DISPOSITION OF PEER REVIEW COMMENTS FOR TOXICOLOGICAL PROFILE FOR METHYL *TERT*-BUTYL ETHER

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

June 2021

Peer reviewers for the third draft of the Toxicological Profile for Methyl tert-Butyl Ether were:

Clifford P. Weisel, Ph.D.
Professor
Environmental and Occupational Health Sciences Institute
SPH - Rutgers University
Piscataway, NJ

Deborah A. Cory-Slechta, Ph.D.
Professor of Environmental Medicine, Pediatrics and Public Health Sciences
Department of Environmental Medicine, Box EHSC
University of Rochester Medical Center
Rochester, NY

James V. Bruckner, Ph.D.
Emeritus
Interdisciplinary Toxicology Program, Professor
Pharmaceutical & Biomedical Sciences, College of Pharmacy
University of Georgia,
Athens, GA

Comments provided by Peer Reviewer #1

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 1: Adverse effects reported in humans exposed to MTBE vapor have been transient and quite limited. Descriptions of self-reported respiratory effects and mild CNS depression are of limited value, due to study limitations, confounding factors and recall bias. Marked gastrointestinal upset, mild liver injury and cholecystitis have resulted from leakage of MTBE during gallstone dissolution procedures

RESPONSE: *No response needed.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 2: Adverse effects observed in animals are not of significant concern in humans due to their generally mild nature and the very high doses/concentrations required to elicit them.

RESPONSE: *No response needed.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 3: Exposure conditions have been well reported for animal studies and two human experiments. No information was provided, however, on ambient MTBE levels in gasoline-associated exposures of study populations in Alaska, New York, Milwaukee, etc.

RESPONSE: *Exposure levels (when available) are reported for these populations in Table 2-1 in Chapter 2.*

Minimal Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 4: I agree that the descriptions of CNS depression in rats following acute ingestion of MTBE are qualitative, and that the male reproductive effects appeared to be transient. Nevertheless, the LOAELs for these effects were quite consistent. I agree that there are not suitable data for derivation of a chronic oral MRL.

RESPONSE: *No response needed.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

a. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 5: Calculation of human equivalent concentrations (HECs) for inhalation exposure is problematic. Use of the ratio of human and rat blood:gas partition coefficients to correct for greater uptake of MTBE into human blood in the alveoli is appropriate. Calculation of the HEC, however, does not take into account other key interspecies factors that determine the extent of systemic uptake and resulting CNS effects. Resting alveolar ventilation rates of rats and mice are as much as 11 and 23 times higher, respectively, than that of humans. Cardiac outputs/pulmonary blood flows are about 6 and 10 times greater than that of humans (Brown et al., 1997). Relative rates of cytochrome P450-mediated metabolism and tissue loading of volatile organic compounds (VOCs) are also important determinants of systemic uptake that are likely higher in rodents (NAS, 2009; Bruckner et al., 2019). The interspecies uncertainty factor (UF) of 3, even for an unlikely pharmacodynamic interspecies difference, is not warranted.

The intraspecies UF of 10 is too large. The National Academy of Sciences adopted an intraspecies UF of 3 for derivation of CNS depression-based human acute exposure guideline level (AEGL) values for toluene, trichloroethylene, tetrachloroethylene and other VOCs. This UF was based upon results of clinical investigations showing limited interindividual (including pediatric and geriatric populations) differences in sensitivity to inhaled VOC anesthetics (NAS/AEGL, 2009).

The method of calculation of the HEC, application of interspecies and intraspecies UFs of 3 and 10, and calculation of a BMCL resulted in an acute inhalation MRL of 2 ppm. This provisional value for acute exposure is unreasonably low, when the NOAEL of Daugherty et al. (1997) for modest reversible CNS effects was 800 ppm.

RESPONSE: *The calculation of the HEC value is consistent with the Agency for Toxic Substances and Disease Registry (ATSDR) and U.S. Environmental Protection Agency (EPA) practice[1]. Compound-specific data pertaining to human and animal toxicodynamics are inadequate to support the Reviewer's statement that pharmacodynamic interspecies differences are unlikely. Therefore, ATSDR has retained the default uncertainty factor of 3 for pharmacodynamic interspecies differences. ATSDR retained the standard uncertainty factor of 10 for human variability, as chemical specific data are not available to support use of a partial uncertainty factor.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 6: My comments about the inappropriate HEC calculation, unnecessary interspecies UF, and excessive intraspecies UF for the acute inhalation MRL apply to the intermediate-duration MRL and the chronic-duration MRL derivations.

RESPONSE: *Please see Response to Comment 5.*

---

[1]EPA. 1994. Methods for derivation of inhalation reference and concentrations and application of inhalation dosimetry. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA600890066F.

QUESTION: In addition, we are requesting feedback on the following for the intermediate oral MRL:

a. If the neurological endpoint is adequate given the quality of the study

COMMENT 7: The observation of hypoactivity in 4 of 10 rats administered >800 mg/kg is imprecise and semiquantitative at best, although there have been similar reports of induction of CNS depression in rodents dosed by (oral) gavage. It should be recognized that ingestion of a similar amount of MTBE in divided oral doses (as in drinking water) would not produce CNS depression, due to MTBE's relatively rapid elimination by metabolism and exhalation.

RESPONSE: *The neurological endpoint was retained as the critical effect for the provisional oral intermediate MRL. The statement below was added to the "Other Additional Studies or Pertinent Information that Lend Support to this MRL" section of the MRL worksheet in Appendix A to address potential role of rapid elimination in central nervous system (CNS) depression following gavage versus drinking water exposure. Since both MTBE and its primary metabolite (*tert-*butanol) are associated with CNS depression, rapid metabolism was not included in this statement.*

Since MTBE elimination is relatively rapid, lack of CNS depression in drinking water studies at similar overall doses may be due to dosing over time (as opposed to bolus gavage doses).

QUESTION:

b. If the use of a modifying factor is appropriate to account for the quality of the neurological study

COMMENT 8: A modify factor is unnecessary. The large intra- and interspecies UFs over-compensate for uncertainty of the LOAEL for hypoactivity.

RESPONSE: *ATSDR has re-evaluated the MRL derivation and has determined that the modifying factor is not needed. The revised provisional intermediate oral MRL is 0.6 mg/kg/day.*

QUESTION:

c. If male reproductive endpoints, specifically the BMDL of increased sperm abnormality, would be a better endpoint for intermediate oral MRL derivation.

COMMENT 9: Increase in abnormal sperm may be a preferable endpoint for calculation of an intermediate duration oral MRL. Li et al. (2008) also described abnormally arranged cells and shedding of cells of the seminiferous epithelium of rats given >800 mg/kg/day. Gholami et al. (2015) reported dose-dependent lesions in the seminiferous tubules of rats administered >400 mg/kg/day. These are more quantitative values than those associated with observations of CNS effects.

RESPONSE: *The neurological endpoint was retained as the critical effect for the provisional oral intermediate MRL despite semi-quantitative nature of data due to database support indicating that the CNS is a target of MTBE toxicity. At this point, support for male reproductive effects is weak, and effects are observed at doses higher than those associated with neurological effects. The MRL based on hypoactivity is expected to be protective of male reproductive effects.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 10: The authors of this chapter have done an excellent job compiling and summarizing study results and reaching overall conclusions about the potential adverse health effects of MTBE in different organ systems.

RESPONSE: *No response needed.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 11: Only a limited number of epidemiology and controlled human studies of MTBE have been conducted. Results of clinical (gallstone dissolution) investigations have largely been unremarkable and do not provide information on dose. Reports of self-reported neurological complaints are compromised by media/recall bias and concurrent exposures to multiple chemical and physical agents. Such limitations are adequately addressed throughout the chapter.

RESPONSE*: No response needed.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 12: I was impressed by the relatively large number of well-designed animal experiments that have been published in the scientific literature. A wide range of exposure levels/doses were utilized. It was often necessary to administer very high/large doses of MTBE to elicit adverse effects.

RESPONSE: *No response needed.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 13: Appropriate animal species (i.e., mice and rats) were used for most toxicity and carcinogenicity studies.

RESPONSE: *No response needed.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 14: Dose-response relationships, or their lack, have been adequately addressed in the descriptions of animal studies. Multiple dosage levels were not employed in the few investigations in humans.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 15: The chapter is quite complete in its coverage of published papers on health effects of MTBE. Li and Han (2006) did publish a report that up to 3,000 ppm MTBE were required to adversely affect mouse spermatogenic cells in vitro.

RESPONSE: *Results of this study were added to Section 2.16:*

Decreased viability, increased plasma membrane damage, and increased ratio of necrotic cells were observed in cultured spermatogenic cells following *in vitro* exposure to MTBE for ≥12 hours; findings were associated with altered sperm morphology (Li and Han 2006; Li et al. 2007).

COMMENT 16: Salimi et al. (2016) published an article relatively recently, in which relatively high concentrations of MTBE were said to generate oxidative species and lipoperoxidative changes, as well as injure intracellular organelles of human blood lymphocytes in vitro. The authors concluded in their abstract that trace concentrations of MTBE were capable of inducing oxidative stress and damage. I questions this, but do not have the complete manuscript to evaluate.

RESPONSE: *Results of this study were added to Section 2.19,* ***Mechanisms of Carcinogenicity*** *(pertaining to possible mechanisms of leukemia in female rats):*

Evidence of decreased viability associated with oxidative stress, lipid peroxidation, damage to mitochondria and lysosomes, and glutathione depletion was reported in human blood lymphocytes following *in vitro* exposure to MTBE (Salimi et al. 2016).

