

**Peer Review Workshop  
for EPA's Draft Toxicological Review of  
Ethyl Tertiary Butyl Ether (ETBE)  
Human Health Assessment**

**Reviewer Post-Meeting Comments**

**February 4, 2010**



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## **IRIS Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE) updated comments**

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### **CHARGE QUESTIONS**

#### ***General Charge Questions:***

*G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?*

In general, the review is logically structured. However, it would benefit from condensation since some summaries and discussion points are repeated. The scientific evidence is acceptably synthesized in some parts of the document, but will require revision in other areas. The major concerns are the PODs and extrapolation factors used in risk assessment, and the cancer hazard assessment. Based on the issues identified in the document regarding study design and study evaluation, and problems identified in other studies performed at the Ramazzini Institute (Maltoni et al., 1999 – Eur. J. Oncol) by a variety of organizations, the carcinogenicity data are not reliable and should not be used for hazard assessment of ETBE. In addition, a weight-of-evidence evaluation well supports a role of  $\alpha_2$ -globulin nephropathy in the renal effects in male rats.

*G2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of ETBE.*

Apparently, the Japanese government and industry have sponsored a number of additional toxicity studies on ETBE. It will be necessary to include the results of these studies to increase the size of the limited database. Depending on the study evaluation, the document may require revision/updating regarding PODS for both inhalation and oral exposures.

Additional studies and datasets to be included/considered:

- a detailed study on the toxicokinetics of ETBE in rats (as cited in McGregor, 2007)
- PBPK models developed for MTBE may be applied to species extrapolation integrating ETBE-specific data
- updated pathology report on lesions in the 90-day inhalation study with ETBE

- de Peyster et al (Tox Letters 2009)

***Chemical-Specific Charge Questions:***

***(A) Oral reference dose (RfD) for ETBE***

*Upon evaluation of the oral database, EPA determined that it was not possible to derive an oral RfD as the proposed composite uncertainty factor (UF) of 10,000 would lead to a value with an unacceptable level of uncertainty (see A Review of the Reference Dose and Reference Concentration Processes, U.S. EPA, 2002 for discussion of UFs). In lieu of deriving an RfD, the available data were used to derive an oral value (i.e., a minimal data value) for limited risk assessment purposes as discussed in Appendix C.*

*A1. The CIT (2004b) two-generation study of reproduction and fertility effects of oral exposure to ETBE was selected as the basis for the derivation of the minimal data value. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.*

Despite some limitations in study design and evaluation, the two-generation reproduction study may be appropriate to derive a point of departure. However, as several additional studies from Japan have been made accessible or are presently performed, the results from these studies may be more suited as principal studies.

*A2. Increased kidney weight in F0 generation male rats (CIT, 2004b) was selected as the critical effect for the minimal data value resulting from oral exposure to ETBE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.*

The selection of this endpoint is not well justified. A “weight of evidence” approach supports an important role of  $\alpha_2$ -globulin nephropathy in the kidney effects of ETBE in male rats. Since  $\alpha_2$ -Globulin nephropathy is not considered relevant for human risk assessment, the male rat data should therefore not be used as critical effect. The small increase in relative kidney weight in female rats is of questionable adversity; however, this endpoint or the increased liver weights may be used as critical effects. The studies performed in Japan should be evaluated since they do not indicate kidney effects in female rats.

*A3. Benchmark dose (BMD) modeling methods were applied to kidney weight data to derive the point of departure (POD) for the minimal data value. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., one standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA’s approach.*

This is not in my direct area of expertise. Apparently, the modeling has been performed using appropriate methodology.

A4. A total composite UF of 10,000 was used to derive a minimal data value for ETBE. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the minimal data value. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

The very high uncertainty factor is justified by absence of a complete database and the many extrapolations required due to absence of appropriate studies. However, the factor to extrapolate from subchronic (90 day) to chronic studies will already cover a less than life time exposure. The additional factor of 10 to account for database deficiencies is not appropriate since it is already included in the subchronic to chronic extrapolation and compensates database deficiencies (absence of 2-year oral study). The POD is based on results of a 2-generation study and small effects on organ weights suggesting that a total UF of 1,000 is sufficiently conservative. The data from the studies performed in Japan should be included since most relevant toxicity endpoints are addressed and, depending on study results, lower UFs may be justified when these studies have been integrated.

**(B) Inhalation reference concentration (RfC) for ETBE**

B1. The Medinsky et al. (1999) 13-week inhalation exposure study in mice and rats was selected as the basis for derivation of the RfC for ETBE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

This inhalation study is a good basis for selection of the RfC. The study was performed according to toxicity testing guidelines and reports sufficient detail. However, the updated pathology evaluation should be included in the assessment.

B2. The occurrence of regenerative foci in the kidneys of male rats (Medinsky et al., 1999) was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Again, as a “weight of evidence evaluation” well support<sup>□</sup> <sub>20</sub>-globulin nephropathy as mechanism for the renal effects in male rats, these effects should not be used as critical for RfC derivation. Absolute liver weights, despite their questionable adversity, may be more suited since they are observed in both genders and in 28 and 90-day inhalation studies. Again, the results from the studies performed in Japan should be integrated.

*B3. An analysis of the mode of action of kidney effects is presented in the Toxicological Review and a determination is made that the mode of action in male and female rats is unknown. Please comment on whether the analysis is scientifically justified.*

There are number of observations suggesting that the renal effects of ETBE in males are mediated by  $\square_{bu}$ -globulin nephropathy. Using a weight of evidence approach, the available information well supports a role of  $\square_{bu}$ -globulin accumulation in the renal effects of ETBE in male rats. The small changes in relative kidney weights in female rats observed in one study do not cast doubts on  $\square_{2u}$ -globulin as the mechanism potentially operating in male rats. Effects on the kidney of female rats have not been reported in the studies from Japan. The effects in one study may be due to a different mode-of-action on the kidney of female rats (unlikely due to identical biotransformation and kinetics), may be incidental or adaptive.

*B4. BMD modeling was applied to data for the mean number of regenerative foci in the kidneys to derive the POD for the RfC. Has the BMD modeling been appropriately conducted? Has the BMR selected for use in deriving the POD (i.e., one standard deviation from the control mean) been scientifically justified? Please identify and provide the rationale for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.*

Apparently, this is ok.

*B5. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).*

A total UF of 3,000 is used. Again, the additional factor of 10 integrated to account for database deficiencies may be questioned (see above) and the additional studies from Japan should be integrated when justifying UFs.

### ***(C) Carcinogenicity of ETBE***

*C1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), the Agency concluded that there is suggestive evidence of carcinogenic potential following oral exposure to ETBE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?*

In my opinion, no conclusion can be made based on the carcinogenicity study due to the peculiar study design, very limited reporting, issues regarding background incidences of the tumors in controls, and possible infections in the animal house. In evaluating the study and its reliability for risk assessment, EPA should also consider the testing results with other chemicals obtained by the Ramazzini Institute. Evaluation of these studies by other organizations also identified many critical issues. For example, see comments by EFSA on the Ramazzini study on aspartame and comments by McGregor et al., 2007 – CritRevTox on the hematological tumors induced by MTBE. Considering these comments and the issues identified by the present review of the ETBE-carcinogenicity study, this study is not reliable to come to conclusions regarding cancerogenicity of ETBE. The carcinogenicity data on the ETBE-metabolite t-butanol can be used to predict potential risks due to ETBE when using PBPK-modelling. However, it needs to be recognized that such extrapolations are highly uncertain due to different study design.

Moreover, the mode-of-action of t-butanol likely is unlikely based on genotoxicity. While acetaldehyde is a metabolite of ETBE, it is also an endogenous intermediate metabolite and is rapidly metabolized. I would question any assessment of ETBE by using acetaldehyde formation rates from ETBE and PBPK-modelling. An extrapolation of the acetaldehyde carcinogenicity data obtained after inhalation to oral dosing is not appropriate due to the high irritancy of acetaldehyde to nasal tissue and the differences in breathing patterns between rodents and humans. Again, the results of the studies from Japan may better support the conclusions regarding absence of carcinogenicity.

*C2. EPA did not derive a quantitative estimate of the carcinogenic potential of ETBE. Do the data support an estimation of a cancer slope factor for ETBE? If a quantitative estimate is proposed, please identify and provide a detailed description of the method(s) and approach(es) for deriving a cancer slope factor.*

Cancer slope factor cannot be derived from the incidences given since there is no dose dependence and the study is not considered reliable.

## **FURTHER COMMENTS**

Page 8, table 3.1: The “percent of dose excreted” column should give the route of excretion.

Page 8, end of first para: The doses received in the Amberg study were not adjusted to body weight and the conclusion of a 500 times higher received doses in rats seems odd.

Page 11: While the metabolic scheme is correct, page 10 states that the hydroxy-group is introduced into the methyl moiety of ETBE which is inconsistent with the figure.

Page 15: TBA unlikely represents an endogenous metabolite

Page 15: What is the consequence of the 37 times higher metabolic capacity of rat olfactory mucosa for ETBE ? Apparently, there are no nasal lesions in the inhalation studies.

Pages 52/53: Units are mM and not nM

Pages 56/57: Studies with mixtures of ETBE and gasoline should not be used in support of conclusions on ETBE because the effects seen in such studies may be due to other components or the mixture itself.

Page 59: MTBE is not a carcinogen as concluded by several organizations (IARC, EU)

Page 71: Considerations of the TBA-inhalation study, more detail should be given.

Page 78: Carcinogenic potential of acetaldehyde. The acetaldehyde data should not be used to support conclusions regarding ETBE since irritation has been identified as a primary mechanism for the nasal tumors observed after inhalation of acetaldehyde.

Page 78: the MTBE study of Ramazzini is also not reliable and the information on MTBE should therefore not be used to support conclusions that there is “suggestive evidence”.

Page 82: The general conclusion not using gene polymorphisms to define potential individual sensitivities in humans toward ETBE is adequate. However, it should be recognized that differences in enzyme activity between individuals does not necessarily permit conclusions on the extent of biotransformation of an agent. Extent of biotransformation depends on kinetic parameters of the enzymatic reaction and the concentration of the agent at the active site of the enzyme at a given exposure.

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**Comments on  
IRIS Toxicological Review of ETBE  
Gary Ginsberg  
Oct 26, 2009; Amended Feb 1, 2010  
ERG Contract No. EP-C-07-024  
Task Order No. 47**

G1. Is the Tox Review logical, clear, concise?

This document is generally well written and easy to follow. However, the rationale for including ETBE on IRIS is not well stated as the Introduction (page 1-2) is boilerplate and the Chemical and Physical information section (p 3-5) has some use and contamination data but is inadequate to document the necessity and utility of an ETBE IRIS profile. Page 3 points out in several places that ETBE use has been stopped and when it was used it was only at a low rate in the US. Page 4 speculates that ETBE “could be produced in very large amounts depending .....”. The LUST site data on p 5 gets further to the point (8.9% of 868 sites with ETBE) but should provide the range of concentrations found, how many of these locations also had MTBE and would MTBE drive the cleanup at these sites? Should more effort be made to update the MTBE IRIS file as that is quite dated and MTBE is much more prevalent in groundwater? More information on ETBE presence and stability in groundwater and likelihood for human exposure would be helpful.

The draft document is not concise in that the same toxicology data are presented 3 or 4 times in only slightly different formats or applications. Substantial shortening is possible by reducing redundancy and certain aspects of the data to make the intended point rather than rehashing the study results numerous times.

As described below, the reproductive and developmental toxicity section is not clearly presented and can be benefited by reorganization and some additional description. In a number of places, a study of very limited utility is presented at the beginning of a section for no good reason while the primary studies are presented thereafter. It would be clearer to start with the best studies and present the weaker studies as either supportive or supplemental information.

Figure 5-1 was not completely legible and so can't really tell which endpoint is represented on the X axis.

G.2. It is apparent that the new Japanese studies presenting oral ETBE animal data are critical for consideration in RfD development and may have implications for MOA. Further, However, the MTBE

database is highly relevant and could have been used more effectively in this document as described below.

A.1. The CIT 2004 rat reproduction study is appropriate as the principal study for RfD derivation.

A.2. Increased kidney weight in the F0 generation is appropriate as the critical effect as it is related to ETBE exposure and is the most sensitive in terms of dose response. It is not clear that this response is alpha-2-mu mediated and so must be considered relevant for human risk assessment. The fact that changes in female kidney weight were also seen is suggestive that ETBE can exert toxicity to the rat kidney outside of the alpha-2-mu mechanism.

A.3. The BMD modeling was appropriate and the BMR selection was reasonable.

A.4. The composite of 10000 could potentially be reduced and an RfD made feasible by using two approaches to decrease uncertainties:

1) More thoughtful evaluation of rat to human pharmacokinetic (PK) differences with possible construction of oral PBPK models in rats and humans based upon the fairly extensive ETBE PK database with assistance from MTBE PK data and models. There is a great deal of ETBE and MTBE PK data with a human ETBE inhalation model already published. MTBE PBPK models for inhalation and I believe also oral dose routes have been published. It may be possible to remove the 3 fold cross-species PK UF via the construction and application of an ETBE oral dosimetry model. Short of this, a qualitative or semi-quantitative approach may be possible to judge the need for this 3 fold factor. The current document does little with the extensive PK information available. See below for more details.

2) Database uncertainties should be reconsidered: there is a 10 fold UF for subchronic to chronic and 10 fold for database deficiencies. A clearer (perhaps tabular) presentation of data available and data gaps would be helpful. One could argue that a 10 fold database deficiency factor to account for the lack of complete histopathology and target organ analysis in the repro/developmental studies could be reassigned as a 3 fold UF. This decision could be informed by considering the extensive database available for MTBE to determine how various endpoints relate (subchronic to chronic, repro/develop to chronic) and to help fill data gaps. While MTBE is not exactly the same as ETBE, there are enough similarities that the MTBE database can be helpful in this uncertainty analysis. The MTBE database is underutilized in this document.

