

1 EPA-SAB-18-0xx

2
3 The Honorable E. Scott Pruitt
4 Administrator
5 U.S. Environmental Protection Agency
6 1200 Pennsylvania Avenue, N.W.
7 Washington, D.C. 20460
8

9 Subject: Review of EPA's Draft Assessments entitled Toxicological Review of Ethyl Tertiary
10 Butyl Ether and Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol)
11

12 Dear Administrator Pruitt:

13
14 The EPA's National Center for Environmental Assessment (NCEA) requested that the Science Advisory
15 Board (SAB) review two draft assessments, entitled *Toxicological Review of Ethyl Tertiary Butyl Ether*
16 (*ETBE*) (*External Review Draft, EPA/635/R-15/016a, June 2017*) and *Toxicological Review of tert-Butyl*
17 *Alcohol (tert-Butanol; tBA)* (*External Review Draft, EPA/635/R-17/015a, June 2017*). The assessments
18 consist of a review of publicly available scientific literature on the toxicity of each chemical. The SAB
19 was asked to comment on the scientific soundness of the hazard and dose-response assessment of ETBE
20 and tBA induced cancer and noncancer health effects.
21

22 In response to EPA's request, the SAB convened a panel consisting of members of the SAB Chemical
23 Assessment Advisory Committee (CAAC) augmented with subject matter experts to conduct the review.
24 The enclosed report provides the SAB's consensus advice and recommendations. This letter briefly
25 conveys the major findings.
26

27 The draft Toxicological Reviews evaluate the available physiologically-based pharmacokinetic (PBPK)
28 models in the literature. The SAB finds that EPA's application of the PBPK model in dose-response
29 characterization of ETBE and tBA is an appropriate way to incorporate science using state-of-the-art
30 methods. However, the overall presentation of the PBPK modeling should be more cohesive, clear, and
31 transparent. Instead of using a default method to calculate the human equivalent dose (HED), the SAB
32 encourages the agency to create an ETBE and tBA model parameterization for humans using the
33 published human PBPK model of Nihlen and Johanson (1999) and data in Amberg et al. (2000).
34

35 Regarding dose metric, the SAB recommends against the EPA's use of the rate of metabolism as the
36 dose metric for extrapolation from inhalation to oral routes of administration of ETBE. The SAB finds
37 that there is no consistent dose-response relationship for ETBE when combining oral and inhalation
38 studies to assess liver tumors. The SAB agrees with the EPA's approach to calculate the dose metric for
39 tBA.
40

41 Regarding noncancer kidney effects, the SAB is unable to reach consensus with respect to how the
42 agency interpreted the ETBE database for noncancer kidney effects. There was considerable
43 disagreement as to whether noncancer kidney effects in rats should be considered a hazard relevant to
44 humans. Although consideration of the role of $\alpha_2\mu$ -globulin in ETBE-induced nephropathy in male rats
45 is thoroughly considered according to the 1991 criteria established by the EPA, the SAB recommends
46 application of the more detailed criteria published by IARC (1999). The SAB also recommends that
47 EPA consider the use of another parameter, such as increases in blood (serum) biomarkers or

1 exacerbation of nephropathy, besides urothelial hyperplasia, as a surrogate for ETBE noncancer kidney
2 effects. For tBA, EPA should provide a more detailed explanation for considering chronic progressive
3 nephropathy (CPN) as a kidney effect relevant to human hazard assessment. The EPA could also
4 consider other indicators besides suppurative inflammation and transitional epithelial hyperplasia as
5 indicators of kidney effects for tBA or provide better justification for their choice.

6
7 For noncancer effects, the SAB agrees that noncancer toxicity at sites other than the kidney should not
8 be used as the basis for deriving an oral reference dose (RfD) or inhalation reference concentrations
9 (RfC) for ETBE or tBA. Nearly all of the possible effects at these sites occurred at much higher
10 exposure levels than did effects observed on the kidney.