COMMENT 17: Sarhan et al. (2019) recently published an account of an experiment in which inhalation of 60 uL MTBE/day (vapor concentration/dose unclear) for 12 months produced lymphoid degenerative changes in the trachea and lungs of rats. An objective of the investigation was to identify an early blood biomarker for MTBE-induced cancer.

RESPONSE: *This study was not added to the profile. Inadequate details on exposure concentration, extremely short daily exposure (3 minutes/day), and qualitative reporting of histopathological findings preclude inclusion of this study in Chapter 2 (Health Effects) section of the profile. The proposed biomarkers of carbonic anhydrase I, carbonic anhydrase II, and peroxiredoxin 2 for early detection of tracheal and lung cancer are of questionable relevance because MTBE has not been associated with tracheal or lung cancer in humans or animals. Additionally, no exposure-related histological alterations of the respiratory tract were observed in intermediate- or chronic-duration inhalation studies in rats or mice exposed to concentrations as high as 8,000 ppm (Bevan et al. 1997b; Biles et al. 1987; Bird et al. 1997; Greenough et al. 1980; Lington et al. 1997).*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 18: I am not aware of any additional studies that may be relevant in deriving MRLs.

RESPONSE: *No response needed.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 19: Appropriate NOAELs and LOAELs, on spot-checking, appear to have been accurately identified.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 20: I agree, in general, with the categorizations of "less serious" and "serious" for the health effects in Chapter 2.

RESPONSE: *No response needed.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 21: The authors of Chapter 2 have done a very nice job discussing mechanisms of action of hepatotoxicity, nephrotoxicity, neurotoxicity and carcinogenicity. I am pleased to see the inclusion of information on mechanisms, including pertinent toxicokinetic and metabolism data.

RESPONSE: *No response needed.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 22: I believe that the chapter's authors have reached reasonable and appropriate conclusions in each subsection of the chapter.

RESPONSE*: No response needed.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 23: Volatile organic chemicals (VOCs) like MTBE exhibit asymptotic time-course profiles during inhalation exposures. Blood levels increase rapidly upon initiation of exposures, then attain near steady-state and continue to rise slowly (asymptotically) thereafter for the duration of continuing exposures.

Were the rats studied by Miller et al. (1997) exposed to neat (undiluted) MTBE? Was evaporation from the skin prevented by an occlusive dressing? High concentrations of VOCs can enhance their own absorption by defatting the skin and increasing dermal blood flow by causing irritation.
It should be kept in mind that the ^{14}C measured following administration of ^{14}C-MTBE represents metabolites as well as parent compound, including ^{14}C incorporated into macromolecules.

RESPONSE*: Text in Section 3.1.1 was revised to include additional information regarding exposure conditions. MTBE and* tert*-butanol levels in blood were directly measured using gas chromatography, so statements regarding MTBE detection and concentration in the plasma were not revised (radioactivity was used to determine distribution in tissues, excreta, and skin). To avoid confusion, the ^{14}C-label was removed (consistent with reporting for other routes from this study). Miller et al. (1997) do not report evidence of skin irritation or defatting. The only dermal irritation data available reports effects at much higher doses (10,000 mg/kg), including slight to severe erythema, blanching, epidermal thickening, acanthosis, or focal necrosis (ARCO 1980). It is unlikely that severe damage to the skin influenced absorption in the study by Miller et al. (1997).*

> In rats exposed to MTBE via 6-hour dermal application at 40 or 400 mg/kg in isotonic saline under occlusive conditions, MTBE was detected in plasma within 10 minutes following the initiation of treatment and peak plasma MTBE concentration was achieved within 2–4 hours after dosing (Miller et al. 1997).

COMMENT 24: Was the decrease in MTBE concentrations in fat from 6-15 weeks at the 50-ppm exposure level in the study of Savolainen et al (1985) attributable to microsomal (P_{450}) enzyme induction?

RESPONSE*: No changes in cytochrome P-450 concentrations in the liver were observed during the study. The only liver enzyme change reported was a transient, dose-related increase in microsomal UDP-glucuronosyltransferase activity at 2 weeks, but not at later timepoints.*

COMMENT 25: Wouldn't metabolic saturation by the higher (8,000 ppm) exposure level result in retention/accumulation of a higher % of the dose in tissues? Was adipose tissue content included/assessed by Miller et al. (1997)?

RESPONSE*: Support for the profile statement "The higher percentage of radioactivity in the tissues after the low dose may be due to shifts in metabolic and elimination pathways as enzyme systems become saturated at high doses" comes from evidence of altered excretion patterns at the high exposure level. At 8,000 ppm, an increased fraction of radioactivity in expired air (53.6%) was observed versus 400 ppm (21.2%), and unmetabolized MTBE accounted for a higher proportion of exhaled radiation at 8,000 ppm, compared to 400 ppm. A clarifying statement was added to Section 3.1.2:*

> The higher percentage of radioactivity in the tissues after the low dose may be due to shifts in metabolic and elimination pathways as enzyme systems become saturated at high doses (e.g., increased exhalation of unchanged MTBE at 8,000 ppm; see Section 3.1.4 for more details).

COMMENT 26: Was adipose tissue content included/assessed by Miller et al. (1997)?

RESPONSE: *Miller et al. (1997) did not report adipose tissue levels (only total tissue/carcass levels). However, the unpublished version of the studies (MTBE Committee 1990a, 1990b) reported radioactivity in various tissues throughout the carcass. Clarification that these data were from the unpublished versions of the studies was made in Section 3.1.2.*

In both single and repeated exposure studies, mean radioactivity in various tissues (e.g., liver, kidneys, lungs, heart, brain, gonads, femur, perirenal fat, muscle) was very low (<1% of the total dose), indicating that MTBE or its metabolites do not accumulate in tissues after short-term exposure (MTBE Committee 1990a, 1990b).

COMMENT 27: Did any research group investigate or determine the relative proportion of MTBE metabolized to formaldehyde and its subsequent products, versus tert-butanol and its products? It would be useful to understand the stoichiometry of the biotransformation of MTBE, particularly the extent of its conversion to potentially toxic or carcinogenic metabolites (e.g., formaldehyde, methanol).

RESPONSE: *Section 3.1.3 indicates that CYP-dependent demethylation of MTBE produces equimolar amounts of* tert*-butanol and formaldehyde.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 28: PBPK models have been adequately described. PBPD models have apparently not been developed for MTBE.

RESPONSE: *No response needed.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 29: It is not clear whether any of the researchers have run their models to simulate/assess the kinetics of MTBE in humans versus rodents.

RESPONSE: *Available application of physiologically based pharmacokinetic (PBPK) models is discussed in Section 3.1.5. The primary PBPK model was developed by Borghoff et al. (1996) in rats; however, the model has been expanded for use in humans (Blancato et al. 2007; Rao and Ginsberg 1997). For it to be used in risk assessment, further refinement is needed to decrease uncertainty in estimated exposure levels, particularly for humans (as discussed in Appendix A).*

Children and Other Populations that are Unusually Susceptible

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 30: I am not aware of any publications with data relevant to potential effects of MTBE on child health and development.

RESPONSE: *No response needed.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 31: Why would people who ingest phenobarbital or ethanol be more susceptible to MTBE? Does CYP2B1 play a major role in MTBE metabolism? CYP2E1 would likely be responsible for mediating the hydroxylation of tert-butanol, which may hasten the overall biotransformation of MTBE and urinary excretion of its major metabolites.

RESPONSE: *Section 3.2 (Children and Other Populations that are unusually susceptible) was revised to clarify the potential increase in susceptibility in individuals with exposure to CYP inducers.*

Studies in rats (Brady et al. 1990; Snyder 1979) indicate that exposure to microsomal inducers of CYP2B1 and CYP2E1 enhances metabolism of MTBE, suggesting that people who are exposed to inducers of CYP2B1 (e.g., phenobarbital) or CYP2E1 (e.g., acetone, alcohol) may be more susceptible to toxic effects mediated via MTBE metabolites. However, because the toxicity of MTBE relative to the toxicities of its metabolites is unknown, the relative susceptibility cannot be determined.

COMMENT 32: It is mentioned that the elderly may be more susceptible to MTBE nephrotoxicity. This seems unlikely, as levels found in drinking water are far lower than those that produced chronic progressive nephropathy in rats.

RESPONSE: *The intent of the Section 3.2 is to identify populations that, if exposed, may have increased susceptibility compared to an "average" individual exposed to the same concentration. The section does not state or imply that susceptible individuals are likely to observe effects at current levels found in drinking water (or other environmental media).*

Biomarkers of Exposure and Effect

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 33: It is clearly explained in the document that MTBE, tert-butanol and/or 2-hydroxybutyric acid could be monitored in human expired breath, blood or urine, but that this would have to be done soon after exposure, since they are so rapidly eliminated.

RESPONSE: *No response needed.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 34: There are no specific biomarkers of effect, as stated in the document.

RESPONSE: *No response needed.*

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 35: The discussion of interactive effects with other substances is adequate. It might be pointed out, however, that microsomal inducers would be expected to hasten MTBE metabolism and

diminish/shorten its CNS effects. Competitive metabolic inhibitors would be anticipated have the opposite effect.