B.1. I agree with Medinsky et al. 1999 as the principal study.

B.2. I agree with regenerative foci in the kidneys of male rats as the critical effect for RfC derivation. This effect represents a toxic response of the kidney to cellular damage involving hyperplasia and represents good quantitative data for dose response modeling.

B.3. The relevance of male rat kidney effects vis-à-vis the potential for alpha-2mu-globulin MOA is reasonably handled in this document. Male rat kidney endpoints should be considered for RfD and RfC derivation given that the alpha-2-mu MOA has not been proven for ETBE and in fact some renal toxicity occurs in female rats. This mechanism for MTBE has also not been proven in spite of extensive research and the metabolite TBA also causes renal changes in female rats. Therefore, it is unlikely that alpha-2-mu can explain all of the male renal effects and to the extent that it can account for some of the effects, a useful way to assess this is that alpha-2-mu related toxicity occurs in control male rats. Thus it represents a background condition in this population that ETBE can enhance. There are likely to be numerous background processes leading to renal disease in humans. Even though these do not involve alpha-2-mu, the fact that ETBE can accelerate a background disease or aging process in the male rat kidney is a cause of concern for its possible effects on human renal disease processes. Therefore, until ETBE's mechanism can be shown to be centrally based upon a direct interaction of ETBE with this protein leading to its impairment of lysosomal degradation, ETBE-induced male rat renal pathology should be considered relevant to human health.

My criticism of the document along these lines is that it leaves the issue hanging into dose-response assessment when it should be fully vetted and clearly decided in the synthesis section so that it doesn't keep coming up as an uncertainty and there isn't this inconsistency between the RfD and RfC treatment of the issue (see below for specific comments on this).

B.4. BMD and BMR look appropriate for these datasets.

B.5. The UFs selected for RfC derivation are questionable on the basis that there is a UF of 10x for subchronic to chronic extrapolation and also for database deficiencies, most notably the lack of reproductive and developmental studies by the inhalation route. However, there are oral studies by this dose route and there are a variety of reproductive and developmental (and chronic) studies for MTBE. Greater use of the MTBE database may be possible with a careful comparative analysis between the two structural analogues given that MTBE is something of a prototype for the series of oxygenate structures.

As mentioned above and detailed below, the PK database for ETBE and MTBE is underutilized and more should be done with this. The default RfC methodology approach should be superseded by at least a semi-quantitative if not full PBPK model description for ETBE similar to what has been done for MTBE. In particular, one is concerned by statements in the document that rats have lower blood concentrations of ETBE than humans when similarly exposed in a chamber atmosphere (Amberg et al. 2000) and yet the RfC methodology basically assumes very similar kinetics across species (just minor partition coefficient changes that do not explain the Amberg data). This raises the possibility that the RfC methodology is inadequate for extrapolating internal dose and risk and that a more formal and comprehensive PK analysis is needed. This would appear to be feasible for ETBE, especially when bringing in the extensive MTBE modeling experience. See below for more details.

- 1) C1. The cancer weight of evidence of “suggestive” is appropriate although I would have arrived at that decision much differently. The draft document underappreciates the fact that MTBE is a well tested oxygenate that can to some degree serve as a surrogate for ETBE. In fact the draft is dismissive of the MTBE database because the Maltoni study did not find the same cancer targets for ETBE as found for MTBE. However, as stated elsewhere in the document, there are many limitations and irregularities with the Maltoni study (excessive mortality, incomplete reporting of toxicology or animal-specific data, unusual dosing vehicle – olive oil, unusual dosing regimen – 4 days per week; lack of effects on kidney or liver, classical targets of ETBE and MTBE; questionable interpretation of results – e.g., “dysplasias of oncological interest”). Thus it shouldn’t be used as evidence to rule out a cancer target site for ETBE. I hesitate to rely on Maltoni et al. as stand alone evidence and would deemphasize this single study, even though it is the only oncogenicity study for ETBE. The best approach is to describe the structural, metabolism, PK, genotox, and tox profile similarities between ETBE and MTBE and then consider whether to use MTBE as a surrogate for ETBE – MTBE has been thoroughly tested and can offer good perspective.
- 2) Rather than so much weight placed upon the difficult to interpret Maltoni study, the better approach is to key off the tumor findings for TBA and MTBE as they have some degree of site concordance and their respective results are logically connected from a comparative dose-response perspective. TBA is a key metabolite from both MTBE and ETBE and in fact will achieve greater AUC than either parent compound. Therefore, at least some of the toxic and carcinogenic effects of ETBE are likely mediated by TBA or its metabolites.

The discussion on Page 78 is wrongheaded as it places primacy on the Maltoni results with ETBE and views the results with MTBE or TBA as not particularly supportive because of differing target sites for cancer. This should be flipped around so that the MTBE and TBA tumor sites and dose response are highlighted as indicative of what ETBE might cause if similarly tested and then use the Maltoni evidence as generally supportive of an oncogenic potential.

Acetaldehyde carcinogenicity, is dismissed on page 78 as only being carcinogenic above doses that cause irritancy. This ignores the fact that acetaldehyde is a well known mutagen and so it likely has a genotoxic mode of action at sites where it reaches or is generated. Further, ethanol's carcinogenic potential at g.i. tract sites is believed to be due to in situ generation of acetaldehyde. Therefore, the in situ generation of this genotoxic metabolite from ETBE should not so easily be dismissed. Further, the cancer mode of action section is very brief and could say a lot more about MTBE, TBA and acetaldehyde MOAs, the lack of genotoxicity of ETBE and the likelihood for a variety of non-genotoxic mechanisms to be at play for ETBE but with no empirical evidence to distinguish between them.

C.2. I agree that a quantitative estimate of ETBE carcinogenicity is not feasible. However, it is unclear from this document how the equivocal carcinogenic evidence for ETBE should be considered for risk assessment. In some cases, an additional risk assessment/risk management decision is to view this as another area of uncertainty for which an additional uncertainty factor may be appropriate. Alternatively, one can consider ETBE to be a "Group C" carcinogen and look at the database of Group C carcinogens with slope factors to determine whether the proposed RfC and oral reference value would be close to de minimis risk if ETBE were as potent as the average Group C carcinogen. When there is no attempt at quantitation, there is a hidden default of zero potency, with ETBE carcinogenicity discounted in the risk assessment. The NAS Science and Decisions report (2008) recommends the use of screening toxicity values to avoid the default assumption of zero potency for chemicals with suggestive but suboptimal datasets. One possible screening approach is to derive a slope factor for TBA and then apply that to ETBE with PBPK adjustment for percent conversion of parent compound to TBA.

### **Specific Comments**

Toxicokinetic studies – the Nihlen and Amberg studies are critical to understanding how dosimetry may compare across species, especially given the lack of a PBPK model. From these data, the following information should be reported for both rats and humans: cumulative exposure dose per kg body weight

(calculated doses were reported in umoles but these need to be normalized to body weight to compare across species), peak concentration, time to peak, AUC, total body clearance and  $t_{1/2}$ . Similar parameters for TBA should be presented. A table showing these data for each exposure concentration is needed to determine how the 2 human studies line up and to evaluate cross-species differences in ETBE kinetics after inhalation exposure. Such a table could also be inspected for any evidence of saturation of clearance processes. Instead the draft document presents only some of this information and it occurs in scattered sections making it difficult to compare across species or across human studies. EPA should make at least a tentative determination as to the degree of similarity in ETBE/TBA kinetics across species. If the data are insufficient for such an assessment, the deficiencies in the data should be clearly stated. The potential importance of the finding that blood levels of ETBE were lower in rats than humans in the Amberg study should be explored in terms of the relative inhalation doses received by both species (rats receiving approx 4 times more per body weight based upon Table 3-1 and approx body wts).

MTBE toxicokinetics have been extensively studied in rats and humans and as I recall there were no major across species differences. These are rapidly cleared compounds with very similar metabolic profiles. Drawing more heavily upon MTBE toxicokinetics would likely be wise use of the available data. Drawing on the Dekant data for MTBE provides little additional perspective on ETBE kinetics as Table 3-1 is focused on percent dose excreted and not on more imp kinetic parameters.

Page 7: is the rat retention factor in the lungs of 0.3 extrapolated from the human data or are there empirical data to support the rat value. What are the uncertainties if this is an extrapolation?

Page 7: The Dekant aqueous dosing of MTBE and TAME – was that gavage? What is the dose in units of kg body wt?

Page 8: Table 3-1 – dose received – should be expressed per body wt – it looks like humans got a much bigger dose than rats which of course is not true. Percent of dose excreted – over what time frame. Partition coefficient terminology is backward – should be blood:air – terminology is correct on page 9. Use of Kow is confused and possibly inappropriate – Kow is a primarily a predictor of Vd (a different section of the report), which in turn can affect rate of excretion. This language needs to be clarified.

Page 10: partition coefficient terminology is again backward – it should be tissue:blood in the table.

The distribution section (p 9-10) has redundant information not germane to distribution – e.g., the Nihlen

description which is primarily about chemical uptake in the lungs. The most pertinent information is the Vd estimate for ETBE and MTBE – the method in which these values were derived should be included. Does the greater Vd for ETBE make a kinetic difference – does it delay clearance? The data are probably available to assess this but aren't presented. The data in table 3-1 show little excretion difference but this is not an expression of the kinetics (rate) of excretion, just some static result at an unreported time point.

Page 10 – Metabolism: why does it say “methyl moiety of the molecule” – there is no methyl in ETBE.

Page 12 – when inspecting dose-metabolite profiles, one is looking to see if there is a shift indicative of metabolic saturation – what is the significance of the metabolite ratio apparent shift on this page? Are these dose differences statistically significant?

Page 13-14 – there is a smattering of Michaelis-Menten parameter data, and that is primarily for MTBE in human microsomes. More importantly what is it for ETBE in contrast to MTBE and for ETBE in the isolated (expressed) isozymes that metabolize ETBE in humans (mostly 2A6) and rodents (??? Isozymes). The Vm and Km inform which isozyme is most active towards ETBE and which are active at lower concentration. It may be that 2A6 is most active at high but not low concentration. When Vm and Km values are finally given they are for enzymes induced by ETBE with the substrate not defined for the M-M kinetic test (pg 16).

Page 18 – the Sun and Beskitt reports have a disclaimer that they are not peer reviewed so are of limited quality and so are provided as additional information only. Yet 4 pages with substantial detail are dedicated to these data; given this presentation, more should be done with these data. For example, the inhaled concentration range is much higher than in the Amberg study which affords more opportunity to demonstrate the presence of kinetic saturation phenomena. For example Tables 3-3 and 3-4 demonstrate non-linearities in the disposition of radiolabeled ETBE that could be used to predict potential non-linearities in the dose-response in rats.

Overall, the PK section could have been more thorough and it needs to have better organization in pulling together key PK data from across species, in describing the implications of these data for extrapolation of potency across species, and in comparison to MTBE. Right now this section does not provide an overall picture of ETBE PK. The fact that a PBPK model exists for ETBE in humans but not rats (page 24) is not reason to ignore the cross-species comparisons possible when considering the data that do exist for ETBE and the extensive cross-species work done on MTBE.

Cancer Bioassay of Maltoni et al. (1999) – originally described on Page 27-31 and in various subsequent sections:

- 3) there is no mention of renal or hepatic tumor incidence – it would be very good to show these data given that these are the main target organs for subchronic ETBE toxicity and also these are cancer targets for MTBE.

Table 4-2, Page 34 – need to indicate whether the elevated organ weights were absolute or relative weights.

Reproductive and Developmental Section (p 40 and onward)

This section is not cohesive or easy to follow. It starts with an unorthodox drinking water study of limited utility (e.g., what dose was given to the animals in mg-kg-d; how do these endpoints relate to other more traditional endpoints). Then at the top of p 42 there is a vague description of a study apparently of hybrid design (a one gen repro with some developmental aspects?), followed on p 43 by a description of a rat developmental study and then bottom of p 43 back to a 2 gen repro study which takes up the rest of this section. It would be easier to follow if there was an introductory paragraph stating the 3 or 4 study types available for ETBE in this area and then segregating them out with subheadings starting with the main study for risk assessment (the classical 2 gen rat repro), followed by the one gen, then the one developmental study and then the unorthodox study. Finally, a summary paragraph is needed stating the data gaps (e.g., no rabbit developmental study) and limitations in the available data.

One further point regarding repro/tox is that Page 37, Table 4-3 raises the issue of possible spermatotoxic effect of ETBE based upon the dose response from inhalation exposure. However, subsequent studies (e.g., page 44- middle para) do not seem to bear this out. The document needs to address this conflicting data at some point and make a determination as to whether ETBE is likely to affect male reproductive capacity. That would be good to bring up as a point of possible disagreement on page 44 and then have this issue addressed in a separate subsection in the MOA and synthesis presentation on some later page (e.g., in the 60s or 70s).

Page 50 – Inhalation studies – the first paragraph reports on a 1950 study of very little utility. This paragraph should be shortened and this study deemphasized with limitations mentioned. The 2<sup>nd</sup> paragraph describes a somewhat more useful study and so should appear first.

Page 52 – Neurological Studies – the first paragraph goes into detail on an in vitro study of little direct utility in setting the RfD. The mechanistic relevance of the endpoints examined is also not well described. This study should be deemphasized and brought in at the end of the section.

Page 56 – ETBE-Gasoline Mixtures – nearly 3 pages are dedicated to describing this mixture study which may be useful for answering certain questions about oxyfuel but has little relevance to setting an RfD for ETBE. This study description should be greatly curtailed and put into proper context.

Page 59 - SAR – computer generated SAR is only one approach. This section fails to describe the relationship between structure, lipid solubility and potency. It fails to evaluate whether the more heavily studied MTBE is a good prototype for ETBE. That argument can be developed on a number of fronts including chemical structure, properties, metabolic fate and toxic effects. This issue has direct relevance to the ETBE risk assessment since there are major datagaps for ETBE which might be filled by the better studied MTBE.