11
12 Regarding noncancer kidney outcomes, the SAB did not reach consensus regarding the oral reference
13 dose for noncancer kidney outcomes from exposure to ETBE. The difference in opinion is based on the
14 extent of confidence in a CPN-based mechanism for these ETBE effects. Similarly, the SAB did not
15 reach a consensus regarding the oral reference dose for noncancer kidney outcomes for tBA. The
16 difference in opinion relates to the extent of confidence in CPN and/or $\alpha_2\mu$ -globulin based mechanisms
17 for these tBA effects.

18
19 The SAB finds that the EPA's conclusion that liver tumors in male rats from exposure to ETBE are
20 relevant to human hazard identification and are scientifically supported. The SAB also agrees that the
21 ETBE mode of action (MOA) for the rat liver tumors remains, at this point, undetermined. A consensus
22 was not reached for tBA concerning the scientific support for the conclusion that male rat kidney tumors
23 are relevant to human hazard identification. The SAB did find that there is scientific support for the
24 EPA's conclusion that thyroid follicular cell tumors in mice for tBA are relevant to humans. However,
25 the SAB finds that there is uncertainty as to whether an increase in thyroid follicular cell tumors for tBA
26 was demonstrated in male mice.

27
28 The SAB concludes that the descriptor should be retained as "Suggestive Evidence" of the carcinogenic
29 potential for both ETBE and tBA. The SAB finds no rationale provided for the EPA's decision to
30 perform a quantitative analysis of carcinogenic potential for either ETBE or tBA. The SAB noted that it
31 is highly unlikely that performing a quantitative assessment of the potential carcinogenic data would be
32 useful for providing a sense of the magnitude and uncertainty of potential risks, ranking potential
33 hazards, or setting research priorities for either ETBE or tBA.

34
35 The SAB agrees that the oral slope factor chosen by the agency is scientifically supported for both
36 ETBE and tBA. No consensus, however, was reached regarding the EPA's calculation of inhalation unit
37 risk for ETBE. Some members conclude that the data are not suitable for developing a cancer inhalation
38 unit risk (IUR) due to a potential lack of biological relevance for ETBE. Other members note that the
39 data are appropriate for dose-response analysis for ETBE. The SAB concludes that the tBA drinking
40 water study is not suitable for developing an IUR. The SAB's concerns include the lack of biological
41 relevance due to the magnitude of the high dose, the lack of a mouse tBA PBPK model and the
42 possibility of nonlinear metabolism kinetics at that dose.

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This draft is a work in progress, does not reflect consensus advice or recommendations, has not been reviewed or approved
by the chartered SAB and does not represent EPA policy.

1 The SAB appreciates this opportunity to review the Toxicological Reviews of ETBE and tBA and looks
2 forward to the EPA's response to these recommendations.

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7 Sincerely,
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14 Enclosure:
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NOTICE

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This report has been written as part of the activities of the EPA Science Advisory Board, a public advisory committee providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters related to problems facing the Agency. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use. Reports of the EPA Science Advisory Board are posted on the EPA website at <http://www.epa.gov/sab>.

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1 **U.S. Environmental Protection Agency**
2 **Science Advisory Board**
3 **BOARD**