RESPONSE: *Section 3.4 currently discusses potential effects of microsomal inducers. A statement regarding competitive metabolic inhibitors was added:*

> ". . . acetone and phenobarbital, as well as other inducers of these enzymes, would be expected to enhance the metabolism of MTBE. Conversely, competitive metabolic inhibitors may slow the metabolism of MTBE. Whether alterations in metabolism of MTBE would lead to greater or lesser toxicity is not clear, because the toxicity of MTBE relative to the toxicities of its metabolites is not known."

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 36: The text briefly addresses the potential of MTBE, through its capacity to induce CYP2B1 and CYP2E1, to potentiate the toxicity of other chemicals that are metabolically activated by these isoforms. Elovaara et al. (2007) demonstrated that MTBE did not potentiate the acute hepatoxicity of several chemicals. Induction of CYP2E1 my be of concern for some other agents such as nitrosodimethylamine. Nevertheless, MTBE and its metabolites are quickly eliminated and its P450-induction soon subsides.

RESPONSE: *Studies evaluating potential interactions between MTBE and other compounds metabolically activated by CYP2B1 or CYP2E1 (e.g., nitrosodimethylamine) were not identified.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 37: I am not aware of any incorrect or missing values.

RESPONSE: *No response needed.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 38: Information is presented on the liquid and gas phases/forms of MTBE.

RESPONSE: *No response needed.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 39: Considerable detail is provided on production, export, use and disposal of MTBE. I do not have additional references on these subjects.

RESPONSE: *No response needed.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 40: Detailed information is provided on release of MTBE to environmental media, and subsequent routes and sources of human exposure. Figures 51 and Table 56 provide details on the location of NDL sites where MTBE was detected and mean/median levels measured in water, soil and air at these sites. I do not have any additional references.

RESPONSE: *No response needed.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 41: Adequate information on transport, partitioning, transformation and degradation in environmental media is covered in the text.

RESPONSE: *No response needed.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 42: Data are provided in the text and tables on MTBE levels measured in ambient air, groundwater, soil and sediment in different location in the U.S.

RESPONSE: *No response needed.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 43: Sources and pathways of human exposure are adequately described. It appears that occupationally-exposed individuals involved in manufacture, formulation, storage and transport of MTBE are currently the only persons with the potential for exposure to toxicologically-relevant amounts of the chemical.

RESPONSE*: No response needed.*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 44: I was able to locate a few additional publications that provide results to fill a health effects data gap. These paper are referenced at the end of this report.

RESPONSE: *Please see responses below under suggested references.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 45: It was concluded that additional studies are needed to evaluate the ability of MTBE to cause injury of a number of organs/systems. In several cases oral studies were recommended, due to the lack of oral data. In most instances (e.g., gastrointestinal upset, CNS depression, male gonadotoxicity, nephrotoxicity) it is quite doubtful that levels of MTBE found in drinking water could even approach toxicity thresholds. Currently, persons with the highest potential exposure are workers who handle, transport or use MTBE-supplemented fuels. As inhalation is the major route of exposure, experiments might be conducted in which human volunteers are exposed in different workday scenarios to a series of vapor concentrations. The objectives would be to determine the thresholds for and dose-dependency of CNS effects, ocular/mucus membrane irritation, GI complaints, etc. under controlled laboratory conditions.

RESPONSE: *The highest potential exposure is for workers who produce, handle, or transport MTBE (or MTBE-containing fuels), and refinement of toxicity thresholds are needed. This concern is addressed in Section 6.2, "Epidemiology and Human Dosimetry Studies" section, which states that experimental studies in volunteers are needed to establish thresholds for irritation and CNS effects. However, there is also concern for potential exposure to the general population via drinking water, which is the reason low-dose oral studies are listed as a data need . Oral toxicity thresholds are needed in order to determine if levels of MTBE in drinking water are of concern.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 46: The data needs appear to be presented in a neutral, unbiased manner. It might be worthwhile, however, to indicate which data needs have the highest priority under present-day usage and exposure scenarios.

RESPONSE: *The purpose of Section 6.2 is to identify data gaps; the profile does not include a prioritization of the data needs. The Agency prioritizes the data needs for a particular compound in a separate document.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 47: I am not aware of any additional regulations or guidelines that should be included or removed.

RESPONSE: *No response needed.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 48: I am not aware of any additional regulations or guidelines that should be included or removed.

RESPONSE: *No response needed.*

Comments provided by Peer Reviewer #2

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 1: YES I AGREE WITH THE EFFECTS AS STATED

RESPONSE: *No response needed.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 2: NO OPINION

RESPONSE: *No response needed.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 3: NO. The indoor air levels are not adequately reported and there is no discussion of potential vapor intrusion of MTBE in homes above aquefiers that continue to be contaminated with MTBE.

RESPONSE: *ATSDR added studies to the profile regarding indoor air levels and vapor intrusion suggested by the Reviewer (see Responses to Comments 35 and 36 for details).*

Minimal Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 4: MRLs have been derived for neurotoxiciological endponts, which appear to be the most sensitive, so for other endpoints where data are sparce it is reasonable to not derive additional MRL. Further, inhalation exposure appears to be the most relevant route for which the MRLs are derived, while the data base for oral exposure route was deamed inadequate for acute or chronic exposures to derive MRLs. This seems appropriate.

RESPONSE: *No response needed.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

a. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 5: The MRLs and UF (generally 30 – 10 for animal to human and 3 for database – or – in one case 10 for human variability) appear to be justified within the document.

RESPONSE: *No response needed.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

In addition, we are requesting feedback on the following for the intermediate oral MRL:

a. If the neurological endpoint is adequate given the quality of the study
b. If the use of a modifying factor is appropriate to account for the quality of the neurological study
c. If male reproductive endpoints, specifically the BMDL of increased sperm abnormality, would be a better endpoint for intermediate oral MRL derivation.

COMMENT 6: These are outside my expertise to comment on

RESPONSE: *No response needed.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 7: Appears appropriate

RESPONSE: *No response needed.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 8: YES. The tables adequate describe the human studies.

RESPONSE: *No response needed.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 9: YES. The tables adequate describe the animal studies.

RESPONSE: *No response needed.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 10: Study species were overwhelming rat and mouse, with several rabbits. These species are commonly accepted for the endpoints being reported

RESPONSE: *No response needed.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 11: Appears adequate.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 12: No

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 13: No.

RESPONSE*: No response needed.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 14: No suggested changes

RESPONSE: *No response needed.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 15: No opinion.

RESPONSE: *No response needed.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 16: Outside my expertise to comment on

RESPONSE: *No response needed.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 17: *Appears appropriate but some sections are outside my area of expertise*

RESPONSE: *No response needed.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 18: Overall there is adequate discussion of ADME. Two additions that should be considered are: 1) in the metabolism or excretion section: human studies have shown difference in metabolism/ expiration by exposure route (as found for most compounds) with near complete metabolism following oral ingest with greater expiration following inhalation and intermediate for dermal (Amberg et al 2001, DeKant et al 2001, Prah et al 2004) These papers also provide information on the differences in how three compartment model pseudo half lives.

RESPONSE: *The Reviewer's suggestion regarding differences in extent of metabolism and a three-compartment model for elimination are supported by Prah et al. (2004). Conclusions of Amberg et al. (2001) and Prah et al. (2004) regarding extent of first-pass metabolism with oral exposure are in conflict. These data and discrepancies were added to the profile:*

Section 3.1.3: Prah et al. (2004) indicated that the degree of metabolism in humans differs based on exposure route, with increased metabolism to *tert*-butanol following oral ingestion (compared to inhalation or dermal exposure) due to first-pass metabolism. However, Amberg et al. (2001) indicated that MTBE biotransformation observed following oral exposure in humans is similar to what they observed for inhalation exposure (Amberg et al. 1999), with no evidence of significant first-pass metabolism. Higher exposure levels in the studies by Amberg et al. (1999, 2001), compared to the study by Prah et al. (2004) may contribute to this discrepancy (15 versus 2.8 mg in oral studies, 40 versus 3.1 ppm in inhalation studies).

Section 3.1.4: However, Prah et al. (2004) indicated that excretion via all routes in humans follows a three-compartment model. Half-lives for the first, second, and third compartment for blood were calculated to be 14.9, 102.0, and 417.3 minutes, respectively, for oral exposure to 2.8 mg; 1.9, 59.0,

and 313.7 minutes, respectively, for inhalation exposure to 3.1 ppm for 1 hour; and 5.5, 126.6, and 403.1 minutes, respectively, for dermal exposure to 51.3 µg/mL for 1 hour. For breath, first-, second-, and third-compartment half-lives following oral exposure were 13.0, 63.1, and 254.0 minutes, respectively. Half-lives in breath following inhalation and dermal exposure were only reported for the first and second compartment and were 30.2 and 265.7 minutes and 58.4 and 256.0 minutes, respectively.

COMMENT 19: 2) there are two USEPA Reports on MTBE Distribution in Humans after controlled exposures, one for inhalation and one for dermal exposure that should be considered.

Gordon, S. 2003 Inhalation Exposure to Methyl Tert-Butyl Ether (MTBE) and Dibromochloromethane (DBCM) Using Continuous Breath Analysis, EPA/600/R-05/095 https://nepis.epa.gov/Exe/tiff2png.cgi/P1004PZG.PNG?-r+75+-g+7+D%3A%5CZYFILES%5CINDEX%20DATA%5C00THRU05%5CTIFF%5C00001369%5CP1 004PZG.TIF

Gordon, S 2003 Human Exposure to Methyl tert-Butyl Ether (MTBE) While Bathing with Contaminated Water EPA/600?R-05/094 https://clu-in.org/download/contaminantfocus/mtbe/mtbe_dermal_report.pdf

These studies use isotopically labeled MTBE (D12) to remove background contributions to the biomarker measurements along with a real time instruments to track continuous breath concentrations.The latter provides additional data on dermal absorption.