ETBE renal effects and possibility for alpha-2-mu mechanism:

In several places the statement is made that females were negative for alpha-2-mu immunoreactivity in the kidneys (e.g., page 72). This statement shows lack of understanding of this mechanism – alpha-2-mu is a male rat protein so female kidney should not exhibit it unless there was a change in gene expression, which is not the classical response to kidney toxicants and thus unlikely. So, its an obvious statement that can be omitted. The more important question is what does the evidence of female renal effects mean – kidney weight and labeling index are increased without any histopathology evidence of effect. From my vantage point it signifies an ETBE effect on the kidneys that is independent of alpha-2-mu but its possible that the latter mechanism may contribute to the kidney damage in male rats, which is more severe. However, as with MTBE, there is insufficient evidence to determine to what extent ETBE effects on male rat kidney are due to alpha-mu-2-globulin and so all of that dose response should be considered relevant for human risk assessment.

### **Synthesis of Cancer Evidence:**

Page 79 – Susceptible Populations – this section has some useful material on metabolism enzyme ontogeny but misses the mark when describing early life risk issues. It states in several places that there are insufficient data to determine ETBE teratogenicity or developmental toxicity. Yet there is a standard rat developmental toxicity study and a hybrid one gen study which both indicate a lack of fetotoxicity or

teratogenicity. What's missing is a rabbit developmental study. However, MTBE is also not particularly toxic in this regard (EPA should check on what MTBE developmental studies are available). However, to blandly state that the data are insufficient for is to dismiss evidence this document mentions earlier and other evidence (for MTBE) which could be brought in. In my estimation, ETBE is unlikely to be a developmental toxicant.

The enzyme ontogeny discussion lacks perspective as there is no MOA discussion regarding what slower metabolism in early life might mean. It could be protective (less metabolism to TBA and acetaldehyde) or it could be potentiating (slower removal of parent compound which may be neurotoxic in its own right; slower removal of acetaldehyde or TBA).

This section should comment on two major endpoints of toxicological concern for early life – neurotoxicity and cancer. ETBE is neurotoxic at high doses. This section should state whether developmental neurotoxicity studies have been done for ETBE (answer is no) and whether this is a significant datagap. The lack of genotoxicity implies that the supplemental guidance for children's cancer risk would not apply to ETBE.

Overall, this section has very limited utility.

Dose-Response Assessment – page 85 forward

When entering this section, the document's position on the relevance of ETBE-induced renal pathology should be clearly stated in a previous (synthesis) section. That way it is clear whether the male rat endpoints are to be considered and why. The oral dose response (pgs 87-88) uses the male rat kidney data without any concern over possible alpha-mu-2 involvement. However, when the inhalation dose response is presented, then the issue is reopened (page 95). The document should not go back and forth on this issue but resolve it before the dose-response assessment.

RfD Derivation – Page 93 – this should be presented fully in the body of the text rather than in the appendix, with the 10000 fold UF clearly justified and the oral minimal value presented. Further, the uncertainties involved with this value should be presented in the text rather than appendix.

RfC development- I generally agree with what is presented. However, it should be more transparent. The dose response for the key endpoint (regenerative foci in male rat kidney tubules) is not described, and

instead the dose response for male rat kidney changes is and to some extent so is the change in LI. This is misleading and incomplete. The key dose response data should be highlighted and then followed through in terms of dose conversions to HEC and PK adjustments and BMD analysis to document how the RfC was derived. For example, the LOAEL and NOAEL for regenerative foci that feeds into Table 5-2 is not shown and one has to hunt through the Appendix to find how the animal dose response converts to a POD of 17 mg/m<sup>3</sup>. This should all be part of a cohesive RfC derivation which concludes on Page 103 with a final RfC calculation.

The proposed RfC of 6 ug/m<sup>3</sup> should be put into context with MTBE – its RfC is much higher (3000 ug/m<sup>3</sup>) – why is that? Does this differential make sense?

Uncertainties Section (pg 105 and beyond):

The PK uncertainty and extrapolation across species could have been informed by the experience with MTBE. Further, ETBE blood levels in rats was lower than in human subjects when exposed to the same concentration (Amberg et al. 2000). This section should describe how these data affect the way we think about cross-species extrapolation (e.g., humans have higher levels of parent compound so may have greater uptake or slower clearance and thus potential for greater toxicity than in rats?). Does the MTBE PK data support this cross-species difference in internal dose? Does the RfC methodology adequately address this potential PK issue (probably not if it is real) – is a 3 fold animal-to-human extrapolation sufficient?

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**Lawrence H. Lash, Ph.D. (Chair)**

Professor

Department of Pharmacology

Wayne State University

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Dr. Lash is Professor of Pharmacology at Wayne State University School of Medicine in Detroit, Michigan, where he has been a faculty member since 1988. Prior to that, Dr. Lash earned his B.A. in Biology at Case Western Reserve University in Cleveland, Ohio in 1980, his Ph.D. in Biochemistry at Emory University School of Medicine in 1985, and did a postdoctoral fellowship in Pharmacology and Toxicology at the University of Rochester School of Medicine from 1985 to 1988 with Professor M.W. Anders. At Rochester, he studied the enzymology of cysteine conjugate  $\beta$ -lyase in the bioactivation of the cysteine conjugate of trichloroethylene, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), and studied mechanisms of renal cellular injury induced by DCVC. Since coming to Wayne State, Dr. Lash's research program has focused on assessment of factors that determine or regulate renal susceptibility to chemically induced injury, including membrane transport, Phase I and Phase II metabolism, gender and species differences, signaling pathways that modulate response to toxicity, and disease.

Some key findings over the past twenty years have included the following: 1) Discovery and characterization of  $\text{Na}^+$ -coupled glutathione (GSH) transport across the renal basolateral plasma membrane and identification of the organic anion transporter 3 (Oat3) as one carrier mediating the transport; 2) development of *in vitro* cell models to study nephron segment-specific mechanisms of injury; 3) identification of mitochondria as a major subcellular site of action for nephrotoxic cysteine *S*-conjugates of halogenated solvents, such as trichloroethylene; 4) extension of *in vitro* cell models for primary culture of rat proximal and distal tubules to those from human kidneys; 5) quantitation of metabolism by both GSH conjugation and cytochrome P450 pathways for trichloroethylene and perchloroethylene in liver and kidney from rats, mice, and humans, providing information that can be used to improve pharmacokinetic models that are part of human health risk assessment efforts; 6) demonstration that DCVC induces a range of responses in human proximal tubular cells, including cell death by necrosis (oncosis) and apoptosis, growth arrest, and repair and proliferation, depending on the concentration and time of exposure; 7) showed that human kidney is less susceptible than rat kidney to cytotoxicity induced by DCVC; 8) showed that compensatory renal hypertrophy and diabetic nephropathy induce oxidative stress in proximal tubules and changes in mitochondrial GSH status. Dr. Lash has published more than 100 original, peer-reviewed research papers and more than 50 reviews and book chapters, and has edited or co-edited four books and a special issue of *Toxicology and Applied Pharmacology* on membrane transport in toxicology.

Dr. Lash has consulted for the U.S. Environmental Protection Agency on their human health risk assessments for trichloroethylene, perchloroethylene, trimethylpentane, and barium and the National Academy of Sciences on their report on biomarkers in urinary toxicology. He has served on study sections and review panels for the National Institutes of Health, National Science Foundation, Super Fund review panel of the National Institute of Environmental Health Sciences, and other organizations. He is an Associate Editor for three major peer-reviewed journals in pharmacology and toxicology, *The Journal of Pharmacology and Experimental Therapeutics*, *Toxicology and Applied Pharmacology*, and *Pharmacology and Therapeutics*, and is on other editorial boards.

## IRIS Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE)

### General Charge Questions:

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?

### RESPONSE:

The EPA draft document is presented in the standard style of these types of reports. It is generally well written, concise, and logically presented, although there is some degree of repetition and there is a scattering of typographical errors. Overall, however, the document is very thorough and presents all the evidence for both the noncancer and cancer hazards. The major concern I have with the document is its extensive reliance on “unpublished” data. Two studies from the Centre Internationale de Toxicologie (CIT) are extensively cited. Regarding these studies, the document states the following: “An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through the EPA’s IRIS Hotline, at (202) 566-1676 (phone), (202) 566-1749 (fax), or [hotline.iris@epa.gov](mailto:hotline.iris@epa.gov) (e-mail address) and on the IRIS website ([www.epa.gov/iris](http://www.epa.gov/iris)).” In this reviewer’s opinion, such a peer-review should have been included or at least summarized in the present document. There is no indication here that the CIT studies were approved by the EPA or that any modifications were sought in its presentation.

The document also makes reference to several other unpublished reports. While the document explains in some cases that these data are presented for completeness because they exist, this reviewer still has a concern that by presenting them and discussing the findings, the report in effect gives a validation of these studies. My suggestion is that while it is appropriate to list these studies because they are known to exist, they should be segregated from other studies and publications that have been scientifically peer-reviewed in the traditionally accepted manner. There are also several places in the document, particularly when referring to clinical or epidemiological studies, where findings are reported as not being statistically significant. In this reviewer’s opinion, such statements are inappropriate; if two parameters are not statistically different from one another, then no effect can be claimed. There is no such thing as a “non-significant increase.” Although it may be appropriate to discuss the lack of statistical significance between two parameters, noting that the means do not differ from one another, the implication of using the terms “increase” or “decrease” is that there is an effect. There are also examples where the document states that a particular parameter was “slightly increased” or “slightly decreased”

without any reference to the statistical significance of these differences.

Although there is much discussion of the MTBE database, particularly with respect to metabolism, minimal use is made of the MTBE database for ETBE toxicity. Appropriate use of PBPK modeling to extrapolate from MTBE to ETBE may provide useful insight. One caution to note, however, is that overzealous extrapolation should be avoided; although the two chemicals are structurally similar, the differences in pharmacokinetics and pharmacodynamics may have a greater impact than may be readily appreciated.

A final concern involves use of data on the ETBE (and MTBE as well) metabolite TBA. It is certainly reasonable to assume that some of the effects observed with ETBE could be due to its metabolite. Of course, the key to proper use of the TBA database will be to have reasonably validated (accurate) dosimetry so that the amount of TBA generated at a target tissue for a given dose of ETBE could be determined.

G2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of ETBE.

**RESPONSE:**

The document states that references up to January, 2009 were included. However, a PubMed search conducted on 28 October, 2009 using the search term “ethyl tertiary butyl ether” revealed a total of 75 citations of which 2 were published in 2009 so could not have been included in the document. However, 2 other references from 2007 were noted. One is a comprehensive toxicological review by D. McGregor and the other is a methods paper that shows measurements of ETBE in human urine, using gas chromatography and mass spectrometry. Again, while the two 2009 references could not have been included in this document because of the timing of their publication, the two references from 2007, particularly the review by McGregor, need to be summarized. Thus, there are a total of 4 additional papers that need to be included:

*Additional References to be Considered:*

McGregor, D. (2007) Ethyl tertiary-butyl ether: a toxicological review. *Crit. Rev. Toxicol.* 37, 287-312.

Scibetta, L., Campo, L., Mercadante, R., Foa, V., and Fustinoni, S. (2007) Determination of low level methyl *tert*-butyl ether, ethyl *tert*-butyl ether and methyl *tert*-amyl ether in human urine by HS-SPME gas chromatography/mass spectrometry. *Anal. Chim. Acta* **581**, 53-62.

de Peyster A, Stanard B, Westover C. (2009) Effect of ETBE on reproductive steroids in male rats and rat Leydig cell cultures. *Toxicol. Lett.* **190**, 74-80.

Yamaki, K., and Yoshino, S. (2009) Inhibition of IgE-induced mast cell activation by ethyl tertiary-butyl ether, a bioethanol-derived fuel oxygenate. *J. Pharm. Pharmacol.* **61**, 1243-1248.

Additionally, the Japanese government has conducted a detailed toxicological review of ETBE. Once a full English translation is available, and not just a summary, this document should be evaluation for its inclusion for consideration by the EPA.

**Chemical-Specific Charge Questions:**

**(A) Oral reference dose (RfD) for ETBE**

Upon evaluation of the oral database, EPA determined that it was not possible to derive an oral RfD as the proposed composite uncertainty factor (UF) of 10,000 would lead to a value with an unacceptable level of uncertainty (see *A Review of the Reference Dose and Reference Concentration Processes*, U.S. EPA, 2002 for discussion of UFs). In lieu of deriving an RfD, the available data were used to derive an oral value (i.e., a minimal data value) for limited risk assessment purposes as discussed in Appendix C.

- A1. The CIT (2004b) two-generation study of reproduction and fertility effects of oral exposure to ETBE was selected as the basis for the derivation of the minimal data value. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

**RESPONSE:**

The document properly and carefully explains the basis for not being able to calculate an RfD. The document also carefully explains the choice of the CIT-2004b study as the principal study on which to base the minimal data value. As stated above, the only concern about use of this study is that it has not been published in the traditional, scientific literature and thus has not undergone the standard, peer review. While the document states that the EPA conducted a peer review of the study, no summary or evidence of this review is presented, making it difficult for the reader to fully judge the appropriateness of the study as a choice for principal study.

A2. Increased kidney weight in F0 generation male rats (CIT, 2004b) was selected as the critical effect for the minimal data value resulting from oral exposure to ETBE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

**RESPONSE:**

The document makes it clear from presentation of the database that increased kidney weight is the most sensitive and consistently changing parameter to use as the critical effect for the minimal data value resulting from oral exposure to ETBE. The rationale for exclusion of other endpoints is clearly and appropriately presented. Although there is concern that the increased kidney weight is a male rat-specific response due to the  $\alpha 2u$  phenomenon, my interpretation of the database is that it is unclear that  $\alpha 2u$  accumulation can fully explain the response, particularly in light of the similar (albeit at different doses) response observed in female rats.