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5 **[to be added]**

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ACRONYMS AND ABBREVIATIONS

1		
2		
3	$\alpha_2\mu$ -globulin	alpha-2-micro-globin
4	ALD	Approximate lethal dosage
5	ALP	Alkaline phosphatase
6	ALT	Alanine aminotransferase/transaminase
7	AST	Aspartate aminotransferase/transaminase
8	ATSDR	Agency for Toxic Substances and Disease Registry
9	atm	Atmosphere
10	BMD	Benchmark dose
11	BMDL	Benchmark dose lower confidence limit
12	BMDS	Benchmark Dose Software
13	BMR	Benchmark response
14	BUN	Blood urea nitrogen
15	BW	Body weight
16	CA	Chromosomal aberration
17	CAAC	Chemical Assessment Advisory Committee
18	CASRN	Chemical Abstracts Service Registry Number
19	CI	Confidence interval
20	CIIT	Chemical Industry Institute of Toxicology
21	CL	Confidence limit
22	CNS	Central nervous system
23	CPN	Chronic progressive nephropathy
24	CYP450	Cytochrome P450
25	DAF	Dosimetric adjustment factor
26	DNA	Deoxyribonucleic acid
27	EPA	Environmental Protection Agency
28	ETBE	Ethyl Tertiary Butyl Ether
29	FDA	Food and Drug Administration
30	FEV ₁	Forced expiratory volume of 1 second
31	GD	Gestation day
32	GDH	Glutamate dehydrogenase
33	GGT	γ -glutamyl transferase
34	GLP	Good Laboratory Practices
35	GSH	Glutathione
36	GST	Glutathione-S-transferase
37	Hb/g-A	Animal blood:gas partition coefficient
38	Hb/g-H	Human blood:gas partition coefficient
39	HEC	Human equivalent concentration
40	HED	Human equivalent dose
41	HERO	Health and Environmental Research Online
42	IRIS	Integrated Risk Information System
43	JPEC	Japan Petroleum Energy Center
44	KO	Knockout
45	LC ₅₀	Median lethal concentration
46	LD ₅₀	Lethal dose median

1	LOAEL	Lowest observed adverse effect level
2	MNPCE	Micronucleated polychromatic erythrocyte
3	MOA	Mode of action
4	MTD	Maximum tolerated dose
5	MTBE	Methyl tertiary butyl ether
6	NIOSH	National Institute for Occupational Safety and Health
7	NOAEL	No observed adverse effect level
8	NRC	National Research Council
9	NTP	National Toxicology Program
10	ORD	Office of Research and Development
11	PBPK	Physiologically based pharmacokinetic
12	PND	Postnatal day
13	POD	Point of departure
14	POD _[ADJ]	Duration-adjusted POD
15	RD	Relative deviation
16	RfC	Reference concentration inhalation
17	RfD	Reference dose oral
18	SAB	Science Advisory Board
19	SAR	Structure activity relationship
20	SE	Standard error
21	tBA	Tert-Butyl-Alcohol
22	UF	Uncertainty factor
23	UF _A	Animal-to-human uncertainty factor
24	UF _H	Human variation uncertainty factor
25	UF _L	LOAEL-to-NOAEL uncertainty factor
26	UF _S	Subchronic-to-chronic uncertainty factor
27	UF _D	Database deficiencies uncertainty factor
28	U.S.	United States
29	WT	Wild type

1. EXECUTIVE SUMMARY

The Science Advisory Board (SAB) was asked by the EPA's National Center for Environmental Assessment (NCEA) to conduct a peer review of two draft assessments, entitled *Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE)* (*External Review Draft, EPA/635/R-15/016a, June 2017*) and *Toxicological Review of tert-Butyl Alcohol (tert-Butanol; tBA)* (*External Review Draft, EPA/635/R-17/015a, June 2017*) (hereafter referred to as the draft ETBE assessment or draft tBA assessment, respectively). The assessments consist of a review of publicly available scientific literature on the toxicity of each chemical.

The draft tBA and ETBE assessments were developed simultaneously by EPA because they have several overlapping scientific aspects. Specifically, 1) tBA is one of the primary metabolites of ETBE, and some of the toxicological effects of ETBE are attributed to tBA. Therefore, data on ETBE are considered informative for the hazard identification and dose-response assessment of tBA, and vice versa; 2) the scientific literature for the two chemicals includes data on $\alpha_2\mu$ -globulin-related nephropathy; therefore, a common approach was employed to evaluate these data as they relate to the mode of action for kidney effects; and 3) a combined physiologically-based pharmacokinetic (PBPK) model for ETBE and tBA in rats was applied to support the dose-response assessments for these chemicals. Given the overlapping data and features of ETBE and tBA, the SAB conducted a peer review of both chemicals simultaneously.

The SAB was asked to comment on the scientific soundness of the hazard and dose-response assessment of ETBE and tBA induced cancer and noncancer health effects. The SAB panel charged with conducting the review included members of the SAB Chemical Assessment Advisory Committee (CAAC) augmented with additional subject matter experts. An overview of the SAB's recommendations and advice on how to improve the clarity and strengthen the scientific basis of each assessment are presented below and discussed in greater depth in the body of the report.