RESPONSE: *These reports were added where applicable in Section 3.1.*

Section 3.1.1: Results from human studies that employed single inhalation exposures to MTBE at concentrations in the range of 0.5–75 ppm for time periods ranging from 30 minutes to 8 hours indicate that inhaled MTBE is rapidly absorbed from the respiratory tract (e.g., Amberg et al. 1999; Cain et al. 1996; EPA 2003a; Johanson et al. 1995; Lee et al. 2001; Nihlén et al. 1998b; Prah et al. 2004; Vainiotalo et al. 2007). For example, in a study of volunteers exposed at rest to airborne MTBE for 4 hours at 4 or 40 ppm, mean blood MTBE concentrations measured 1.9 and 6.7 µM, respectively, immediately following cessation of exposure (Amberg et al. 1999). Another study showed that following a 30-minute exposure to 0.5 ppm, the mean fraction of absorbed MTBE dose was 0.73, with blood levels of 0.9–2.5 µg/L at the end of exposure (EPA 2003a). The mean uptake residence time was 5.7 minutes.

Section 3.1.1: Another study evaluated dermal uptake in volunteers showering or bathing with water containing 150 µg/L MTBE for 30 minutes using continuous breath analysis (EPA 2003b). Small increases in breath concentrations of MTBE indicated dermal absorption from bath water, with the mean uptake residence time of 21.2 minutes. No measurable increase in exhaled MTBE was observed following a 30-minute shower (EPA 2003b).

Section 3.1.4: Two studies evaluated exhalation of isotopically labeled MTBE in volunteers during and after 30-minute inhalation exposure to 0.5 ppm or dermal exposure to 150 µg/L (EPA 2003a, 2003b). In the inhalation study, the fraction of exhaled $MTBE_{d12}$, compared to air concentrations, was 0.29 (EPA 2003a). The breath decay phase data, which fit a two-compartment model, estimated decay residence times of 3.8 and 61 minutes for the first and second compartments, respectively. In the dermal study, the fraction of exhaled $MTBE_{d12}$, compared to water concentrations, was 0.00011 (EPA 2003b). The mean residence time for decay (assumed one-compartment model) was 41.5 minutes.

COMMENT 20: *There is also an older study of toxicokinetics that was not cited though does not change any of conclusions.* Nihlen A, Lof A, Johanson G. Experimental Exposure to Methyl tertiary-Butyl Ether. *Toxicology and Applied Pharmacology*. 1998;148(2):274-280.

RESPONSE: *This Nihlen study (cited as Nihlén et al. 1998b) was already included in the profile.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 21: Is complete, though there is a recent thesis (2018) entitled SMITH, NIKKI SHAVON. A Comparison of Physiologically-Based Pharmacokinetic (PBPK) Models of Methyl-Tertiary Butyl Ether (MTBE). (Under the direction of Hien T. Tran and Marina V. Evans.) https://repository.lib.ncsu.edu/bitstream/handle/1840.20/35212/etd.pdf?sequence=1&isAllowed=y that examines the issue of kidney metabolism for renal tumors. However, I was unable to find the results from that dissertation published in the peer review literature.

RESPONSE: *The primary objective was to determine if added complexity to the lung compartment of the Blancato et al. (2007) model (adding the upper respiratory tract [URT]) resulted in better model predictions. The thesis concluded that adding the URT did not result in better fit of the data. A secondary objective of the thesis was to determine if adding kidney metabolism parameters contribute significantly to the model fit to male rat kidney tumor data, which is not relevant to human health because male rat tumors are mediated via the α2u-globulin-mediated carcinogenic mode-of-action. These non-peer-reviewed data do not critically impact PBPK model discussion or MRL derivation. ATSDR will search for results of this dissertation in the peer-reviewed literature during the post-public update literature search.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 22: Seems adequate (Section 3.1.6) but the amount of data available is limited

RESPONSE: *No response needed.*

Children and Other Populations that are Unusually Susceptible

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 23: Am not aware of any studies on children.

RESPONSE: *No response needed.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 24: Am not aware of population at a higher risk of susceptibility.

RESPONSE: *No response needed.*

Biomarkers of Exposure and Effect

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 25: MTBE, and t-butanol in expired breath and blood and the urinary metabolites of MTBE are appropriate biomarkers.

RESPONSE: *No response needed.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 26: As indicated in the text there are no specific biomarkers of effect for MTBE, though the effects listed when combined with an appropriate exposure characterization are appropriate.

RESPONSE: *No response needed.*

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 27: This section, 3.4, does not address interactive effects that might occur at hazardous waste sites. However, I did not find literature on that interaction at hazardous waste site, though it is likely that MTBE would exist at waste sites with other chemicals, especially compounds in gasoline but not exclusively. If waste leaches into the ground water MTBE moves at a different rate in the water aquifer than less water soluble compounds, such as aromatic and aliphatic hydrocarbons in gasoline, so the ratio of the components can change from what is present in the waste site compared to what individuals are exposed to. As mention in the document, if the MTBE percentage is high enough then it can increase the solubility of other non-polar compounds (such as BTEX) in the water so the change in relative compositions may be slowed. A sentence pointing to the discussion in Chapter 5 about the prevalence at hazardous waste sites that contain other chemicals would be appropriate.

RESPONSE: *The purpose of Section 3.4 is to evaluate potential interactions that would affect the toxicity of MTBE. Interactions in the environment that would result in alterations in chemical compositions or transport are outside the scope of the profile.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 28: The text mentions enhanced metabolism of MTBE when CYP2B1 and CPY2E1 are induced due to previous exposures to compounds metabolized by these enzyme systems. Induction of these enzymes are likely workers who are exposed to gasoline routinely, where acetone is used

extensively such as nail salons and other occupations that use these chemicals, and for individuals who consume alcohol and cigarettes regularly, though the entire impact of the induction of these enzymes on MTBE metabolism or toxicity is not fully known. It might be worth mentioning the groups that are most likely to have CYP2B1 and Cyp2E1 induction from an exposure perspective. I did not locate additional references that specifically mention the role of enzyme induction on MTBE metabolism or health.

RESPONSE: *Section 3.2 (Children and Other Populations that are unusually susceptible) was revised to clarify the potential increase in susceptibility in individuals with exposure to CYP inducers.*

> Studies in rats (Brady et al. 1990; Snyder 1979) indicate that exposure to microsomal inducers of CYP2B1 and CYP2E1 enhances metabolism of MTBE, suggesting that people who are exposed to inducers of CYP2B1 (e.g., phenobarbital) or CYP2E1 (e.g., acetone, alcohol) may be more susceptible to toxic effects mediated via MTBE metabolites. However, because the toxicity of MTBE relative to the toxicities of its metabolites is unknown, the relative susceptibility cannot be determined.

COMMENT 29: The paper Fiedler N, Kelly-McNeil K, Mohr S, et al. Controlled human exposure to methyl tertiary butyl ether in gasoline: symptoms, psychophysiologic and neurobehavioral responses of self-reported sensitive persons. *Environ Health Perspect*. 2000;108(8):753-763. doi:10.1289/ehp.00108753 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1638278/ notes differences in response between individuals exposed to gasoline containing 11% MTBE, gasoline containing 15% MTBE, and gasoline without MTBE. This paper is discussed in section 3.2 under susceptible populations. It is not clear whether this is an interaction effect since an exposure to just MTBE at the same concentration as MTBE in gasoline was not done, but a sentence alerting to this paper could be useful since the effects did not seem to follow a purely dose response effect based on what was seen in other studies on MTBE exposures and therefore may be related to an interaction effect.

RESPONSE: *The study design in the paper by Fielder et al. (2000) is inadequate to evaluate potential interaction between MTBE and other gasoline components because an MTBE-only exposure group was not included. It is unclear how effects in this study, which is primarily focused on "sensitive" individuals, can be compared to other MTBE studies to evaluate a "purely dose response effect" or lack thereof.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 30: Appears complete, though adding a soil water distribution coefficient would be useful since MTBE adsorption onto soils is important in understanding its fate in the environment. One source of information is Mark H. Greenwood, Ronald C. Sims, Joan E. McLean, William J. Doucette & Jeffrey Kuhn (2007) Sorption of Methyl **tert** -Butyl Ether (MTBE) and **tert** -Butyl Alcohol (TBA) to Hyporheic Zone Soils, Soil and Sediment Contamination: An International Journal, 16:4, 423-431, DOI: 10.1080/15320380701404672
https://www.tandfonline.com/doi/abs/10.1080/15320380701404672?scroll=top&needAccess=true&journalCode=bssc20

RESPONSE: *The log K_{oc} of 2.13 (Greenwood et al. 2007) was added to Table 4-2.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 31: Don't understand what is being asked relative to forms. The typical data are provided for MTBE as a chemical.

RESPONSE: *No response needed.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 32: Appears complete

RESPONSE: *No response needed.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 33: The report gives general information about the extent and distribution of NPL sites. I don't know if it is available, but providing information on potential contamination of drinking water sources by NPL sites and the proximity of sites to residential areas (for the discussion below about vapor intrusion) would be a helpful addition to the report. That information can provide more insight on where potential current exposures might occur.