A3. Benchmark dose (BMD) modeling methods were applied to kidney weight data to derive the point of departure (POD) for the minimal data value. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., one standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

**RESPONSE:**

The BMD modeling applied to the kidney weight data seems to be done appropriately, considering the limitations of the database. The rationale for selecting the BMR value for use in deriving the POD was also clearly explained and is scientifically justified. Because of the limited database, I do not see any alternative, appropriate methods or models to use to estimate these risk parameters.

On page 92, the document states that "the power model was selected to estimate the BMDL or POD for body weight gain in the pregnant dams, the linear model was selected to estimate the POD for net body weight gain in the pregnant dams, and the power model (with the highest dose group dropped) was selected to estimate the POD for body weight gain in the F0 generation males during the last quarter of treatment from CIT studies (2004a, b, unpublished reports)." It is unclear why the highest dose was dropped from the model; this needs to be explained.

A4. A total composite UF of 10,000 was used to derive a minimal data value for ETBE. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the minimal data value. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

**RESPONSE:**

The document authors use the standard criteria for assigning uncertainty factor (UF) values. In the case of the total composite UF for derivation of the minimal data value, a maximum value of 10,000 has been chosen based on 4 areas of maximum uncertainty. Considering the insufficiencies in the database, the lack of chronic oral studies, and UF values to account for animal-to-human extrapolation and human interindividual variation, this choice of a maximum composite UF of 10,000 seems appropriate. However, one may consider the composite UF value to be overly conservative in that there is overlap in considering the lack of chronic oral studies and database deficiencies. Hence, an overall or composite UF value of 3,000 may be more appropriate.

An additional concern is the presentation of a minimal data value (MDV) instead of an RfD for ETBE. First, this is not a standard practice with any precedent in previous IRIS evaluations. Second, it is unclear what use would be made of this MDV in lieu of an RfD. Although the EPA may revise the document and make it clearer that the MDV does not carry as much weight as an RfD because of the high degree of uncertainty, users of this information may treat the MDV as a de facto RfD. Thus, substantially more justification for presenting any value in lieu of an RfD needs to be presented.

**(B) Inhalation reference concentration (RfC) for ETBE**

B1. The Medinsky et al. (1999) 13-week inhalation exposure study in mice and rats was selected as the basis for derivation of the RfC for ETBE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

**RESPONSE:**

Selection of the Medinsky et al. (1999) study is appropriate for derivation of the RfC for ETBE because it is a reasonably comprehensive and well-controlled study. The effects indicating the kidneys as a target organ are consistently observed and exhibited both a dose dependence and higher incidence in male rats. Because of the limitations in the literature database, this is really the only appropriate study to consider as the basis for deriving the RfC.

- B2. The occurrence of regenerative foci in the kidneys of male rats (Medinsky et al., 1999) was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

**RESPONSE:**

As explained above in the choice of the Medinsky et al. (1999) study as the principal study for deriving an RfC value, similarly the choice of occurrence of regenerative foci in the kidneys of male rats as the critical effect among the several renal effects observed is also appropriate and clearly justified in the text. None of the other renal endpoints would seem to be either as sensitive or as toxicologically relevant. The major unknown factor in using data from male rats is the potential contribution of  $\alpha$ 2u accumulation to the response. Additional, more mechanistic studies are needed to clarify this issue.

- B3. An analysis of the mode of action of kidney effects is presented in the Toxicological Review and a determination is made that the mode of action in male and female rats is unknown. Please comment on whether the analysis is scientifically justified.

**RESPONSE:**

I would certainly agree that there are significant data gaps in our knowledge of how ETBE and its metabolites produce renal injury. The document spends a significant amount of text assessing the hyaline droplet – alpha-2u response in male rats. Unfortunately, I find the presentation to be somewhat confusing and possibly misleading. For one thing, I believe there is uniform consensus that the alpha-2u response has no direct relevance for human health risk assessment because the response is male rat-specific. While the document at one point actually does state this point, it then goes on to discuss the response at some length and makes a statement about it being relevant (page 69, top) that seems contradictory to other statements.

- B4. BMD modeling was applied to data for the mean number of regenerative foci in the kidneys to derive the POD for the RfC. Has the BMD modeling been appropriately conducted? Has the BMR selected for use in deriving the POD (i.e., one standard deviation from the control mean) been scientifically justified? Please identify and provide the rationale for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

**RESPONSE:**

Here, as with the modeling done for the minimal data value (in lieu of an RfD), the modeling procedure used to derive the POD for calculation of an RfC is explained clearly and in sufficient detail. I do not see any other parameter or modeling method, based on the limited database, as an alternative approach to that proposed by the EPA.

- B5. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

**RESPONSE:**

Unlike the UF values proposed for calculation of the minimal data value (default for an RfD), in which maximum values are used for 4 areas of uncertainty, here the document chooses a composite UF value of 3,000 because a UF of 3 is chosen to account for extrapolation of data from laboratory animals to humans. The rationale for this choice is clearly explained and seems consistent with normal practice. No changes are suggested in any of the UF values.

**(C) Carcinogenicity of ETBE**

- C1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), the Agency concluded that there is *suggestive evidence of carcinogenic potential* following oral exposure to ETBE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

**RESPONSE:**

I agree with the overall conclusion that there is suggestive evidence of carcinogenic potential following oral exposure to ETBE. While the document describes some of the limitations of the studies, such as the limited number of positive studies and the absence of any data among multiple species showing target organ concordance. Some studies are described that show a significant increase in overall tumor incidence but lack significance when certain specific targets are excluded. Although the document raises some questions about the importance of such results, I do not feel as though it is as critical of these studies as it could be. Overall, however, the basic conclusion seems valid. The EPA should be clearer, however, in what their conclusion actually means. By stating that there is "carcinogenic potential," they are not implying that there is evidence for ETBE being a carcinogen. While this terminology is consistent with their 2005 revised guidelines, it may not be clear to some readers of the document.

C2. EPA did not derive a quantitative estimate of the carcinogenic potential of ETBE. Do the data support an estimation of a cancer slope factor for ETBE? If a quantitative estimate is proposed, please identify and provide a detailed description of the method(s) and approach(es) for deriving a cancer slope factor.

**RESPONSE:**

I agree with the conclusion expressed in the EPA document that a cancer slope factor for ETBE cannot be calculated. The basic fact is that there are not enough data or not enough strong, quantitative data to support such a calculation.



**Melanie A. Marty, Ph.D.**  
Supervising Toxicologist and Branch Chief  
Office of Environmental Health Hazard Assessment  
California Environmental Protection Agency

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Dr. Marty received an A.B. in Biological Sciences in 1976 from the University of California Berkeley, and a Ph.D. in Pharmacology and Toxicology in 1983 from the University of California Davis. She has worked for the State of California since 1987, starting as Staff Toxicologist in the Department of Health Services and is currently Supervising Toxicologist and Branch Chief of the Air Toxicology and Epidemiology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. As the Branch Chief, she functions as the Office of Environmental Health Hazard Assessment's Lead for risk assessment in the Criteria Air Pollutant program, Air Toxics Hot Spots program and the Toxic Air Contaminant program in Cal/EPA. This responsibility also includes acting as Departmental Lead on children's environmental health issues, particularly as they relate to air pollution. Her duties include supervising about 20 staff consisting of toxicologists, epidemiologists and physicians responsible for evaluating public health impacts of air contaminants, and conducting epidemiological investigations of health effects of criteria air pollutants, as well as clerical and administrative staff. Dr. Marty's work includes addressing key risk assessment issues such as those related to children's environmental health; use of mechanistic data in risk assessment of both carcinogens and noncarcinogens; evaluation and refinement of use of uncertainty factors in noncancer risk assessment; evaluation of risk assessment of complex mixtures; and incorporating new data into setting ambient air quality standards.

Dr. Marty is a member of such professional societies as the Society of Toxicology, Society for Risk Analysis, and the Genetic and Environmental Toxicology Association of Northern California (GETA). She has presented a large number of seminars and invited lectures on a wide variety of topics, mostly related to health effects of airborne toxicants. She has presented poster sessions at many SOT annual meetings.

Dr. Marty has published research articles in such professional journals as the *International Journal of Toxicology*, *Risk Analysis*, *Journal of Exposure Analysis and Environmental Epidemiology*, *Human and Ecological Risk Assessment*, *Regulatory Toxicology and Pharmacology*, as well as others.

## Post-Meeting Comments on USEPA' draft Toxicological Review of Ethyl Tertiary Butyl Ether

G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?

The review is complete and logical, although in a few places could be clearer (e.g., page 102, discussion of HEC adjustment, see note below). Another example is on page 55 in describing genotoxicity, it is not stated which *Salmonella* strains were used. EPA has fairly clearly synthesized the scientific evidence they had as of the review date on the toxicity of ethyl tertiary butyl ether (ETBE). Despite having been in commerce for many years, there are relatively little data on ETBE for EPA to use in risk assessment. The document can also be made more concise with some editing. There are a number of places where a study description is repeated. This could be edited down referring the reader to the previous description. The section on reproductive and developmental toxicity describes a study starting at the top of page 42 which does not have a citation.

I agree with the suggestion by another reviewer to add in an Executive Summary, and a better description of the main study relied upon for the oral assessment (CIT 1994). In addition, another reviewer suggested attaching the summary of the peer review that was conducted by EPA on the CIT 1994 study.

G2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of ETBE.

A PubMed search revealed a new article which EPA needs to include. The article is:

Toxicology Letters 190(2009):74-80

Effect of ETBE on reproductive steroids in male rats and rat Leydig cell cultures, Ann de Peyster,

Bradley Stanard, Christian Westover

The investigators used Isolated rat Leydig cells to compare hCG-stimulated testosterone production following exposure to ETBE, methyl tertiary butyl ether (MTBE), and their common main metabolite, tert-butanol (TBA). In addition, they gavaged male Fischer 344 rats daily with 600 mg/kg, 1200 mg/kg or 1800 mg/kg ETBE in corn oil ( $n = 12$ ) for 14 days.

All three compounds produced an inhibition of testosterone production at equimolar concentrations in the in vitro assay. TBA was more potent than MTBE and ETBE, which had about the same potency. In the in vivo study, no significant plasma testosterone reduction was seen 1 h after the final 1200 mg/kg ETBE

dose. (In a previous study, 1200 mg/kg MTBE significantly lowered plasma testosterone in Sprague–Dawley rats). The authors note that the group treated with 1800 mg/kg ETBE had high variability in testosterone levels; group average testosterone level was 66% of corn oil vehicle control ( $p > 0.05$ ).  $17\beta$ -Estradiol was elevated in the 1200 and 1800 mg/kg ETBE dose groups ( $p < 0.05$ ), both groups also experiencing significantly reduced bodyweight gain. An apparent NOAEL was 600 mg/kg/day ETBE for these effects in this study. No changes were observed in accessory organ weights, and no testicular pathology was observed after 14 days in a small subset of 1800 mg/kg ETBE-treated animals. ETBE altered reproductive steroid levels in peripheral blood sampled 1 h after the high dose treatment.

No subchronic or chronic studies useful for deriving an RfD or RfC were found.

As noted at the peer review meeting, observers indicated that a study conducted in Japan is soon to be available. EPA will be evaluating that study for its utility in risk assessment.

#### **Chemical-specific Charge Questions:**

##### **(A) Oral reference dose (RfD) for ETBE**

Upon evaluation of the oral database, EPA determined that it was not possible to derive an oral RfD as the proposed composite uncertainty factor (UF) of 10,000 would lead to a value with an unacceptable level of uncertainty (see *A Review of the Reference Dose and Reference Concentration Processes*, U.S. EPA, 2002 for discussion of UFs). In lieu of deriving an RfD, the available data were used to derive an oral value (i.e., a minimal data value) for limited risk assessment purposes as discussed in Appendix C.

- A1. The CIT (2004b) two-generation study of reproduction and fertility effects of oral exposure to ETBE was selected as the basis for the derivation of the minimal data value. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

The selection of the CIT (2004b) study as the principle study is justified in view of the limited available data that EPA has to work with for this chemical. This study was a reproductive and developmental toxicity study and provides the most data for a chronic RfD derivation of any of the oral studies (of which there are few). There are no typical subchronic or chronic toxicity studies available for ETBE following oral exposure, which evaluate a range of organs. Further, the CIT (2004b) had very limited histopathological evaluation of organs, so did not provide much information regarding the effects on kidney and liver other than the noted relative weight increases. Thus, while certainly not an ideal study, it is all that EPA has to work with at this point. As noted above, additional description of the CIT study would be useful, as would a summary of the peer review of the study. The 2 year carcinogenicity bioassay conducted by Maltoni did not provide data on noncancer effects useful for an RfD derivation.

- A2. Increased kidney weight in F0 generation male rats (CIT, 2004b) was selected as the critical effect for the minimal data value resulting from oral exposure to ETBE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

The increased kidney weight in F0 generation male rats is an appropriate endpoint. It is the most sensitive endpoint yielding the lowest BMDL. As noted above, unfortunately there are no available studies that provide thorough histopathological evaluations following exposure to ETBE by the oral route. In the CIT (2004b) study, which was designed as a two generation reproductive and developmental study, only animals with gross morphological abnormalities were evaluated histopathologically. There is some information from the inhalation study (Medinsky et al, 1999) indicating that the kidney is a target of ETBE toxicity. Since the kidney of males was more affected than females at lower exposures, it is appropriate to use the kidney weight data from male rats as the critical effect for deriving the RfD (or in this case the “minimal data value”).

Although there is some evidence for an alpha-2 $\mu$  globulin mode of action of ETBE in inducing kidney toxicity in the male rats, the existing data are not sufficient for a definitive conclusion. In addition, some kidney toxicity is also seen in females (elevated kidney weight in female rats in the CIT study, and some evidence of proliferation at least in the earlier time points in the Medinsky inhalation study). Thus, there could be multiple modes of action for nephrotoxicity, not just the male rat specific alpha-2 $\mu$  globulin mode of action. Further, the main metabolite TBA also produces adverse kidney effects in both males and female rats, and may be involved in the effects seen after ETBE exposure. More discussion of this issue appears below in response to other comments.