Literature Search Strategy/Study Selection and Evaluation

In general, the SAB finds that the structure and strategy for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods are clearly presented and appropriate with a few exceptions for both chemicals. Several points of clarification/correction are necessary within this section of draft tBA and ETBE assessments, and specific recommendations are provided for each assessment. The SAB is not able to provide advice on whether the EPA's evaluation of study methods and quality were applied objectively for each assessment because of the lack of documentation within the EPA's *ETBE* and *tBA Toxicological Review (2017)* documents. There also is no clear documentation on the comparative quality evaluation for each of the studies (e.g., by providing the information for each study/quality criterion in the publically available Health and Environmental Research Online - HERO - database). On the other hand, the SAB finds no evidence that quality evaluation criteria for each Toxicological Review were applied in a non-objective manner.

Chemical Properties and Toxicokinetics

Chemical properties

The SAB observed several inconsistencies between the chemical properties provided in both the draft ETBE and tBA assessments and the chemical property values actually reported in the cited source or

1 found in other sources. Because multiple values for a given parameter could be found in the literature,
2 EPA should use only primary data sources for citation.

3 4 *Toxicokinetic modeling*

5 The SAB strongly supports the EPA's application of the PBPK model in dose-response characterization
6 of ETBE and tBA as an appropriate way to incorporate science using state-of-the-art methods.
7 However, the SAB offered a few points of correction/clarification regarding the EPA's application of
8 the PBPK model. The SAB noted that the overall presentation of the PBPK modeling should be more
9 cohesive, clear, and transparent. Instead of using a default method to calculate the human equivalent
10 dose, the SAB recommended an ETBE and tBA model parameterization for humans be created using the
11 published human PBPK model of Nihlen and Johanson (1999) and data in Amberg et al., (2000).
12 Furthermore, the EPA could also maximize the potential benefit for dose-response and mechanistic
13 analyses using appropriately selected dose metrics. In future modeling efforts, the EPA could assess
14 whether fits to data would benefit from consideration of capacity-limited blood binding of ETBE or sex
15 differences in metabolism.

16 17 *Choice of dose metric*

18 The SAB finds the choice of the rate of metabolism of ETBE to be a reasonable dose metric; however, it
19 is not recommended for extrapolation from inhalation to oral routes of administration of ETBE. This
20 consensus is reached because there is no consistent dose-response relationship for ETBE when
21 combining oral and inhalation studies to assess liver tumors. The SAB agrees with the EPA's approach
22 of using blood concentration to calculate a dose metric for tBA.

23 24 **Hazard Identification and Dose-Response Assessment – Noncancer**

25 *Noncancer kidney toxicity*

26 The SAB is unable to reach a consensus with respect to how the agency interpreted the ETBE database
27 for noncancer kidney effects. There is disagreement within the SAB as to whether noncancer kidney
28 effects for ETBE should be considered a hazard relevant to humans. Although consideration of the role
29 of $\alpha_2\mu$ -globulin in ETBE-induced nephropathy in male rats is thoroughly considered according to the
30 criteria established by the EPA (1991), the SAB recommends application of the more detailed criteria
31 published by IARC (1999). The SAB also recommends that the EPA consider using another parameter,
32 such as increases in blood (serum) biomarkers or exacerbation of nephropathy, besides urothelial
33 hyperplasia, as a surrogate for noncancer ETBE kidney effects.

34
35 For tBA, the SAB suggests that the EPA provide a more thorough explanation for considering the
36 enhancement of CPN as a kidney effect relevant to human hazard assessment. The SAB notes that the
37 EPA could also consider other indicators besides suppurative inflammation and transitional epithelial
38 hyperplasia as indicators of tBA kidney effects or provide better justification for their choice.