RESPONSE: *Although EPA provides a map of NPL sites nationwide, the information requested by the Reviewer is not readily available.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 34: There are some additional studies examining potential biodegradation by bacteria in soils using laboratory based techniques that should be included. These may reflect degradation process over long time period or methods for controlled biodegradation as part of remediation responses. e.g.

Alfonso-Gordillo, Guadalupe et al. " Biodegradation of Methyl Tertiary Butyl Ether (MTBE) by a Microbial Consortium in a Continuous Up-Flow Packed-Bed Biofilm Reactor: Kinetic Study, Metabolite Identification and Toxicity Bioassays." PloS one vol. 11,12 e0167494. 1 Dec. 2016, doi:10.1371/journal.pone.0167494 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5132332/

Li S, Zhang D, Yan W. Enhanced Biodegradation of Methyl tert-butyl-ether by a Microbial Consortium. Current microbiology. 2013;68(3):317-323. doi:10.1007/s00284-013-0480-9

Bianchi E, Censabella I, Fascetti E. Aerobic biodegradation of MtBE in an upflow fixed bed reactor. Journal of Chemical Technology & Biotechnology. 2009;84(6):871-876. doi:10.1002/jctb.2133

Also studies have shown than MTBE can biodegrade in microaerobic or mild aerobic conditions as opposed to anaerobic conditions.

e.g. Martienssen et al Determination of naturally occurring MTBE biodegradation by analysing metabolites and iodegradation by-products, Journal of Contaminant Hydrology, 87(1–2), 37-53, 2006, https://doi.org/10.1016/j.jconhyd.2006.04.007.

Also under anaerobic conditions when isotopic labeling was used for confirming degradation products

Kuder T, Wilson JT, Kaiser P, Kolhatkar R, Philp P, Allen J. Enrichment of Stable Carbon and Hydrogen Isotopes during Anaerobic Biodegradation of MTBE: Microcosm and Field Evidence. Environmental Science & Technology. 2005;39(1):213-220. doi:10.1021/es040420e

My examination of the literature on biodegradation is not all inclusive as this is fairly extensive and well studied area. The references provide are just a subset of that published, particularly if remediatioin processes are to be included in the document.

RESPONSE: *ATSDR has added data from Alfonso-Gordillo (2016), Bianchi et al. (2008), and Li et al. (2014) to Sections 5.2.4 (Disposal) and 5.4.2 (Transformation and Degradation) to address Reviewer's concerns. Please note that while the Li et al. (2014) paper suggested by the reviewer was available online in 2013, the publication date for the citation is 2014. ATSDR has also added data from Martienssen et al. (2006) and Kuder et al. (2005) to Section 5.4.2.*

Section 5.2.4: Enhanced biodegradation of MTBE can be accomplished using degrading bacterial consortiums which can then be used to clean up contaminated soils or water (Li et al. 2014). Bioremediation methods have been used to remove MTBE from aqueous solution such as gasoline-contaminated waters using continuous up-flow packed-bed biofilm reactors (Alfonso-Gordillo et al. 2016; Bianchi et al. 2009).

Section 5.4.2: Li et al. (2014) used mixed microbial cultures to identify and isolate various strains of bacterium that could use MTBE as a sole carbon source. Other investigators studied the aerobic biodegradation of MTBE in a microbial consortium using a continuous up-flow packed-bed biofilm reactor (Alfonso-Gordillo et al. 2016). While MTBE was shown to be toxic to the microbes at high loading rates, lower levels of MTBE could be degraded in the bioreactor with a theoretical chemical oxygen demand of up to 90%.

Section 5.4.2: Kuder et al. (2005) utilized a novel approach using compound-specific stable isotope Analysis (CSIA) to study the anaerobic biodegradation mechanisms of MTBE in enrichment cultures and field studies. Following the isotopic fractionation allows for a better understanding of the degradation of MTBE in gasoline plumes since following the concentration of MTBE's main metabolite, *tert*-butyl alcohol is confounded in plumes since it is often a constituent in gasoline anyway. Martienssen et al (2006) studied the degradation of MTBE in a contaminated groundwater plume located in Leuna (eastern Germany). They determined that degradation occurred primarily under microaerobic conditions with little or no degradation under anoxic conditions.

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 35: The text is incomplete on reporting indoor air measurements of MTBE. Since people spend the majority of their time indoors these studies should be included. There are differentials in the

concentration dependent upon whether a car was parked or gasoline was stored in a garage attached to a home when MTBE was in the fuel. Reports of indoor levels include, but are not limited to :

C.P. Weisel, J. Zhang, B.J. Turpin, M.T. Morandi, S. Colome, T.H. Stock, *et al.* **Relationships of Indoor, Outdoor, and Personal Air (RIOPA): part I. Collection methods and descriptive analyses,** HEI Report No. 130 (Pt. 1) Health Effects Institute, Boston, MA (2005) NUATRC Report No. 7, Houston, TX: National Urban Air Toxics;

Hun DE, Corsi RL, Morandi MT, Siegel JA. Automobile proximity and indoor residential concentrations of BTEX and MTBE. *Building and Environment*. 2011;46(1):45-53. doi:10.1016/j.buildenv.2010.06.015,

S.N. Sax, D.H. Bennett, S.N. Chillrud, P.L. Kinney, J.D. Spengler **Differences in source emission rates of volatile organic compounds in inner-city residences of New York City and Los Angeles** J Expo Anal Environ Epidemiol, 14 (2004), pp. S95-S109,

Dodson RE, Levy JI, Spengler JD, Shine JP, Bennett DH (2008) Influence of basements, garages, and common hallways on indoor residential volatile organic compound concentrations. Atmos Environ 42(7):1569–1581. https://doi-org.proxy.libraries.rutgers.edu/10.1016/j.atmosenv.2007.10.088

NYSDOH (New York State Department of Health). 2006. Study of Volatile Organic Chemicals in Air of Fuel Oil Heated Homes. In: Final NYSDOH Soil Vapor Intrusion Guidance. Appendix C.1. http://www.health.state.ny.us/environmental/investigations/soil_gas/svi_guidance/docs/svi_appendc.pdf.

and outside of the US in Canada

Zhu, J., R. Newhook, L. Marro, and C. Chan. 2005. Selected volatile organic compounds in residential air in the City of Ottawa, Canada. *Environmental and Science Technology* **39**, no. 11: 3964–3971.

and in S. Korea

Jo WK, Kim KY, Park KH, Kim YK, Lee HW, Park JK. Comparison of outdoor and indoor mobile source-related volatile organic compounds between low- and high-floor apartments. *Environ Res*. 2003;92(2):166-171. doi:10.1016/s0013-9351(03)00013-6

Another potential source of MTBE to indoor that will be discuss in the next comment is vapor intrusion into buildings over MTBE contaminated aquifers.

There is a report of air monitoring done in Detroit, MI, Detroit Air Toxics Initiative, Risk Assessment Report, DEQ, 2005 that includes measurement of MTBE in ambient air https://www.michigan.gov/documents/DATI_-_COMPLETE_FINAL_REPORT_11-9-05_142053_7.pdf

RESPONSE: *ATSDR has revised the Air monitoring Section 5.5.1 to include information from all eight suggested citations.*

Section 5.5.1 (outdoor Air): The Michigan Department of Environmental Quality monitored air samples at nine locations around the city of Detroit to gather air quality data in 2001–2002 (Michigan DEQ 2005). MTBE was detected in 72 out of 480 samples with a maximum concentration of 2.11 µg/m^3 (0.584 ppbv). Outdoor MTBE levels were measured in New York City, New York and Los Angeles, California (Sax et al. 2004). Median levels in the winter and summer months in New York were 10.0 and 10.9 µg/m^3 (2.77 and 3.02 ppbv), respectively. In Los Angeles, the median levels were 16.0 and 13.0 µg/m^3 (4.43 and 3.60 ppbv) in the winter and fall, respectively.

Section 5.5.1 (Indoor Air): A research initiative by the Health Effects Institute (HEI) measured indoor, outdoor, and personal exposure concentrations of pollutants between the summer of 1999 and the spring of 2001 that included measurements obtained from 100 homes in Los Angeles, California; Houston, Texas; and Elizabeth New Jersey (HEI 2005). The mean concentration of MTBE in indoor air samples was 11.8 µg/m^3 (3.27 ppbv) with 93% of all samples (N=553) at or above the detection limits. Hun et al. (2011) also analyzed indoor air data collected from the HEI and determined that homes with attached garages had higher indoor air levels as compared with homes that did not have attached garages, presumably due to automobile exhaust that infiltrated the residences. The mean MTBE levels in garages of residences located in Boston, Massachusetts were reported as 131 µg/m^3; (36.3 ppbv), whereas the ambient outdoor levels were 1.2 µg/m^3 (0.33 ppbv) (Dodson et al. 2008). Indoor air levels in randomly sampled homes located in Ottawa, Canada during the winter of 2002–2003 ranged from 0.025 to 3.32 µg/m^3 (0.0069–0.920 ppbv) and the detection frequency was 9% (Zhu et al. 2005). MTBE was detected in indoor air of homes that heat with fuel oil at a mean concentration of 11.8 µg/m^3 (3.27 ppbv) in air sampling conducted by the New York State Department of Health (NYSDOH) from 1997 to 2003. (NYSDOH 2006). Jo et al. (2003) analyzed outdoor and indoor air MTBE levels as a function of height in 56 high-rise apartment buildings located in South Korea and found levels were significantly greater for lower floor apartments than for the higher floor apartments most likely due to closer proximity to mobile sources. These indoor air levels are not likely to be relevant to exposure levels currently in the United States since MTBE is no longer used as a gasoline additive.