- A3. Benchmark dose (BMD) modeling methods were applied to kidney weight data to derive the point of departure (POD) for the minimal data value. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., one standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA’s approach.

It appears that the BMD model has been appropriately conducted, using continuous data models. The output provided in the appendices indicates that EPA evaluated all possible endpoints with the BMD method before choosing a Point of Departure (POD). I was able to reproduce the key effect BMD and BMDL. The Hill model had a good fit to the data (both visually and by Chi-square). In the end, EPA chose the data set that provides the lowest BMDL as the critical endpoint for the “minimal data value” derivation. This is appropriate and consistent with their guidance on risk assessment. The POD of one

standard deviation from the control mean is a standard assumption to apply in the face of a lack of specific biological criteria for when a continuous variable (such as relative organ weight) is considered adverse.

A4. A total composite UF of 10,000 was used to derive a minimal data value for ETBE. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the minimal data value. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

The total composite UF of 10,000 consisted of 10X for interspecies extrapolation, 10X for intraspecies extrapolation, 10X for subchronic to chronic exposure extrapolation, and 10 X for database deficiency. These appear reasonable given the paucity of available data on the toxicity of ETBE. The lack of a chronic study, oral or inhalation, and the unusual circumstance of having just a two-generation reproductive and developmental study without histopathology for most organs underscores the need for a subchronic to chronic uncertainty factor. The lack of any chronic studies or even subchronic studies with adequate histopathological analysis argues for a 10X factor.

On the other hand, it might be useful to consider a 3X UF for subchronic to chronic extrapolation. The subchronic study was for 120 days (17 weeks), or about a month longer than a typical subchronic 90-day study. However, the EPA's definition of chronic exposure has been an exposure lasting for more than 12% of a lifetime (which for a rat is around 12.5 weeks assuming a 2 year lifespan; or about 18.7 weeks if you assume a 3 year lifespan). EPA also applied a database deficiency factor of 10, which could be viewed as accounting at least partially for the lack of a chronic study. It would be useful to provide some additional discussion of these issues if EPA decides to choose a 10X factor rather than an intermediate factor (e.g.,  $\sqrt{10}$  or  $\sim 3$ ).

Since the EPA guidelines preclude establishing an RfD if the cumulative UF is  $> 3000$ , the result of a subchronic to chronic UF of 10 is that no RfD is available. If they chose a total UF of 3000 by applying an intermediate UF of 3 for subchronic to chronic exposure, the RfD, by my calculations would be 47  $\mu\text{g}/\text{kg}\cdot\text{d}$ . The 2-generation reproductive and developmental toxicity study available showed relatively little in the way of reproductive and developmental toxicity, so that is somewhat comforting.

It is not clear how EPA (or others) would use a "minimal data value". It may be more useful to develop an RfD which has established use, and which could be done with a reconsideration of the uncertainty factors.

There also may be some room for exploring route-to-route comparisons, since there is a draft RfC for ETBE in this document. The toxicity is systemic so the concern about the inappropriateness of route-to-route extrapolations for point-of-contact toxicants is not relevant for ETBE.

**(B) Inhalation reference concentration (RfC) for ETBE**

B1. The Medinsky et al. (1999) 13-week inhalation exposure study in mice and rats was selected as the basis for derivation of the RfC for ETBE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

The Medinsky et al, (1999) inhalation subchronic exposure study appears to be the only study available to use to derive an RfC, given the lack of a chronic inhalation exposure study.

Again, EPA has limited choices for deriving an RfC for ETBE. Limitations of the Medinsky study include relatively small numbers of animals per dose group for histopathological evaluation.

B2. The occurrence of regenerative foci in the kidneys of male rats (Medinsky et al., 1999) was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

The occurrence of regenerative foci in male rat kidney was chosen as the critical endpoint for derivation of the RfC. This endpoint is justified. It is the most sensitive endpoint based on available data, having the lowest modeled BMDL. Although there is some evidence that the mechanism of action may be related to alpha<sub>2</sub>μ-globulin precipitation in the kidney, available data are insufficient to conclusively demonstrate this mode of action. Further, there was an increase in the labeling index in female rat kidneys after 1 and 4 weeks of exposure (but not at the 13 week time point), indicating nephrotoxicity in female rats. Thus, there are likely multiple mechanisms of action involved in the increased kidney weight, and cell death and subsequent regeneration observed. Although the document states that the increased cell proliferation observed in female rats is of questionable biological significance, I disagree with that. The transient nature could be the result of increased metabolism of the ETBE following induction of catabolic enzymes, or some other change in kinetics. The increased labeling index at the earlier time points is an indication that renal cell death occurred at least in the first weeks of the study.

Other endpoints were considered by EPA, but resulted in higher BMDL values, and thus were not the most sensitive endpoint. Given that, regenerative foci in the kidney of male rats is a reasonable endpoint to choose for the derivation of the RfC.

Based on discussion at the peer review meeting, it would probably be better to use a different term than regenerative “foci” when describing the toxicological endpoint that is the basis for the RfC. The study observed regenerative hyperplasia, so that term is more appropriate for describing the toxicological endpoint. The BMDL is based on numbers of regenerative “foci” per animal in each dose group. So, the word foci is useful in that context, but the critical endpoint should be referred to as regenerative hyperplasia.

- B3. An analysis of the mode of action of kidney effects is presented in the Toxicological Review and a determination is made that the mode of action in male and female rats is unknown. Please comment on whether the analysis is scientifically justified.

As noted above, I agree that the mode of action data are insufficient to presume that the kidney toxicity is occurring solely through the accumulation of alpha<sub>2</sub>μ-globulin. Data on the sequential pathology one would see if this were the only mode of action are unavailable. Female rats also experienced elevated kidney weight and there was some increase in labeling index in the first month or so of exposure to ETBE via inhalation (Medinsky et al., 1999). This is indicative of cell death and subsequent regeneration in female rat kidney, and the alpha<sub>2</sub>μ-globulin mode of action is restricted to males. Thus, something else is happening to produce this effect in females.

It would be helpful to discuss the fuller criteria for alpha<sub>2</sub>μ-globulin MOA laid out by IARC, rather than just the three criteria laid out on page 69. The data on ETBE do not meet the fuller criteria.

In addition, a primary metabolite of ETBE is tertiary butanol (TBA). In the NTP (1995) study of TBA in drinking water, renal toxicity was also noted in the female rats in a dose-dependent fashion (severity of nephropathy, transitional cell hyperplasia of the kidney). Further, the NTP notes that hyaline droplet accumulation in male rat kidneys was minimal at doses producing significant increases in renal tumors. Thus, the observed nephrotoxicity of ETBE may involve the metabolite TBA, whose MOA is not entirely consistent with the alpha<sub>2</sub>μ-globulin MOA.

Finally, the related compound MTBE induces nephropathy in females as well as males. Both ETBE and MTBE are metabolized to TBA. EPA used the nephropathy data in female rats to derive an RfD for MTBE.

- B4. BMD modeling was applied to data for the mean number of regenerative foci in the kidneys to derive the POD for the RfC. Has the BMD modeling been appropriately conducted? Has the BMR selected for use in deriving the POD (i.e., one standard deviation from the control mean) been scientifically justified? Please identify and provide the rationale for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

The BMD analysis appears to have been properly conducted. EPA evaluated the regenerative foci as continuous data using the mean number of regenerative foci per animal per dose group as input to the model. The benchmark response rate chosen was 1 standard deviation change relative to the control mean, which is the default approach that EPA uses. The software output (both that in the appendices and when I ran the BMDS model with the data) indicates that a non-constant variance model is appropriate. Of the three non-constant variance continuous data models chosen, the Hill model produced the lowest BMD and BMDL. Thus, this is appropriate for the POD. They also evaluated the regenerative foci as dichotomous data and obtained similar BMC and BMCL values with the logistic model, using a 10% benchmark dose response. However, lower BMCL were obtained with other dichotomous models that had similar goodness of fit. It would be helpful to clearly explain their choice of using a continuous model rather than a dichotomous model.

I was able to reproduce the BMC analyses for this endpoint except for the BMCL from the regenerative foci continuous data that was chosen as the POD. I got the same BMC as EPA but the modeled failed to calculate the BMCL. I am not sure why the model failed, but it could be related to differences in the model version or some other model input I am unaware of. If they have not yet done so, EPA scientists should recheck their analysis.

The adjustment from the BMCL to a  $BMCL_{HEC}$  appears to have been done correctly, although the equation on page 102 has a mystery 0.1368 in the calculation. This obviously wasn't applied or the  $BMCL_{HEC}$  would have been much lower. I was able to reproduce their  $BMCL_{HEC}$

- B5. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

The choice of the UFs applied to the POD appears to be based on the standard EPA methodology, and are reasonable given the data. However, it should be noted that a HEC adjustment is primarily a dosimetric adjustment and does not, in my opinion, give adequate consideration to other kinetic differences between species. One could rationalize an additional UF of 2 for the remaining toxicokinetic uncertainty in extrapolating from animals to humans. I also do not think it is justifiable to state (page 102, section

5.2.3., third paragraph) that part of the toxicodynamic difference between animals and humans is accounted for by what is in essence a dosimetric adjustment (the HEC).

The database deficiency factor of 10 appears reasonable given the lack of data on ETBE. One could also argue for a half-log UF here. Although there is not a developmental toxicity study by inhalation, there is one by the oral route, and it does not reveal much if any developmental toxicity. It does not appear that EPA gave this much weight in their discussion of the database deficiency UF.

There is a typo in the final RfC on page 102 (the 10 is missing before the -3).

## C Carcinogenicity

C1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), the Agency concluded that there is *suggestive evidence of carcinogenic potential* following oral exposure to ETBE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

I agree with the cancer weight of evidence interpretation by USEPA, although the wording on page 75 needs to be fixed. I agree with the conclusion as worded in the charge question - the Agency concluded that there is *suggestive evidence of carcinogenic potential*. The wording on page 75, "there is suggestive evidence of human carcinogenicity" is not correct.

There is only one study available of the carcinogenicity of ETBE (Maltoni et al., 1997) conducted in one strain of rats. This study supports the conclusion that there is suggestive evidence of carcinogenic potential. I agree with EPA that the reporting of the data was unusual. In particular, lumping together precancerous lesions with tumors makes it difficult to impossible to use the data for a quantitative evaluation of the cancer potency factor. I am somewhat less concerned that vaginal schwannomas and uterine carcinomas are lumped together. There is an argument to evaluate all statistically significant tumors together to derive a cancer slope factor for a chemical (e.g., by adding distributions of the slope factors). There were elevations, although not statistically significant as noted by EPA, in other tumors as well. Thus, the study supports the conclusion of suggestive evidence of carcinogenic potential. Note this is not the same as EPA deciding this compound is a carcinogen.

As noted on pages 77 and 78 in the document, there is evidence from a well-conducted NTP carcinogenicity bioassay that TBA, a main metabolite of ETBE, is carcinogenic in rodents. Further MTBE, the methyl version of ETBE, is a multi-site carcinogen in animals.

- C2. EPA did not derive a quantitative estimate of the carcinogenic potential of ETBE. Do the data support an estimation of a cancer slope factor for ETBE? If a quantitative estimate is proposed, please identify and provide a detailed description of the method(s) and approach(es) for deriving a cancer slope factor.

It is unfortunate that the data in the Maltoni et al (1999) study were not more precisely reported; lumping precancerous lesions with tumors makes it difficult to use the data in a quantitative analysis. Other reviewers noted that tumor types may have been lumped together inappropriately. It is also unfortunate that the mortality in the Maltoni study was not quantified more precisely and that time to tumor data are not reported.

Another way to estimate a cancer slope factor for ETBE is to base it on the metabolite TBA. As noted earlier, there is a 2 year cancer bioassay of TBA conducted by NTP (1995). The NTP reported elevated tumor incidences in both rats and mice; renal tubule adenoma and carcinoma was elevated in male rats, and thyroid follicular cell adenoma was elevated in female mice as a result of TBA exposure. California EPA developed a slope factor for TBA of  $3 \times 10^{-3} \text{ (mg/kg-d)}^{-1}$  from the NTP male rat kidney tumor data, which was applied in their drinking water program. One could also cross-extrapolate to an inhalation exposure. Assuming a 70 kg person breathes  $20 \text{ m}^3$  per day, and a 50 percent fractional absorption of TBA via inhalation, allows an estimate of the inhalation slope factor of about  $5 \times 10^{-6} \text{ (}\mu\text{g/m}^3\text{)}^{-1}$ . (The 50% absorption rate has been applied to a number of volatile small molecules based on a study by Raabe et al (1989) on fractional absorption of small VOCs across the lung at low environmental exposures). If EPA estimated ETBE cancer potency in this fashion there would have to be a toxicokinetic analysis to evaluate the rate of formation of TBA from ETBE.

Although there is some argument for assuming that male rat kidney tumors seen in the TBA NTP study are the result of alpha $2\mu$ -globulin accumulation, cell death, and subsequent proliferation, the data to conclusively establish this are insufficient. Borghoff et al (2001) exposed male rats to 0, 250, 450, or 1750 ppm TBA 6h/d for 10 days, and demonstrated a dose-dependent accumulation of protein droplets in the renal proximal tubules, and an increase in renal alpha $2\mu$ -globulin in the 1750 ppm group. There was not a dose-dependent increase in alpha $2\mu$ -globulin staining, however. In this study, TBA induced increased cell proliferation at exposures below that which induced alpha $2\mu$ -globulin accumulation. Thus, it is not clear that the only mode of action involved in TBA renal toxicity is the alpha- $2\mu$  globulin accumulation MOA. Further, there are a few positive studies of TBA genotoxicity, although several studies were negative. TBA induced mutations in Salmonella strain TA 102, a strain that is susceptible to oxidative DNA damage (Williams-Hill et al., 1999). In addition, TBA was positive in a Comet assay in

HL-60 cells (Tang et al., 1997), and was found by accelerator mass spectrometry to produce DNA adducts in mice (Yuan et al, 2007). Another investigator reported that TBA caused DNA fragmentation (COMET assay) and induction of oxidative DNA damage (8-OH-dG adducts) in rat fibroblasts in vitro (Sgambato et al, 2009).

