ was based on crude incidence rates of hepatocellular adenoma and carcinoma in female mice. Adjusted incidence rates, based on the Kaplan-Meier estimated tumor incidence at the end of the study adjusted for intercurrent mortality, are presented in Table 5-5. An adjusted tumor rate of 46.3% is given for controls. That is, the adjusted rate is the number of control animals with tumors divided by the effective number of control animals, n_{adj} , with an adjustment for intercurrent mortality in the controls. That is, the adjusted rate of $0.463 = 17/n_{adj}$. Hence, for the controls the effective number of animals is $n_{adj} = 17/0.463 = 36.7$. Similarly, the adjusted numbers of animals are 39.2, 37.6, and 44.1 for the 200, 600, and 1800 ppm groups, respectively. **These are the sample sizes that should be used in model fitting and the calculation of the BMC_{10} and $BMCL_{10}$.** Using these adjusted sample

sizes in BMDS Version 1.4.1 gave the following results for hepatocellular adenoma or carcinoma in female mice. Experimental concentrations were converted to human equivalent concentrations (HECs) expressed as mg/m³. Hence, BMC₁₀ and BMCL₁₀ are expressed as HEC mg/m³.

<u>Model</u>	<u>AIC</u> ^a	<u>P-value</u> ^b	<u>BMC</u> ₁₀	<u>BMCL</u> ₁₀
Multistage	178.8	0.48	61.2	35.5
Probit	177.0	0.73	79.3	60.8
Logistic	176.9	0.74	72.9	53.9

^a Akaike Information Criterion

^b Goodness-of-fit test. Larger P-value indicates better statistical fit of model to data.

Several choices arise for selecting the appropriate model(s) for the BMCL₁₀ to use for the estimation of the inhalation unit risk.

(1) Since the multistage model, perhaps, has the best biological basis and has been widely used for cancer risk assessments, use only the multistage model. Also, from the precautionary standpoint, here, the multistage model produces the highest inhalation unit risk.

(2) Use the best fitting model. In this case the Logistic model.

(3) Since the Logistic and Probit models produced almost equally good fits to the data, average the BMCLs from these two models.

(4) Use a model averaging procedure that weights the BMCL₁₀ based on a function of the AICs. See e.g., Kang, S-H, Kodell, RL, and Chen, JJ. Incorporating model uncertainties along with data uncertainties in microbial risk assessment.

Regulatory Toxicology Pharmacology 32: 68-72 (2000).

The BMCL₁₀ and inhalation unit risk = 0.1/BMCL₁₀ for these four options are listed below:

<u>Option</u>	<u>BMCL</u> ₁₀	<u>Inhalation unit risk</u>
(1)	35.5 mg/m ³	0.0028 per mg/m ³
(2)	53.9 mg/m ³	0.0019 per mg/m ³
(3)	57.4 mg/m ³	0.0017 per mg/m ³
(4)	53.7 mg/m ³	0.0019 per mg/m ³

Options (2)-(4), based on statistical goodness of fit for the models, give similar results for the inhalation unit risk. From a precautionary perspective, the multistage model provides the highest estimate for the inhalation unit risk of 0.0028 per mg/m³.

Response from Nancy Kerkvliet

No response was provided to this question.

Response from Lisa Peterson

The discussion of the decisions leading to the derivation of the inhalation unit risk is clearly presented with appropriate scientific justification. I agree with the decision to select a linear low dose extrapolation since there is no data to support other choices. This is the EPA's default in the absence of data indicating non-linearity. It is possible that this approach is over estimating cancer risk for this chemical as it appears to be a weak carcinogen.

Response from Karl Rozman

This is entirely unjustified. The threshold approach using UFs to provide a margin of safety like the $R_f D$ for noncancer end points does achieve perfect protection. The rationale for this opinion is that in instances when cancer is not the most sensitive endpoint of toxicity such as is the case with many very low potency carcinogens, lowering exposure to below levels causing noncancer toxicity will provide protection also from cancer risk.

QUESTION D5

THF induces a spectrum of effects consistent with both Category 1 and Category 3 gases. Therefore, for the purposes of calculating human equivalent concentrations, respiratory tract effect levels were calculated using the default equations for Category 1 gases and extrarespiratory tract effect levels were calculated using default equations for Category 3 gases. Please comment on the explanation for the dosimetry choice in the derivation of the inhalation unit risk. Has the rationale been scientifically justified and transparently described?

Response from John Christopher

I agree with the authors' designation of THF as a Category 1 gas when calculating human equivalent concentrations for systemic toxic effects. Category 1 gases are those which reach the deep lung for gaseous exchange with blood.

Response from George Corcoran

The explanation for the dosimetry choice in the derivation of the inhalation unit risk has been scientifically justified and transparently described. Again, this reviewer does not subscribe to the rationale or methodology of using linear low-dose extrapolation for THF based on the previously stated considerations.

Response from David William Gaylor

No response was provided to this question.

Response from Nancy Kerkvliet

No response was provided to this question.

Response from Lisa Peterson

The rationale for the decision to use the default equations for Category 1 gases for the respiratory tract effects and those for Category 3 gases for the extra-respiratory effects is logical and clearly presented. The available experimental evidence supports this decision.

Response from Karl Rozman

No comment.

ADDITIONAL COMMENTS

David Gaylor

Conclusions, Section 6.1

Page 121, lines 7-8. Change to read: The RfD of 0.6 mg/kg-day is based on a BMDL_{1SD} of 601 mg/kg-day for decreased pup body weight gain (BASF, 1996).

Page 124, line 4 from end. Replace 52 with 35.5.

Page 124, last line. Replace $0.0019 \text{ (mg/m}^3\text{)}^{-1}$ with 0.0028 per mg/m³.