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 36*:* One potential exposure pathway that still exists but is not discussed in the document is vapor instrusion from contaminated aquifiers below buildings, particularly residences. Penetration of soil gases into homes is most commonly documented for radon, but also exists for volatile organic compounds that have contaminated soil or aquifiers such as MTBE (Review of models Ma J, McHugh T, Beckley L, Lahvis M, DeVaull G, Jiang L. Vapor Intrusion Investigations and Decision-Making: A Critical Review. Environmental science & technology. 2020;54(12):7050-7069. doi:10.1021/acs.est.0c00225, *A* Review of this issue by ATSDR Investigators is: Burk T, Zarus G. Community exposures to chemicals through vapor intrusion: a review of past agency for toxic substances and Disease Registry public health evaluations. J Environ Health. 2013;75(9):36-41.)

If soil gas vapors penetrate in to homes they will be mixed throughout the house exposing all residents, though there will be differential exposures depending upon where an individual spends the majority of his or her time while at home and the ventilation conditions within the home (Du L, Batterman S, Godwin C, Rowe Z, Chin JY. Air exchange rates and migration of VOCs in basements and residences. Indoor Air. 2015;25(6):598-609. doi:10.1111/ina.12178).

Two papers that specifically discuss MTBE associated with vapor intrusion are: Sanders PF, Hers I. Vapor Intrusion in Homes over Gasoline-Contaminated Ground Water in Stafford, New Jersey. Groundwater Monitoring & Remediation. 2006;26(1):63-72. doi:10.1111/j.1745-6592.2006.00048.x who reported elevated MTBE indoor air concentration in home above a contaminated aquifer in NJ and due to the slower degredation of MTBE in soil and water than other petroleum constitutents, it was elevated to a greater extent than BTEX compounds; and Ma J, Xiong D, Li H, Ding Y, Xia X, Yang Y. Vapor intrusion risk of fuel ether oxygenates methyl tert-butyl ether (MTBE), tert-

amyl methyl ether (TAME) and ethyl tert-butyl ether (ETBE): A modeling study. Journal of Hazardous Materials. 2017;332:10-18. doi:10.1016/j.jhazmat.2017.02.057 which presents a vapor instrusion mathematical model to predict indoor MTB and other fuel ether oxygenates. This potential exposure pathway will expose a much smaller number of people to MTBE than when it was being uses as a gasoline additive. However, it potentially will result in current exposures to subpopulation and while the peak exposures will be lower than previously encounter, the exposure is expected to be over a longer duration since people spend the majority of their time indoors at home. This is particularly true for newborne through toddlers and the elderly, to populations that are susceptible to neurological toxicants.

RESPONSE: *ATSDR has added MTBE-specific vapor intrusion model data and monitoring data from Ma et al. (2017), Sanders and Hers (2006), and Burk and Zarus (2013) to Chapter 5. ATSDR did not add the general references regarding vapor intrusion (Du et al. 2015; Ma et al. 2020) because they do not have MTBE-specific data. Additionally, monitoring data from the EPA (2011) report (Background indoor air concentrations of volatile organic compounds in North American residences (1990–2005): A compilation of statistics for assessing vapor intrusion) was added to address the Reviewer's concern.*

Section 5.1: Exposure to MTBE from indoor air by vapor intrusion can occur if the residence is near a contaminated aquifer.

Section 5.5.1: Vapor intrusion may also be a potential source of MTBE exposure, as vapor intrusion has been observed for several VOCs with similar properties. EPA's compilation of four studies of background indoor air concentrations found a 9–70% detection rate for MTBE in 502 U.S. resident samples between 1990 and 2005 (EPA 2011). The background medians ranged from 0.025 to 3.5 µg/m^3, 95th percentiles ranged from 71 to 72 µg/m^3, and maximum values ranged from 3.3 to 470 µg/m^3. ATSDR did not find MTBE to exceed any ATSDR vapor intrusion comparison values from air, soil gas, or groundwater in a review of 148 public health assessments published between 1994 and 2010 (Burk and Zarus 2013). Ma et al. (2017) developed a numerical model that used groundwater monitoring data from the EPA Underground Storage Tank program to estimate the potential vapor intrusion into buildings depending upon the characteristics of the buildings, soils, and MTBE level in groundwater. Their findings indicated that indoor air concentrations can exceed the EPA indoor air screening level for MTBE for highly contaminated groundwater plumes. Sanders and Hers (2006) analyzed indoor air in buildings potentially affected by contaminated groundwater due to a leaking underground gasoline storage tank in Stafford Township, New Jersey. Groundwater levels of MTBE ranged from 0.370 to 590 mg/L at five sampling locations. Indoor air was sampled on the main floor, in the basement, and under the foundation slab. In the location with the highest groundwater MTBE level, the indoor air concentrations were reported as 130 and 52.0 µg/m^3 (36.0 and 14.4 ppbv) in the basement and main floor, respectively. The vapor concentration of MTBE 2 m below the slab was 18,000 µg/m^3 (~5,000 ppbv).

Section 5.6: Vapor intrusion of MTBE into buildings and residences from contaminated groundwater may result in indoor air inhalation exposure.

COMMENT 37*:* In the discussion of the NHANES data it would be appropriate to reference Silva LK, Espenship MF, Pine BN, Ashley DL, De Jesús VR, Blount BC. Methyl Tertiary-Butyl Ether Exposure from Gasoline in the U.S. Population, NHANES 2001-2012. Environmental health perspectives. 2019;127(12):127003-. doi:10.1289/EHP5572 even though the tables presented in the report include more recent data than presented in that paper. The data for 2013-2014 and 2015-2016 no longer show measurable concentration of MTBE in blood. Thus, the MTBE blood levels in the latest NHANES data are below detection even at the 95th percentile. This is encouraging and suggests that the number of people in the US still being exposed to MTBE from previously contaminated soil and water is low.

RESPONSE: *ATSDR added the data requested by the Reviewer from Silva et al. (2019) to Section 5.6:*

Silva et al. (2019) analyzed NHANES data prior to the discontinued use of MTBE and afterwards. They determined that the unweighted proportion of the individuals with MTBE blood levels above the limit of detection (LOD) for years 2001–2002 was 93%; this dropped to 25.4% of the population for the period 2011–2012.

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 38: No

RESPONSE: *No response needed.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 39: As described in the above answers, there is a data gap in knowledge of potential on-going population exposure via increases in indoor air from vapor intrusion into homes. This can be addressed by better identifying locations where contaminated soil and aquifers exists near residential areas, measurements of indoor air in homes predicted to be impacted, and measurements of blood levels of the potentially impacted populations. As part of the distribution analysis of locations of contaminated aquifers and soil an evaluation of present or future potential impacts on drinking water supplies, both public and private well, should be done to predict and avert potential exposures.

RESPONSE: *ATSDR has added this as a data gap to Section 6.2, Exposure Levels in Environmental Media:*

There is a data gap in knowledge of potential ongoing population exposure via increases in indoor air from vapor intrusion into homes or buildings that are near contaminated groundwater (e.g., NPL sites, leaking underground storage tanks).

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 40: None were noted.

RESPONSE: *No response needed.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 41: No

RESPONSE*: No response needed.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 42: No

RESPONSE: *No response needed.*

COMMENT 43: No further comments.

RESPONSE: *No response needed.*

Annotated Comments

The Reviewer suggested a number of editorial revisions. The suggested revisions were made to the profile. Responses to Reviewer comments that were not considered editorial or stylistic are presented below.

COMMENT 44: Referring to a typographical error, the Reviewer commented "Line 2702 '0*tert*-butanol appeared' should be '*tert*-butanol appeared '"

RESPONSE: *The typographical error was fixed.*

COMMENT 45: Referring to a typographical error, the Reviewer commented "Line 2740 'Modeling of selected scenarios to allows for' should be 'Modeling of selected scenarios allows for'"

RESPONSE: *The typographical error was fixed.*

COMMENT 46: Referring to a typographical error, the Reviewer commented "Line 3082 'coefficient (K_{oc}) of MTBE indicates that it possess high mobility in soil' should be 'coefficient (K_{oc}) of MTBE indicates that it possesses high mobility in soil'"

RESPONSE: *The typographical error was fixed.*

COMMENT 47: Referring to the statement in Section 5.4.2, line 3212— While acetone would be relatively resistant to further OH radical degradation, there is very little research on the reactivity of the *tert*-butyl formate degradation products, with available research suggesting atmospheric residence times of up to 15 days (Cox and Goldstone 1982). — the Reviewer commented "The paper Pimentel AS, Tyndall GS, Orlando JJ, et al. Atmospheric chemistry of isopropyl formate and tert-butyl formate. *International Journal of Chemical Kinetics*. 2010;42(8):479-498. doi:10.1002/kin.20498 provides more updated information."

RESPONSE: *No change was made based upon this comment. The reference cited provided the reaction rate for tert-butyl formate with Cl rather than hydroxyl radicals and thus was not included.*

COMMENT 48: The Reviewer made the following comment referring to in Section 5.4.1 regarding Transport and Partitioning: "Lines 3188 -3189 In reviewing data on MTBE levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable."