Thus, there is some concern about TBA carcinogenicity, and there is some logic to using it as a basis for estimating a cancer slope factor for ETBE applying pharmacokinetic considerations to the metabolism of ETBE to TBA.

**References used in the reviewer comments:**

Borghoff SJ, Prescott JS, Janszen DB, Wong BA, Everitt JI. (2001)  $\alpha$ 2u-Globulin nephropathy, renal cell proliferation, and dosimetry of inhaled tert-butyl alcohol in male and female F-344 rats. *Toxicol Sci* 61:176-86.

NTP (1995) NTP Toxicology and Carcinogenesis Studies of t -Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). *Natl Toxicology Program Tech Rep Ser* 1995 May;436:1-305.

Sgambato A, Iavicoli I, De Paola B, Bianchino G, Boninsegna A, Bergamaschi A, Pietroiusta A, Cittadini A (2009) Differential toxic effects of methyl tertiary butyl ether and tert-butanol on rat fibroblasts in vitro *Toxicology and Industrial Health* 25:141-51.

Tang G, Wang J, Zhuang Z (1997) Cytotoxicity and genotoxicity of methyl tert-butyl ether and its metabolite to human leukemia cells *Chin J Prev Med* 31:334-337.

Williams-Hill D, Spears CP, Prakash S, Olah GA, Shamma T, Moin T, Kim LY, Hill CK. (1999) Mutagenicity studies of methyl tert-butyl ether using the Ames tester strain TA 102. *Mutat Res* 446:15-21

Yuan Y, Wang HF, Sun HF, Du HF, Xu LH, Liu YF, Ding XF, Fu DP, Liu KX. (2007) Adduction of DNA with MTBE and TBA in mice studies by accelerator mass spectrometry. *Environmental Toxicology* 22:630-635.

**Additional comments :**

1. Check the document for typos. Page iii Epidemiology is misspelled in the TOC and on page 25 in the header.
2. Page 8. This section could be written more clearly. Should be blood:air partition coefficient, not air:blood. The last sentence indicating that doses were 500 times higher in rats than humans needs to be more clearly explicated. How did this get calculated?

3. pp8 and 9 discuss dermal absorption of ETBE. The text seems to downplay the results of Prah et al (2004) which measured higher dermal penetration in humans than would be predicted by log Kow. It is inappropriate to downplay these data which were experimentally determined. The last sentence of the first paragraph on p.9 points out the reasons why experimental absorption could be higher than calculated based on log Kow..
4. page 9 section 3.2, This section flips the tissue:blood partition coefficient as blood:tissue. Please fix.
5. Section 4.2.2. p. 28 top paragraph indicates that in Maltoni et al (1999), the MTD may have been exceeded. The last sentence contradicts this as there were no major effects on food and water intake or body weight. So, that needs clarification.
6. p. 30-31. Is there any indication that mortality played a role in the dose-response for uterine tumors in Maltoni et al (1999) (e.g., early mortality reduced apparent tumor incidence?)
7. It is interesting to note that White et al (1995) report statistically elevated increase in white blood cell count in female rats at 2000 and 4000 ppm, and that the CIIT study (Medinsky et al) noted bone marrow congestion in female rats. The report indicates these are of questionable toxicological significance. Yet the methyl analogue of ETBE, MTBE induces leukemias in rats.
8. p. 42. What is the citation for this study?
9. Page 46, Table 4-7. At the meeting we discussed the question what body weight was used to calculate a relative organ weight (for the females in the 2-gen repro study). This should be clarified.
10. Suggest adding some headings to the section on repro tox – it will make it easier to follow.
11. page 54, 2<sup>nd</sup> full paragraph. The statement in the last sentence that in spite of transient ataxia, the authors conclude ETBE is not a neurotoxicant is strange. Ataxia is a neurological effect. Perhaps it could be worded that no apparent neurotoxicity was observed at lower concentrations. As it is worded, the sentence makes no sense. Also, in the next paragraph, in discussing the CIT 2004b study, the last sentence indicates there was no effect of treatment on neurobehavioral parameters, please add as measured in this study. Assessment was fairly limited. Locomotor activity was only evaluated at one time point, and they did not do histological evaluations of the brains (it seems).
12. What strains of Salmonella were used? Did anyone evaluate strains sensitive to oxidative DNA damage?
13. page 95, mid-page. The text indicates that the increased Labeling Index noted at 1 and 4 weeks in female rat kidney was of questionable biological significance. I disagree with this – it indicates kidney damage and compensatory proliferation.

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College of Medicine and Public Health  
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Dr. Pereira received a B.Sc. in Microbiology in 1967 and a PhD in Pharmacology and Toxicology in 1971 from Ohio State University. He then received a Damon Runyon Cancer Research Fellowship (1971-1973) to work at NIH. He is currently an Emeritus Professor of Medicine, Division Hematology and Oncology at Ohio State University. Dr. Pereira has over 40 years of experience in toxicology and carcinogenesis. This includes research performed as an employee of the US government (US EPA), private industry (EHRT) and academia (New York University, Medical College of Ohio and Ohio State University). As an employee of the US EPA, he was responsible for the evaluation of the genotoxicity and carcinogenic of chemicals found in water, especially drinking water and for the determination of their human health hazard. At EHRT, he was the Vice-President for Toxicology and PI of numerous grant and contracts with NCI and NIOSH. He has continued in academia to be PI of three grants from NCI, of two grants from EPA, one grant from NIEHS, and over 30 contracts with NCI. He was also the PI of a contract with the City of Tampa to perform an evaluation of the toxicity of effluents from their wastewater reuse pilot plant. He has over 230 publications in peer-reviewed journal that are related to toxicology and cancer. Since leaving the US EPA in 1986 he has reviewed numerous documents for the agency. Some of the other committees he has been a member of include the NCI SBIR Study Section, NCI Study Section for Program Project, NCI SPORE in Lung-GU Cancer Review Committee (P50 applications), and NCI Chemo/Dietary Prevention Study Section.

**Pereira: Review**

Contract No. EP-C-07-024

Task Order No. 47

January 29, 2010

**IRIS Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE)**

**General Charge Questions:**

**G1.** Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?

**Response:**

The Toxicological Review is logical, clear, and easily to read with a complete review of the literature. However, the document is not concise having a lot of redundancies, and discussion of possible mode of action for which there is no associated toxicity or very limited, if any evidence.

Example of redundancies:

1) Page 10 The first sentence of the first paragraph is identical to the first sentence of Section 3.3.1.1 on page 11 and Section 3.4.1 on page 16.

The inclusion of an Executive Summary at the beginning of the document would be useful. It need not include any actual values but state what values were calculated.

Each section of the document that includes data pertaining to MTBE and TBA should contain a summary that compares and contrasts the results of ETBE to those of MTBE and TBA. Tables comparing the three chemicals would be very useful. The comparison should also clearly and in very short and simple sentences present the extent to which ETBE is metabolized to TBA and thus extent to which TBA could account for affects observed for ETBE. Although, this is given to some extent in the document it is hidden by the details of the studies and by the redundancies. Therefore, the extent to which TBA could account for affects observed for ETBE needs to be separated in a separate paragraph, if not subsection.

With respect to  $\alpha_2\mu$ -globulin nephrology, the ETBE document needs to be consistent with other EPA documents of MTBE and TBA, especially as to its relevance to humans and to the calculation of RfD.

A more extensive and detailed discussion of the CIT study, including actual results with SE or SD and not percent change, needs to be included since it is hard to find needed information the 1,724 page document.

**G2.** Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of ETBE.

**Response:**

There are no additional published studies.

The needs to include the results of the Japanese studies and needs to use the results of these studies in the calculation of the RfD and RfC, when appropriate. The summary of these studies indicate that they would be the most appropriate studies for the calculation of the RfD and RfC. Also, English translations of the write-up of these studies as well as English drafts of the publications of these studies are likely to be obtained from the authors. Hence, without these studies the document is incomplete and premature.

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**Chemical-Specific Charge Questions:****(A) Oral reference dose (RfD) for ETBE**

Upon evaluation of the oral database, EPA determined that it was not possible to derive an oral RfD as the proposed composite uncertainty factor (UF) of 10,000 would lead to a value with an unacceptable level of uncertainty (see *A Review of the Reference Dose and Reference Concentration Processes*, U.S. EPA, 2002 for discussion of UFs). In lieu of deriving an RfD, the available data were used to derive an oral value (i.e., a minimal data value) for limited risk assessment purposes as discussed in Appendix C.

- A1.** The CIT (2004b) two-generation study of the reproduction and fertility effects of oral exposure to ETBE was selected as the basis for the derivation of the minimal data value. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

**Response:**

1. Although, this is not a peer reviewed study a 1,724 page complete description of this study is presented on the internet, so that its selection as the basis for the derivation of the minimal data value is justified. However, only percent changes are given (Table 4-7) and not the actual values, which makes it hard to critically review the study as presented in the IRIS Toxicological review. Please, include the actual values and either SD or SE with an indication of the number of animals evaluated. Also please address the following:
  - a) Delete the incidence data from Table 4-7 since only specimens with an observable effect were evaluate by histopathology. Hence the incidence of an effect in specimens with an effect should be 100%, i.e. 3/3. The incidence is really 3/25. Thus the data presented as incidence in the table are not the incidence of the effect but rather a description of the effect and should be discussed as such in the text.
  - b) Why was histopathogy performed on only a few of the rats and how were they chosen? Due to the length of the write-up of the study on the internet, it is very hard to find this data, although it is presented. More detail of the study should be given in the document.
2. Include the EPA review of the CIT study. The text of the document could include a detail and in depth summary of EPA's review with the complete review presented in the Appendix.

Additional Note: What is the reference for the study discussed in the three paragraphs on page 42?

- A2.** Increased kidney weight in F0 generation male rats (CIT, 2004b) was selected as the critical effect for the minimal data value resulting from oral exposure to ETBE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

**Response:**

The choice of the increased male kidney weight is not justified since all evidence supports the increase being related to  $\alpha_2\mu$ -globulin. Also, only percent changes are given.

The small change in the relative kidney weight in female rats was presented as relative to body weight. However, the terminal body weight for the female rats was not obtained at sacrifice. Therefore, how was the kidney weight to body weight ratio obtained? Since the increase in female rat kidney weight was only 10% and obtained only at the high dose (1,000 mg/kg-day), only for the F1 generation, without any histopathologic evaluation and without being relative to the terminal body weight, it is likely not biologically significant or reproducible. Furthermore, the Japanese studies, oral and inhalation did not observe any kidney pathology in female rats. **Thus, the small affect report in the CIT, 2004b study was not reproducible. However, the apparent, not reproducible kidney affect in F1 female rats is more justified as the critical effect than that in male rats.** It should be used as a NOAEL and not a LOAEL, without be relative to the terminal body weight it is more likely a suggestion of an affect rather than an observed effect.

It should be noted that the studies discussed on pages 68-72 as to whether the kidney effects are related to  $\alpha_2\mu$ -globulin do demonstrate that the kidney effects in male rats are the result of this nephropathy and thus not related to humans. Thus, I disagreed with the documents' conclusion that a determination cannot be made as to whether  $\alpha_2\mu$ -globulin is the mode of action. Furthermore nothing is presented in the document to suggest that  $\alpha_2\mu$ -globulin is not the mode of action.

This leaves the increased liver weight at 1,000mg/kg as a possible toxicity. However, it is not clear that an increase in liver weight is a toxic response and not an adaptive response to increased metabolism of ETBE. No histopathologic evidence for liver toxicity was found in either the CIT 2004b or Maltoni studies. Thus, 1,000 mg/kg would appear to be a NOAEL for oral exposure to ETBE.

**A3.** Benchmark dose (BMD) modeling methods were applied to kidney weight data to derive the point of departure (POD) for the minimal data value. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., one standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

**Response:**

The modeling methods are appropriate and justified.

**A4.** A total composite UF of 10,000 was used to derive a minimal data value for ETBE. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the minimal data value. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

**Response:**

The UF should be 30.

a) The UF for animal to humans (UFA) should be 0.1. This is because the ETBE in the oral studies were given by oral gavage. This administration results in a much higher blood concentration and greater toxicity than potential human exposure administered over 16 hours. This difference in animal versus human exposure would result in a much greater toxic response in the animal than any hypothetical increase in human sensitivity to ETBE toxicity. Furthermore, the UFA should be 0.1 if the increased kidney weight in F0 generation male rats is selected as the critical effect because humans are much less, if at all sensitive.

b) The UF for human intraspecies variability UFH should be 3 since this variable is purely hypothetical without any support, there is nothing to suggest there is any sensitive human subpopulation.

- c) It is noted that the UF for database deficiency and for subchronic to chronic exposure extrapolation are redundant and only one, i.e. the UF for database deficiency based on subchronic to chronic exposure extrapolation should be used.

**(B) Inhalation reference concentration (RfC) for ETBE**

- B1.** The Medinsky et al. (1999) 13-week inhalation exposure study in mice and rats was selected as the basis for derivation of the RfC for ETBE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

**Response:**

The selection of Medinsky et al. (1999) 13-week inhalation exposure study in F-344 rats and CD-1 mice is a very good study and justified as the basis for derivation of the RfC for ETBE. **However, the Japanese studies need to be included in the discussion and in the decision, since it is possible that they are better justified as the basis for derivation of the RfC for ETBE. Furthermore, they could also be used to support and justify the choice of the Medinsky et al. (1999) 13-week inhalation exposure study.**

- B2.** The occurrence of regenerative foci in the kidneys of male rats (Medinsky et al., 1999) was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

**Response:**

The occurrence of regenerative foci in the kidneys of male rats is not justified as the critical effect for the RfC. These foci were associated with protein droplet accumulation shown to be  $\alpha_2\mu$ -globulin. Hence, this response is not related to humans. Furthermore, the regenerative foci are likely not focal and thus not foci but rather are evidence of regeneration. The histopathologic evaluation of the slides by Gordon Hard should be included in the discussion and decision to use regeneration as the critical effect.