39 40 *Noncancer toxicity at other sites*

41 The SAB agrees that noncancer toxicity at sites other than the kidney should not be used as the basis for
42 deriving an oral reference dose (RfD) or inhalation reference concentrations (RfC) for ETBE. Nearly all
43 of the possible effects at these sites occurred at much higher exposure levels than did ETBE effects
44 observed on the kidney. Similarly, the SAB agrees that noncancer toxicity at sites other than the kidney
45 should not be used as the basis for deriving an RfD for tBA. Nearly all of the possible effects at these
46 sites occurred at much higher exposure levels than did tBA effects on the rat kidney.

1 *Oral reference dose for noncancer kidney outcomes*

2 The SAB has not reached consensus for ETBE regarding the RfD for noncancer kidney effects. The
3 difference in consensus is based on the extent of confidence in a CPN-based mechanism for these
4 effects. The SAB notes that if urothelial hyperplasia in male rats (Suzuki et al., 2012) is used for hazard
5 assessment, then the derivation of an oral RfD of 5×10^{-1} mg/kg-day would be considered to be
6 scientifically supported and its derivation clearly described as currently presented in the draft EPA
7 assessment of ETBE. Similarly, the SAB has not reached a consensus for tBA. The difference in
8 opinion is related to the extent of confidence in CPN and/or $\alpha_2\mu$ -globulin-based mechanisms for tBA
9 effects. If the selection of increases in severity of nephropathy in female rats in response to tBA
10 administration via drinking water remains the basis of the oral reference dose, then the SAB considers
11 the derivation of the oral reference dose of 4×10^{-1} mg/kg-day to be scientifically supported and its
12 derivation clearly described.

13
14 *Inhalation reference concentration for noncancer outcomes*

15 The SAB concludes that the derivation of all RfC candidate values is described clearly. The SAB also
16 states that if EPA's assertion is accepted about the human relevance of the increased urothelial
17 hyperplasia in the male rat kidneys (Saito et al, 2013), then the derivation of the RfC of 9×10^0 mg/m³ is
18 scientifically supported for ETBE. The SAB has examined the issues surrounding the human relevance
19 of the kidney endpoints reported in the rat. The SAB agrees that if the severity of tBA-induced
20 nephropathy in the female rat is accepted as relevant to humans, then the estimated 5×10^0 mg/m³ RfC is
21 scientifically defensible, with some caveats that are presented in this report.

22
23 **Hazard Identification and Dose–Response Assessment – Cancer**

24 *Cancer modes-of-action*

25 Regarding ETBE, the SAB finds that there is scientific support for the EPA's conclusion that liver
26 tumors in male rats are relevant to human hazard identification. The SAB also agrees that the
27 mechanism of action (MOA) for the rat liver tumors remains, at this point, undetermined. Under these
28 circumstances, tumor responses in animals are assumed to be relevant to human hazard identification.
29 While supporting the EPA's decision regarding the human relevance of the male rat liver tumors, the
30 SAB finds that improvement is needed for aspects of the discussion of MOA for hepatic effects of
31 ETBE which are detailed in this report.

32 For tBA, the SAB notes divergent views regarding whether the conclusion, that male rat kidney tumors
33 are relevant to human hazard identification, is scientifically supported. While some tumors might be
34 attributable to $\alpha_2\mu$ -globulin nephropathy augmented by CPN, others could be due to other unspecified
35 processes that are assumed to be relevant to humans. The SAB did not reach a consensus because some
36 members are in agreement with the draft EPA assessment, while others think that renal tumors could be
37 explained by CPN, and are therefore not relevant to humans. Further discussion on this point is
38 presented in this report. The SAB did find the conclusion that thyroid follicular cell tumors in mice are
39 relevant to humans to be scientifically supported. However, there is uncertainty as to whether an
40 increase in thyroid follicular cell tumors was in fact demonstrated in male mice.