RESPONSE: *ATSDR added the following statement to Section 5.4.1:*

It is also true that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

COMMENT 49: Referring to the discussion in Section 6.2, Bioavailability from Environmental Media (Lines 3622-3688), and the references (Fujiwara et al. 1984; Mackay et al. 1993), the Reviewer made the following comment: "I find the discussion about bioavailability at little confusing. Bioavailability and bioconcentrate are not the same. Unfortuantely, I was unable to review the two references for bioconcentrate given as one was in Japanese and the second in a book I could not access. Bioavailability refers to whether an agent is readily absorbed into the body and potentially interacts with the body systems, such as being metabolized or absorbed, as opposed of be eliminated from the body attached to a physical or chemical agent without being aborbed. That does not seem to be the case for MTBE. Certainly all MTBE inhaled or consume in water is bioavailable. Some MTBE could be adsorbed onto soil particles and therefore if ingested not bioavailable, but ingestion of MTBE as part of soil is a very minor pathway for exposure. The above sentences need clarification."

RESPONSE: *ATSDR revised Section 6.2 (Bioavailability from Environmental Media):*

There is no indication that MTBE is a concern in any raw or processed food items. MTBE is highly volatile and shows little tendency to sorb to soil particles; therefore, even if it is in bioavailable form, it is not likely to be found in soils except those contaminated by leaking underground storage tanks.

COMMENT 50: Referring to the statement in Section 5.7, line 3366-3369— The geometric mean urinary MTBE levels for the children living close to the refinery were 0.79 µg/L (evening) and 0.82 µg/L (morning). The geometric mean urinary MTBE levels for the population living far-removed from the refinery were 0.56 µg/L (evening) and 0.59 µg/L (morning) — the Reviewer commented, "These sentences suggest that there is a difference between the evening and morning in addition to living close to more distant from refineries. Providing the geometric standard deviation with those values and whether any of the differences were statistically different should included to strengthen the sentences."

RESPONSE: *The study used two sampling times during the course of the day. The statistical analysis showed significantly greater exposure to the population near the facility. ATSDR added the following sentence to Section 5.7 for clarification:*

Levels of urinary MTBE and other compounds consistent with gasoline exposure were significantly higher at two sampling times (morning and evening) for the group residing near the refinery as compared to the group 70 km away.

COMMENT 51: Referring to the statement in Section 6.2, Epidemiology and Human Dosimetry Studies, line 3523— Experimental studies of volunteers exposed to realistic exposure levels for longer durations are needed to establish the threshold for irritation and mild CNS effects (available controlled exposure studies are of brief duration and do not identify a threshold) — the Reviewer commented " Rather than using the words "brief duration" specify the range of durations studied."

RESPONSE: *Text in Section 6.2, Epidemiology and Human Dosimetry Studies was revised:*
Experimental studies of volunteers exposed to realistic exposure levels for longer durations are needed to establish the threshold for irritation and mild CNS effects (available controlled exposure studies are of brief duration [≤2 hours] and do not identify a threshold).

COMMENT 52: Referring to the statement in Section 6.2, Biomarkers of Exposure and Effect, line 3537-3542—The amount of MTBE in blood or expired air appears to be the most useful biomarker of exposure because much of the absorbed MTBE is excreted unchanged in expired air (MTBE Committee 1990a, 1990b). In addition, expired air or blood levels of its metabolite, *tert*-butanol, can be useful indicators of MTBE exposure — the Reviewer commented "While the above statements about MTBE in blood and expired air as biomarkers can be true, caveats should be provided about its halflife in the body and when the samples are collected relative to when the exposure occurred. Markedly different levels would be measured in both blood and expired air if the samples were taken within seconds, minutes or hours after exposure."

RESPONSE: *The usefulness of these biomarkers are discussed in Section 3.3.1 (Biomarkers of Exposure), specifically how expired air is only useful for recent exposures due to short half-life in the body. Further discussion of this is unwarranted in the Section 6.2, which is focused on identifying data needs pertaining to biomarkers of exposure. Since half-life is known, this is not a data need.*

COMMENT 53: Referring to Table 5-9, the Reviewer made the following comment: "Are the tables for NHANES Mean and Selected Percentiles of MTBE correct for the following entries:
Year 2015-2016, the 90th and 95th percentiles for Total is <LOD while Female (90th and 95th), Mexican Americans (95th), Non-Hispanic blacks (95th), and All Hispanics (95th) percentiles are 10.0 (LOD-18.0)? It is possible to have the since the 'n' for each are different and could shift the percentils."

RESPONSE: *One correction was made in Table 5-9 for data for Year 2015–2016: the 90th percentile for Females was changed from "10.0 (<LOC–12.00)" to <LOD. All other data for Year 2015–2016 were accurate. The 'n' for each group is already reported in the final column (sample size).*

Comments provided by Peer Reviewer #3:

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 1: I do not have any disagreements with the effects known to occur in humans as reported in the text.

RESPONSE: *No response needed.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 2: Numerous places within the text refer to studies in rodents wherein increases in hepatic weight occur. As also mentioned in the text, such changes are often described as effects reflecting homeostatic adaptation and/or rodent specific when they occur in the absence of histopathological and/or enzymatic changes. To support these decisions, there are statements in the text indicating no histopathological observations occurred in conjunction with the increases in weight. Indeed, this has become the default in risk assessment.

However, this raises several questions that are not addressed in the document. First, are the histopathological assessments actually comparable across these studies, e.g., same outcome measures, same in extent of assessment? Secondly, how in-depth are such assessments? Do they involve anything further than gross histology that would not divulge any functional changes? Thirdly, these assessments are typically done at the end of some exposure, but do we actually know anything about consistency of their potential for reversibility or, conversely, for persistence or for delayed toxicity?

RESPONSE: *Histological observations refer to standard histology (e.g., H&E stains), consistent with guidelines put forth by many agencies (National Toxicology Program [NTP], Organisation for Economic Co-operation and Development [OECD], etc.). If recovery groups were included, and showed either recovery or delayed toxicity, this is indicated in the text (e.g., Chun and Kintigh 1993 in Section 2.13; Bird et al. 1997 in Appendix A).*

COMMENT 3: An additional related point is that the analysis appears to treat all studies as of equal quality, while clearly this cannot be the case. Consequently, it is not clear whether studies that differ in outcome differ because of study quality. Further, it isn't clear that the studies from industry were ever peer reviewed.

While recognizing the issues related to this, some recognition of these uncertainties should be added to the document.

RESPONSE: *While formal study quality review was not performed for this profile (i.e., formal systematic review), ATSDR (2018) guidance[2] dictates that profile authors take into consideration study quality while reviewing studies for inclusion in the profile as outlined by the National Research Council's "Guidelines for Assessing the Quality of Individual Studies"[3]. Poor quality studies with major limitations are either excluded from the profile (e.g., if available data are too limited for independent review) or, if included, ATSDR clearly notes major study quality issues in the text. These studies would not be included in LSE table or considered for MRL derivation. Regarding industry studies, Section B.1 in Appendix B indicates: "Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of MTBE have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest".*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 4: Yes, exposure conditions are adequately described

RESPONSE: *No response needed.*

Minimal Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 5: The derivation of MRLs as based on endpoints with sufficient data in each category of derivation and thus seem appropriate based on the parameters of the review.

RESPONSE: *No response needed.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

a. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 6: The MRL values and associated uncertainty factors seem appropriate as derived. It might be useful however, to include the UFs that were applied directly on the figures (e.g., in parentheses) on the Figures in Chapter 1 which would also make the MRLs listed easier to understand.

RESPONSE: *ATSDR will consider the Reviewer's suggestion regarding revised Chapter 1 figures in future versions of the profile guidance.*

---

[2]ATSDR. 2018. Draft guidance for the preparation of toxicological profiles. Atlanta, GA: Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/toxprofiles/guidance/profile_development_guidance.pdf. June 21, 2021.

[3]National Research Council (US) Steering Committee on Identification of Toxic and Potentially Toxic Chemicals for Consideration by the National Toxicology Program. Toxicity Testing: Strategies to Determine Needs and Priorities. Washington (DC): National Academies Press (US); 1984. PMID: 25032410.

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 7: As noted above, and also of course affecting the MRLs, is that all studies are included in the risk assessment, despite unequal quality. This then becomes essentially a binary assessment of the number of yes vs. no studies rather than a quality review of effects. This is troubling because it is hard to imagine that comparable outcomes could occur across dissimilar studies by chance alone, i.e., higher probability of being correct, but easy to imagine numerous reasons for the absence of any effects of an exposure.

RESPONSE: *As indicated in the Response to Comment 3, a formal study quality review was not performed for this profile, but any study of poor quality resulting in inability to independently review results and studies with inadequate evaluation of endpoints were not included in LSE table, and were not considered for MRL derivation. Regarding selection of a critical effect for MRL derivation, the database is reviewed as a whole and expert judgement is used to determine strength and consistency of effects. Any inconsistencies in the database are evaluated to determine potential sources of inconsistency, including differences in study design, endpoints evaluated, etc. (as opposed to stacking up "yes" vs "no" studies), prior to selection of critical effect and principal study.*

QUESTION: In addition, we are requesting feedback on the following for the intermediate oral MRL:

a. If the neurological endpoint is adequate given the quality of the study

COMMENT 8: The finding of CNS depression is highly similar to what has already been reported following exposures in humans, therefore it has plausibility and should be retained, unlike the increased sperm abnormality which is not yet amply demonstrated in human studies.

RESPONSE: *The neurological endpoint was retained as the critical effect for the provisional oral intermediate MRL.*

QUESTION:

b. If the use of a modifying factor is appropriate to account for the quality of the neurological study

COMMENT 9: This question is difficult to address given that quality assessments and or criteria used to assess that feature is not provided in the document, and its not clear it was applied to the assessment at all.