The effect of ETBE in female rat kidney might be used as the critical effect. However, the Japanese study did not find any effect of ETBE in female rat kidney. Thus, the effect of ETBE would appear to be specific to male rat kidney. This needs to be critically discussed in the EPA document related to the mode of action.

The increased liver weight is possible effect of ETBE that could be selected as the critical effect for the RfC. However, it is not clear that an increase in liver weight is a toxic response and not an adaptive response to increased metabolism of ETBE.

- B3.** An analysis of the mode of action of kidney effects is presented in the Toxicological Review and a determination is made that the mode of action in male and female rats is unknown. Please comment on whether the analysis is scientifically justified.

**Response:**

This section strongly indicates that the mode of action of the kidney effects in male rats is due to  $\alpha 2\mu$ -globulin. Nothing except for pure speculation is given to indicate otherwise. Therefore, the conclusion of the document should be changed to the mode of action of the kidney effects is due to  $\alpha 2\mu$ -globulin. It could be added to the conclusion that it is always possible for ETBE to have some other modes of actions in affecting the kidney but that these would be insignificant relative to the  $\alpha 2\mu$ -globulin mode of action.

As stated above, the Japanese study did not find any effect of ETBE in female rat kidney so that this needs to be critically discussed in the EPA document related to the mode of action.

- B4.** BMD modeling was applied to data for the mean number of regenerative foci in the kidneys to derive the POD for the RfC. Has the BMD modeling been appropriately conducted? Has the BMR selected for use in deriving the POD (i.e., one standard deviation from the control mean) been scientifically justified? Please identify and provide the rationale for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

**Response:**

The modeling methods are appropriate and justified. The document should discuss the modeling for female rat kidney and use it as a NOAEL in calculating the RfC, since it was not reproducible by the Japanese study.

- B5.** Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

**Response:**

The UF should be 30 if a kidney affect is used or 300 if liver weight is used.

a) The UF for animal to humans (UFA) should be 0.1 if the occurrence of regenerative foci in the kidneys of male rats is used because humans are much less, if at all sensitive. If liver weight is used then this UFA should be 1, since the increased liver weight is likely not a toxic response. The UF for human intraspecies variability UFH should be 3 since this variable is purely hypothetical without any support, since there is nothing to suggest there is any sensitive human subpopulation.

b) The UF for database deficiency should be 3 since ETBE did not demonstrate reproductive and developmental toxicity when administered orally to rats. The reproductive and developmental study performed by the Japanese should be considered, discussed and used critically in the derivation of the RfC.

**(C) Carcinogenicity of ETBE**

- C1.** Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), the Agency concluded that there is *suggestive evidence of carcinogenic potential* following oral exposure to ETBE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

**Response:**

The conclusion that there is *suggestive evidence of carcinogenic potential* following oral exposure to ETBE is not justified. Rather, that there is suggestive evidence that ETBE is not carcinogenic as follows:

- 1) The only carcinogenic bioassay, (Maltoni et al., 1998) was negative and did not demonstrate carcinogenic activity for ETBE. As listed below there are numerous problems with Maltoni's study. However, the study is sufficiently described and reported to arrive at a bottom line that the high dose level did not demonstrate any suggestive evidence for carcinogenic response to ETBE. Hence, it is extremely unlikely that a repeat of the carcinogenic bioassay in rats would provide evidence for a carcinogenic response to ETBE. Still the results of another carcinogenic bioassay of ETBE in rats will be available to the EPA within a few months. Therefore, the EPA should hold off on any evaluation of ETBE for carcinogenic potential until it critically reviews the Japanese results and include it in this IRIS document.
- 2) ETBE was negative and did not demonstrate mutagenic or genotoxicity in numerous in vitro and in vivo bioassays. The evaluation of ETBE for genotoxicity and mutagenicity performed by the Japanese should also be included in the IRIS document.

Comments on the discussion of Maltoni's chronic carcinogenesis bioassay:

- 1) The Maltoni's study followed a lifetime protocol in which the rats are allowed to die rather than being sacrificed at a given data. This causes a lot of difficulty in analyzing the tumor response because tumor incidence is highly related to the age of the animals. Hence, a small difference in the age at death could greatly affect tumor incidence. Thus without knowing the age of the animals, especially those with tumors it is almost impossible to perform statistical analysis on a small change, up 20% change in tumor incidence.
- 2) The number of rats evaluated by histopathology is not given instead the number of animals at the start of the study is given and presumably used as the N in the statistical analysis. It is very unlikely that all the rats supplied suitable tissue for analysis and that all the histopathologic slides were suitable for analysis, especially since the animals were allowed to die rather than being euthanized. Thus, the actual number of rats evaluated needs to be determined in order to confirm the statistical analysis.
- 3) Page 29. Where dose the 100 rats in the 250 mg/kg-day come from, since there were only 60 rats in this treatment group. Page 31, Line 26 and 27. You can not increase the N to 100 in order to increase the power of an assay and to obtain statistical significance. What was used as the N in the statistics, 60 or 100 for the number of rats?
- 4) Since the ETBE treated rats both the 250 and 1,000 mg/kg dosed groups live longer, it is likely that this is the reason for the slight, not statistically significant increase in oral dysplasias. Furthermore, the increase in oral lesions is found when these are added to the other lesions. Also, when as reported in Maltoni;'s article male and female results are added together, the incidence of total lesions is not significant.
- 5) The data for the uterus is negative. The only positive response reported in Maltoni's article involves the 250mg/kg dose group and requires adding 2 Schwannomas found in the area of the uterus and 4 Schwannomas found in the area of the vagina and uterus to the other tumors found in the uterus. These tumors of neural origin should not be added to the 4 other tumors. Furthermore, the carcinomas should not be added to the 2 Leiomyosarcomas. It is not appropriate to combine these tumors of different origin and etiology.
- 6) None of the effects reported by Maltoni demonstrated any dose relationship.

Section 4.7 needs to be re-written since as written it indicates that ETBE has been demonstrated to be carcinogenic in rats and that it has not been tested for mutagenic and genotoxic activity. For examples:

- 1) Page 73, line 8 states that ETBE has not been shown to act as a genotoxicant. Rather it should state: ETBE has been demonstrated not to be genotoxic or mutagenic in numerous and various in vitro and in vivo assays so that all the evidence demonstrates that ETBE is not genotoxic.
- 2) This section should state that ETBE was evaluated in one chronic carcinogenesis (rats) bioassay and that the results of the bioassay demonstrated that is not carcinogenic in rats. It can then give a very short summary of why the ovary and uterus results support this conclusion.
- 3) Any discussion of a carcinogenic mode of action should be deleted since there is no evidence for any carcinogenic activity, which would have to be target organ specific.

- C2. EPA did not derive a quantitative estimate of the carcinogenic potential of ETBE. Do the data support an estimation of a cancer slope factor for ETBE? If a quantitative estimate is proposed, please identify and provide a detailed description of the method(s) and approach(es) for deriving a cancer slope factor.

**Response:**

Since there is no evidence or data for a carcinogenic potential of ETBE, an estimate of a cancer slope factor can not be perform. Hence, the EPA is correct in not estimating a cancer slope factor and in not deriving quantitative estimate of the carcinogenic potential of ETBE.

**Lisa M. Sweeney, Ph.D., DABT, CHMM**  
Senior Scientist  
Toxicological Excellence for Risk Assessment (TERA)

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Lisa M. Sweeney, Ph.D., DABT, joined Toxicology Excellence for Risk Assessment (TERA) as a senior scientist in 2009. Lisa has a broad range of experience in the application of toxicology, chemistry, and engineering to problems in the health and environmental sciences. She has over 15 years experience in risk assessment, pharmacokinetics, and biochemical engineering from a variety of private sector and non-profit backgrounds. Her experience has focused on the development and refinement of physiologically-based pharmacokinetic (PBPK) models and their application to risk assessment and experimental design. She is an author of over 30 peer-reviewed publications, with 18 as first author. She has previously served as a councilor for the Society of Toxicology's Risk Assessment and Biological Modeling specialty sections.

Dr. Sweeney holds a bachelors of science in Chemical Engineering from Case Western Reserve University and Ph.D. in Chemical Engineering with a minor in Toxicology from Cornell University. She is a Diplomate of the American Board of Toxicology (DABT) and a Certified Hazardous Materials Manager (CHMM).

**Post-meeting comments**  
Prepared by Lisa M. Sweeney

**Responses to charge questions**

**General Charge Questions:**

*G1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?*

No, there are sections that are not “logical, clear and concise.” In particular, there are problems with the clarity of the toxicokinetics section. These concerns are detailed under “Other Comments”.

*G2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of ETBE.*

The Japanese studies noted in the public comments and Gordon Hard’s reanalysis of the kidney pathology slides for Medinsky et al. (1999) should be evaluated and considered by EPA.

**Chemical-Specific Charge Questions:**

***(A) Oral reference dose (RfD) for ETBE***

*Upon evaluation of the oral database, EPA determined that it was not possible to derive an oral RfD as the proposed composite uncertainty factor (UF) of 10,000 would lead to a value with an unacceptable level of uncertainty (see A Review of the Reference Dose and Reference Concentration Processes, U.S. EPA, 2002 for discussion of UFs). In lieu of deriving an RfD, the available data were used to derive an oral value (i.e., a minimal data value) for limited risk assessment purposes as discussed in Appendix C.*

*A1. The CIT (2004b) two-generation study of reproduction and fertility effects of oral exposure to ETBE was selected as the basis for the derivation of the minimal data value. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.*

While the CIT (2004b) study appears to be well-conducted, the recent Japanese 180-day oral toxicity study should also be considered. EPA should also consider route-to-route extrapolation from studies conducted by inhalation using a modification of the Nihlen and Johanson (1999) ETBE PBPK model. An assumption of 100% absorption appears reasonable, and a range of oral uptake rates could be tested, possibly using an absorption rate from MTBE as a baseline, with alternate rates spanning an order of magnitude. The designation of CIT (2004b) as the “principal study” is contingent upon working backward from the lowest potential oral reference value among multiple candidate endpoints and adequate studies, and is therefore contingent upon the proper selection of relevant endpoints, dose-response modeling, and uncertainty factors. If any of these other choices is altered, the standing of this study as “principal” will necessarily have to be reconsidered.

*A2. Increased kidney weight in F0 generation male rats (CIT, 2004b) was selected as the critical effect for the minimal data value resulting from oral exposure to ETBE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.*

Modest changes in bodyweight or tissue weights are generally considered non-adverse, in the absence of other histological changes. Other possible endpoints as delineated in EPA's assessment could be considered.

- A3. *Benchmark dose (BMD) modeling methods were applied to kidney weight data to derive the point of departure (POD) for the minimal data value. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., one standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.*

The most recent version of BMDS should be used.

- A4. *A total composite UF of 10,000 was used to derive a minimal data value for ETBE. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the minimal data value. If changes to the selected UFs are proposed, please identify and provide a rationale(s).*

Regarding the "minimal data value", I have little enthusiasm for the generation of such numbers given their strong potential for misuse and mischaracterization. I concur with the sentiments expressed at the meeting by Dr. Ginsberg that it would be preferable for EPA to provide their analysis on a potential point of departure and thoughts/considerations regarding potential values for the various uncertainty factors, but not actually calculated any minimal data values. The selection of uncertainty factors is also unclear, based on the scanty information provided in Appendix C. Inspection of Figure C-4 indicates that sub-chronic to chronic uncertainty factors were not applied for findings of absolute changes in liver weight in male F1 rats or kidney weight changes (relative and absolute) in F1 female rats, but were applied to relative liver weight for the same male F1 rats and absolute kidney weights for F1 male rats. If true, the lack of consistency requires justification. There appears to be "double dipping", using the lack of chronic studies both for UFS and to justify the value of the database factor.

#### **(B) Inhalation reference concentration (RfC) for ETBE**

- B1. The Medinsky et al. (1999) 13-week inhalation exposure study in mice and rats was selected as the basis for derivation of the RfC for ETBE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

As noted for the oral reference value, the designation of any study as the "principal study" is contingent upon working backward from the lowest potential reference value among multiple candidate endpoints and adequate studies, and is therefore contingent upon the proper selection of relevant endpoints, dose-response modeling, and uncertainty factors. If any of these other choices is altered, the standing of this study as "principal" will necessarily have to be reconsidered.

- B2. The occurrence of regenerative foci in the kidneys of male rats (Medinsky et al., 1999) was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

I am uncomfortable with how male rat kidney "regenerative foci" are treated in this assessment. Clearly, they are increased in ETBE treated animals, but they are also commonly occurring in controls (4/11). They are considered "indirect evidence of necrosis", and referred to as a "biomarker of an adverse effect". I am skeptical that it is appropriate to use this endpoint as the

basis of a toxicity reference value. A PubMed search for kidney and “regenerative foci” produced only 4 hits, and the Medinsky ETBE paper was one of them. I am not convinced that there is an extensive, persuasive literature on how these “regenerative foci” are “biomarkers” of an adverse kidney effect. EPA’s kidney expert should review Gordon Hard’s reanalysis, which indicates that these lesions are not relevant to humans.