RESPONSE: *ATSDR re-evaluated the derivation of the intermediate oral MRL and determined that the modifying factor was not needed. The revised provisional intermediate oral MRL is 0.6 mg/kg/day.*

QUESTION:

c. If male reproductive endpoints, specifically the BMDL of increased sperm abnormality, would be a better endpoint for intermediate oral MRL derivation.

COMMENT 10: No.

RESPONSE: *The neurological endpoint was retained as the critical effect for the provisional oral intermediate MRL.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 11: Yes, the health effects appear to reflect findings in the published literature even to date with an updated search.

RESPONSE: *No response needed.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 12: As noted above, study strengths and limitations are inconsistently applied throughout the document. There were human studies in which the same limitations were noted for every outcome measure for which it was cited, whereas others have no information. I couldn't tell if that meant that other studies were perfect?

RESPONSE: *Epidemiological studies inherently include limitations. ATSDR re-evaluated reporting of human studies in the profile, and it appears that major limitations for epidemiological studies have been consistently reported throughout. No major limitations were identified for controlled exposure studies. Limitations are not discussed for the clinical studies on intracystic MTBE therapy because they were only briefly cited in support of hazard identification.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 13: Very limited information is provided for animal studies and therefore it is not possible to ascertain quality of these studies. There are many features other than those listed above that relate to study quality: age of animals, housing conditions, statistical analysis etc. which are not detailed here. Again, some data quality conditions should be imposed prior to inclusion in the document.

RESPONSE: *It is beyond the scope of the toxicological profile to include extensive summaries of individual animal studies. Some information on the study design (e.g., species and strain, number of animals per group, dose levels) are provided in the LSE tables in Chapter 2. All studies cited in the profile have undergo extensive review to evaluate the quality of the study design (including statistical analysis) and the validity of the results and conclusions. Studies which are considered to be poor quality are typically not included in the profile; if they are included in the profile, the study limitations are noted in the discussion.*

COMMENT 14: One comment on studies related to corticosterone changes, again a reflection of the absence of quality indices for these studies, is that levels of corticosterone are time-dependent and many

of the apparent inconsistencies in outcome could depend upon the timing at which the samples were collected.

RESPONSE: *None of the studies reported at what time of day blood was drawn for corticosterone analysis. A statement was added in Section 2.13 to address this potential issue.*

One potential reason for inconsistent results between studies may be due to time-of-day dependent variations in corticosterone levels; however, studies did not report at what time of day blood was collected.

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 15: While ultimately this is an empirical question that would require significant cross-species studies, the species used in the toxicological studies are appropriate given the extensive information about these species in comparison to humans.

RESPONSE: *No response needed.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 16: Yes, these are examined quite well.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 17: I am not aware of any that were not included.

RESPONSE: *No response needed.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 18: I did a search but did not see anything that was new.

RESPONSE: *No response needed.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 19: Yes, appropriate values are cited as are reasons for exclusions.

RESPONSE: *No response needed.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 20: There are numerous cases where effects are listed as less serious vs. more serious, and the introductory material cites guidelines for this. The terms biologically relevant and irrelevant are also used but it's not clear whether these are intended to be interchangeable. Unfortunately, those guidelines are not described except in the case of hepatic changes and the reason for their exclusion. But whether an effect is serious or not requires several considerations. In the case of neurotoxic endpoints such as e.g., hypoactivity (Daughtry et al., 2000) or lack of a startle reflex (Bevan et al., 1997b) listed as a less serious effect. If you are driving your car, and your reaction time is slowed or your startle reflex inactive, that could be quite serious. These are behaviors that always occur in a context and that does not appear to be considered.

RESPONSE: *Biologically relevant and irrelevant are not intended to be interchangeable with more vs. less serious. Only changes that are expected to be biologically relevant are included in the LSE table. There are a few instances where reported effects are of unclear biological relevance due to direction or magnitude of effect or lack of clear association with an apical endpoint based on available data and/or expert judgement. In those cases, findings are discussed in the text, but the endpoint is not used to establish a LOAEL or included in the LSE table. As per ATSDR (2018) guidance[4], ATSDR defines a LOAEL, or adverse health effect, as described by Chou et al. (1998)[5]: "a harmful or potentially harmful change in the physiologic function, physiologic state, or organ structure that may result in an observed deleterious health outcome [which] may be manifested in pathophysiologic changes in target organs, psychiatric effects, or overt disease." If the observed health effect is serious enough to evoke failure in a biological system and can directly lead to morbidity or mortality, the associated dose is considered a serious LOAEL.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 21: I am not aware of any additional studies related to mechanisms of action that are not included in the document.

RESPONSE: *No response needed.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

---

[4]ATSDR. 2018. Draft guidance for the preparation of toxicological profiles. Atlanta, GA: Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/toxprofiles/guidance/profile_development_guidance.pdf. June 21, 2021.

[5]Chou CHSJ, Williams-Johnson M. 1998. Health effects classification and its role in the derivation of minimal risk levels: Neurological effects. Toxicol Ind Health 14(3):455-471.

COMMENT 22: On. P. 104, line 1673 there is a statement regarding measures of thyroid function that these were not considered to be biologically significant because they were inconsistent between the sexes. This should absolutely be removed. Males ≠ females, and females ≠ males. Sexes differ. Pregnancy doesn't occur in males either, but that doesn't mean it isn't real in females.

RESPONSE: *The statement regarding inconsistency between sexes in Section 2.13 was removed:*

These alterations were not considered to be biologically significant because they were transient and not associated with histopathological thyroid lesions.

<u>Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions</u>

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 23: It seems somewhat surprising given the characteristics of MBTE that there is no real discussion of the routes to the brain (likely olfactory and blood stream) as well kinetics in brain. If the available information is lacking, that should be indicated along with the need for additional information.

RESPONSE: *Two PBPK models (Rao and Ginsberg 1997; Blancato et al. 2007) contain separate compartments for the brain, as indicated in Section 3.15. These models use understanding of kinetics in the body and brain (e.g., partition coefficients) to predict brain concentrations. To emphasize why the brain was first added to the model originally developed by Borghoff et al. (1996), a slight revision was made in Section 3.1.5. No toxicokinetic studies with detailed information regarding the potential for MTBE (or metabolites) to be directly transported from the olfactory mucosa to the brain were identified. Section 6.2 (Absorption, Distribution, Metabolism, and Excretion) was updated to address this data gap.*

Section 3.1.5: Rao and Ginsberg (1997) expanded the model of Borghoff et al. (1996) to include compartments for brain, since it is a known target of MTBE toxicity, and skin (to address dermal exposure).

Section 6.2 (Absorption, Distribution, Metabolism, and Excretion): One area of research that is lacking is evaluation of the potential for direct olfactory transport of MTBE (or its metabolites) to the brain following inhalation exposure.

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 24: To the best of this reviewer's knowledge.

RESPONSE: *No response needed.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 25: To the best of this reviewer's knowledge

RESPONSE*: No response needed.*

Children and Other Populations that are Unusually Susceptible

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 26: Not to my knowledge

RESPONSE: *No response needed.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 27: Yes, it appears to have identified all susceptible subpopulations based on knowledge of effects.

RESPONSE: *No response needed.*

Biomarkers of Exposure and Effect

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 28: No, and would not be expected to be given the chemical nature of the substance

***RESPONSE*:** *No response needed.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 29: No, and would not be expected to be given the chemical nature of the substance.

RESPONSE: *No response needed.*

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 30: It appears to reflect current understanding.

RESPONSE: *No response needed.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 31: I'm not aware of any additional such references.

RESPONSE: *No response needed.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 32: Not to my knowledge

RESPONSE: *No response needed.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 33: Yes

RESPONSE: *No response needed.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 34: It appears to be complete and as up to date as information is available.

RESPONSE: *No response needed.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 35: Yes, the document does a good job of presenting this information. I am not aware of any missing information.

RESPONSE: *No response needed.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 36: Yes, the document does a good job covering this. I am not aware of missing information although this is not my area of expertise.

RESPONSE: *No response needed.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 37: Yes, the document presents this in a very understandable manner; I am not aware of any additional information that should be added but this is not my area of expertise.

RESPONSE: *No response needed.*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 38: Yes, the document does a good job of this, including distinctions between past use and current conditions which involve manufacture and export.

RESPONSE: *No response needed.*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 39: I'm not aware of any other studies that could fill data gaps

RESPONSE: *No response needed.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 40: In general, yes. However, given the nature of the chemical, it does seem that descriptions of effects on brain are very superficial which may be consequence of superficial assessments in the studies themselves. Specifically, it is generally noted that there were no 'histological' lesions/changes in brain, but it is not clear from these descriptions what specifically was examined; are these all just H&E stains? If so, it does not provide very significant information other than on a gross basis

RESPONSE: *As indicated in Response to Comment 2, histological refers to standard histopathological examinations. Since neurological findings associated with MTBE in humans and animals are attributable to CNS depressive effects, it is not surprising that there is no evidence of histopathological lesions in the nervous system or that identified studies did not conduct specialized pathological neurological examinations. Based on observed effects and proposed mechanisms (e.g., interaction with γ-aminobutyric acid [GABA] receptors), such specialized examinations appear unwarranted.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 41: Yes

RESPONSE: *No response needed.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 42: Not that I am aware of.

RESPONSE: *No response needed.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 43: No

RESPONSE: *No response needed.*