- B3. *An analysis of the mode of action of kidney effects is presented in the Toxicological Review and a determination is made that the mode of action in male and female rats is unknown. Please comment on whether the analysis is scientifically justified.*

After providing a wealth of data for comparisons of ETBE to MTBE in earlier portions of the document, the Agency is largely silent on such comparisons throughout the MOA section (4.6.3). I am not a “kidney” expert, but my impression is that the (limited) data available for ETBE are suggestive of an alpha<sub>2</sub>u-related MOA, and that analogy to the more extensive/stronger MOA data for MTBE may tip the scales toward an alpha<sub>2</sub>u-related MOA that would then be “not relevant to humans.” EPA should expand the MOA section to discuss MOA data for this structurally-similar compound so that knowledgeable readers can more readily come to their own conclusions. With regard to the ETBE-, MTBE-, and TBA-specific data, it would be useful to see a summary of the data organized as a table in which the alpha<sub>2</sub>u globulin MOA criteria are listed, and the evidence for each criterion expressed as a scheme such as +++, ++, +, +/-, -, not available, etc. EPA should consider the new analyses by Gordon Hard, provided by LyondellBasell. Chronic progressive nephropathy is much better represented in the literature (65 PubMed hits) than “kidney regenerative foci” and is a reasonable mode of action for ETBE.

- B4. BMD modeling was applied to data for the mean number of regenerative foci in the kidneys to derive the POD for the RfC. Has the BMD modeling been appropriately conducted? Has the BMR selected for use in deriving the POD (i.e., one standard deviation from the control mean) been scientifically justified? Please identify and provide the rationale for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA’s approach.

The latest version of BMDS should be used. Lacking information on the biological significance of regenerative foci in the kidney, it is impossible to say what is an acceptable amount of change to their number.

- B5. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

The characterization of regenerative foci as “a biomarker of an adverse effect” rather than an adverse effect calls into question the use of default uncertainty factors with such an endpoint. The justification of uncertainty factors is very sparse. A 10-fold uncertainty factor for subchronic to chronic extrapolation should be reconsidered in light of (1) the use of an endpoint that is not demonstrably related to subchronic “toxicity” (only a “biomarker”) and (2) subchronic vs. chronic differences for the structurally similar chemical MTBE should also be considered as potentially indicative of what would be expected for ETBE. The 10-fold database factor also seems excessive given the lack of developmental toxicity and multigeneration by inhalation is partially compensated for by the fact that such studies are available for rats by the oral route and the use of the subchronic to chronic uncertainty factor. Findings of immunotoxicity in the G/ETBE mixture study provide minimal support for the database factor because EPA has not provided an assessment of the extent to which co-exposure to gasoline condensates may have elicited the effects observed in the G/ETBE mixture study. This study has not even been published. This reference does not serve as justification for a database adjustment for ETBE.

### (C) Carcinogenicity of ETBE

- C1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), the Agency concluded that there is *suggestive evidence of carcinogenic potential* following oral exposure to ETBE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

The numerous questions surrounding the conduct of studies at the Ramazzini Institute deserve to be directly addressed by EPA in their assessment. It does not appear that the study is suitable for use (qualitative or quantitative) in evaluating the carcinogenicity of ETBE. Should EPA come to a different conclusion on the merits of this study, they should propose at least one cancer mode of action for ETBE and provide an analysis of this (these) mode(s) of action as support for their classification of "suggestive evidence."

- C2. EPA did not derive a quantitative estimate of the carcinogenic potential of ETBE. Do the data support an estimation of a cancer slope factor for ETBE? If a quantitative estimate is proposed, please identify and provide a detailed description of the method(s) and approach(es) for deriving a cancer slope factor.

The data do not support an estimation of a cancer slope factor at this time.

### Other Comments for EPA

#### Section 3: Toxicokinetics

**General comment:** The toxicokinetics section has the feel of a document that was written one way, and then broken into pieces and rearranged, without re-reading the section to verify that it shows logical progression from one thought to another. Some of the specific comments provided below reflect the difficulties in following the narrative in its current construction.

#### Specific comments:

Page 6. If ETBE doses in inhalation studies are to be provided as mmol, it would also be helpful to provide the body weight, so that the reader can convert to a body-weight normalized dose.

Page 6. Acetone is introduced without any mention of it being an ETBE metabolite. I suggest adding a reference to the metabolism section and Figure 3-1 at this point.

Page 8. Table 3-1 and the discussion of this table might be easier to understand if "excretion" was clarified. Does this reflect only urinary excretion, or does it include respiratory excretion?

Page 8. The convention in the literature is to refer to "blood:air" partition coefficients as the equilibrium ratio of the concentration in blood divided by the concentration in air. The author of this section has reversed the nomenclature without reversing the reported values.

Page 8. The text says that "on a body weight basis, doses were about 500 times higher in rats than in humans". Assuming a 70 kg human and a 0.3 kg rat, and the doses listed in Table 3-1, the 4 ppm ETBE dose to the human was  $121 \text{ umol}/70 \text{ kg} = 1.5 \text{ umol}/\text{kg}$  while the rat dose was  $2.3 \text{ umol}/0.3 \text{ kg} = 7.7 \text{ umol}/\text{kg}$ . Thus the ratio of the rat dose was  $7.7/1.5 = 4.4$  fold different, not 500 fold.

Page 10. Table 3-2. The convention in the literature is to present tissue: blood partition coefficients (ratio

of equilibrium concentration in tissue divided by the concentration in blood. Again, the author has apparently reversed the nomenclature without inverting the values. This is evident from the text regarding the volume of distribution—for ETBE to have a higher apparent volume of distribution (as compared to MTBE), the fat:blood ratio needs to be higher for ETBE than MTBE (11.6 vs. 4.98).

Page 12. Second full paragraph. The text states that ETBE and TBA blood levels were measured in human volunteers exposed to ETBE. TBA data are found in the third paragraph, but the ETBE data are found on page 17!

Page 13. On this page there are (at least) 3 instances where “CYP” is misspelled as “CPY”.

Page 14. It is stated that the peak blood levels of ETBE and TBA were “much lower [in rats] than in humans”. As noted for Page 12, the human data have not yet been presented (they are on page 17). The statement is also incorrect, based on the values in the text. At 40 ppm, the peak human TBA level was 13.8 uM, while the peak rat level was 21.7 uM. At 4 ppm, the peak human TBA level was 1.8 uM and the peak rat level was 5.7 uM.

Page 15. On this page there are (at least) 3 instances where “CYP2E1” is misspelled as “CP2E1”.

Page 16. In the last paragraph, the author(s) speculate on the 4 apparent phases of elimination from the (presumably venous) blood after two hours of inhalation. They speculate that “the first phase likely indicate [sic] uptake into highly perfused tissues.” This reviewer disagrees. ETBE is eliminated from the body via metabolism and exhalation. After two hours it is likely that RPT (but not fat) approached equilibrium with blood. Initially, after the end of exposure, elimination from blood will be controlled by those the same process that describe the elimination from the body as a whole, plus distribution from RPT into fat. Once equilibrium with fat is established, the continuing supply of ETBE into the blood (to compensate for losses from exhalation and metabolism) will be from remobilization from SPT, then fat. The sensitivity analyses presented by Nihlen and Johanson (1999) indicate the key parameters (and thus key processes) which determine the post-exposure ETBE blood time course in humans.

Pages 16-17. How can the sum of metabolic clearance (0.39 L/hr-kg) and exhalation clearance (0.35 L/hr-kg) exceed the total body clearance (0.57 L/hr-kg)?

Page 22. It is noted that the Nihlen and Johanson (1999) ETBE PBPK model does not include a “slowly perfused tissues” compartment. This is not really an accurate characterization since the model includes “working muscle” and “resting muscle” compartments that would more commonly make up the majority of the lumped “slowly perfused tissues” compartment. If the volume and blood flow for the lumped RPT compartment are sufficient to account for the inclusion of skin in that compartment, all of the SPT are accounted for in the model structure.

#### **Section 4: Hazard Identification**

Page 29., last sentence. The numbers appear incorrect, or there is a mismatch between the data presented in table 4-1 and the text. The text describes “number of malignant tumors per 100 animals” whereas the table appears to present #s of animals with malignant tumors; if animals have more than one tumor, there could be a greater number of tumors per 100 animals. As is, malignant tumors in female rats dosed with 250 mg/kg ETBE (55 per 100) exceeds the percentage computed from the incidence in the table (21/60 = 35%). EPA should clarify.

Page 31, first full paragraph, 2<sup>nd</sup> to last sentence. Please correct grammar in “a survival analyses”.

Page 36. Page 55, section 4.5.1.2. The potential for evaporative losses of ETBE appears to be overstated. With a water:air partition coefficient of 8.4, ETBE will tend to remain in the test medium, rather than lost to headspace. If the relative volumes of medium and headspace could be determined from the report, a correction for loss to headspace could be approximated.

Pages 56-59. Are there any studies regarding neurobehavioral or immunotoxic effects of gasoline or gasoline condensate that could provide a context for ascertaining the contribution of ETBE condensate to effects observed in tests of gasoline/ETBE condensate? If not, EPA should say so. If yes, EPA should discuss.

Page 61, last full paragraph. CPY2A6 should be CYP2A6.

Page 83. The statement “With respect to ETBE toxicity, higher catalytic activity would signify potentially higher risk” implies a mode or modes of action related to ETBE metabolites rather than parent compound. Nowhere in this document does EPA indicate that they have come to a conclusion that ETBE toxicity is mediated through metabolites or parent compound. EPA should strike the risk-related statement on this page.

### **Section 5: Dose response assessments**

Page 99. If increased regenerative foci (and the related endpoint of Labeling Index) due to ETBE exposure are good biomarkers of renal effects, how come the only other indicators of nephrotoxicity in male rats have BMDLs nearly two orders of magnitude higher? The ability to reliably estimate a dose at which a 10% change relative to controls would occur is limited when the dose-response jumps from 4/11 in controls to 10/11, 11/11, and 11/11 in the dosed groups.

Page 103. This figure is very difficult to read. A higher quality figure should be prepared.

Page 105. How biologically significant is the increase of seminiferous tubules with degenerated spermatocytes, given the lack of effect in the two-generation reproductive toxicity study?



**Appendix A**  
**List of Reviewers**



# **Peer Review Workshop of EPA's Draft Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE) Human Health Assessment**

Holiday Inn Capitol  
Washington, DC  
January 26, 2010

## **Peer Reviewers**

### **Wolfgang Dekant, Dr. Rer.Nat.**

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**NOTE:** Dr. Marty conducted this review as a private consultant and not as a representative of the California EPA.



**Appendix B**  
**List of Observers**





# Peer Review Workshop of EPA's Draft Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE) Human Health Assessment

Holiday Inn Capitol  
Washington, DC  
January 26, 2010

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# **Appendix C**

## **Agenda**





# Peer Review Workshop of EPA's Draft Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE) Human Health Assessment

Holiday Inn Capitol  
Washington, DC  
January 26, 2010

## Agenda

- 8:00 a.m. Registration/Check in
- 8:30 a.m. **Welcome, Introductions, Meeting Purpose & Agenda**..... *Jan Connery, ERG (contractor)*
- 8:40 a.m. **EPA Welcome Remarks** ..... *Karen Hammerstrom, IRIS Deputy Director, EPA NCEA*
- 8:50 a.m. **Public Comment**..... *Jan Connery*
- 9:00 a.m. **General Questions**..... *Lawrence Lash (Chair) & Panel*
- G1.** Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?
- G2.** Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of ETBE.
- 9:30 a.m. **Oral Reference Dose (RfD) for ETBE**..... *Lawrence Lash & Panel*
- A1. Principal Study:** The CIT (2004b) two-generation study of reproduction and fertility effects of oral exposure to ETBE was selected as the basis for the derivation of the minimal data value. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.
- A2. Critical Effect:** Increased kidney weight in F0 generation male rats (CIT, 2004b) was selected as the critical effect for the minimal data value resulting from oral exposure to ETBE. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.
- A3. Point of Departure:** Benchmark dose (BMD) modeling methods were applied to kidney weight data to derive the point of departure (POD) for the minimal data value. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (i.e., one standard deviation from the control mean) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.
- 10:30 a.m. BREAK

10:45 a.m. **Oral Reference Dose (RfD) for ETBE** (cont.).....*Lawrence Lash & Panel*

**A4. Uncertainty Factors:** A total composite UF of 10,000 was used to derive a minimal data value for ETBE. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the minimal data value. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

11:10 a.m. **Inhalation Reference Concentration (RfC) for ETBE** .....*Lawrence Lash & Panel*

**B1. Principal Study:** The Medinsky et al. (1999) 13-week inhalation exposure study in mice and rats was selected as the basis for derivation of the RfC for ETBE. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study.

**B2. Critical Effect:** The occurrence of regenerative foci in the kidneys of male rats (Medinsky et al., 1999) was selected as the critical effect for the RfC. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Noon LUNCH

1:15 p.m. **Inhalation Reference Concentration (RfC) for ETBE** (cont.) .....*Lawrence Lash & Panel*

**B3. Mode of Action Analysis:** An analysis of the mode of action of kidney effects is presented in the Toxicological Review and a determination is made that the mode of action in male and female rats is unknown. Please comment on whether the analysis is scientifically justified.

**B4. Point of Departure:** BMD modeling was applied to data for the mean number of regenerative foci in the kidneys to derive the POD for the RfC. Has the BMD modeling been appropriately conducted? Has the BMR selected for use in deriving the POD (i.e., one standard deviation from the control mean) been scientifically justified? Please identify and provide the rationale for any alternative approaches (including BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

**B5. Uncertainty Factors:** Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

2:15 p.m. **Carcinogenicity of ETBE**.....*Lawrence Lash & Panel*

**C1. Weight of Evidence Determination:** Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), the Agency concluded that there is *suggestive evidence of carcinogenic potential* following oral exposure to ETBE. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

**C2. Quantitative Estimate.** EPA did not derive a quantitative estimate of the carcinogenic potential of ETBE. Do the data support an estimation of a cancer slope factor for ETBE? If a quantitative estimate is proposed, please identify and provide a detailed description of the method(s) and approach(es) for deriving a cancer slope factor.

2:45 p.m. BREAK

3:00 p.m. **Additional Discussion Issues** ..... *Lawrence Lash & Panel*  
3:30 p.m. **Reviewer Final Comments** ..... *Lawrence Lash & Panel*  
3:50 p.m. **Closing Remarks** ..... *Jan Connery & EPA/NCEA*  
4:00 p.m. ADJOURN