41
42 *Cancer characterization*

43 For ETBE, the SAB concludes that the cancer descriptors should be retained as “Suggestive Evidence”
44 of the carcinogenic potential of ETBE which met the minimal criteria for that designation as described
45 in EPA's 2015 Cancer Guidelines. There is a general consensus that “Suggestive Evidence of
46 Carcinogenic Potential” is the proper descriptor for tBA because tBA was found to cause renal tubule

1 adenomas in male F344 rats and thyroid follicular adenomas in female (and possibly male) B6C3F1
2 mice. This cancer descriptor is scientifically supported for oral exposure, though there have apparently
3 been no inhalation bioassays of the chemical.
4

5 *Cancer toxicity values*

6 The SAB notes that no rationale is provided in the EPA's draft assessment for the decision to perform a
7 quantitative analysis in the case of ETBE. The SAB concludes it is highly unlikely that performing a
8 quantitative assessment of the data on ETBE liver carcinogenicity would be useful for "providing a
9 sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research
10 priorities" (EPA 2005).
11

12 Additionally, there is considerable concern about the ability of dose-response modeling to provide
13 meaningful and useful information when there is a flat, unresponsive dose response at all doses except
14 the high dose. Because EPA's policy is to use only the multistage model for benchmark dose modeling
15 of cancer dose-response, only a single estimate of the benchmark dose lower confidence limit (BMDL)
16 is produced. However, while the rationale stated for this policy is to maintain consistency throughout
17 various IRIS assessments, had other models been investigated, it would have been seen that many
18 different models could adequately fit these data, and would likely yield widely divergent BMDL values.
19

20 The SAB agrees that while the Saito et al. (2013) study is well-conducted and well-reported, with a
21 statistically significant increase in tumors at the high dose only, the Saito et al. (2013) data are not
22 sufficiently robust to provide a meaningful quantitative estimate of human cancer risk for ETBE.
23

24 Similarly, there does not appear to be a rationale for performing a quantitative analysis for tBA and it is
25 highly unlikely that performing a quantitative assessment of the data on tBA thyroid carcinogenicity
26 would be useful for providing a sense of the magnitude and uncertainty of potential risks, ranking
27 potential hazards, or setting research priorities. The SAB agrees that the NTP (1995) study is well-
28 conducted and well-reported. However, with a statistically significant increase in tumors at the high dose
29 only, and evidence from other studies supporting a potentially nonlinear mode of action, the NTP (1995)
30 data are not sufficiently robust to provide a meaningful quantitative estimate of human cancer risk for
31 tBA.
32

33 *Cancer modes-of-action*

34 The SAB concludes that the oral slope factor chosen is scientifically supported for both ETBE and tBA.
35 The SAB has no recommendations for alternative approaches for developing the oral cancer slope
36 factor. The SAB has not reached consensus regarding the EPA's calculation of inhalation slope factor
37 for ETBE.
38

39 For some members, the Saito et al. (2013) study is not suitable for developing a cancer inhalation unit
40 risk (IUR) due to a potential lack of biological relevance for ETBE. Other members have expressed that
41 the Saito et al. (2013) study is appropriate for dose-response analysis, and indicate that the ETBE IUR is
42 scientifically supported, given the quality of the Saito et al. (2013) study and the liver metabolism of
43 ETBE. The SAB has no recommendations for alternative approaches for developing an IUR for ETBE.
44

45 The SAB concludes that the NTP (1995) tBA drinking water study is not suitable for developing an
46 IUR. Concerns include the lack of biological relevance due to the magnitude of the high dose, the lack

1 of a mouse tBA PBPK model and the possibility of nonlinear metabolism kinetics at that dose. The
2 SAB also has concerns as to whether modeling a single positive concentration would produce a
3 meaningful oral slope factor. An alternative approach for developing an IUR for tBA would be to
4 perform a route-to-route extrapolation from the oral cancer slope factor using default human body
5 weight and inspiration rate values.

6 7 **Susceptible Populations and Life Stages**

8 The SAB agrees that there is “plausible evidence” for a vulnerable subgroup to ETBE exposure. The
9 SAB recommends that EPA should provide additional details with respect to differences in expected
10 outcomes, vulnerable life stages, and the possibility of other potentially vulnerable population
11 subgroups, such as individuals that have non-coding region variants in ALDH2 (Aldehyde
12 dehydrogenase gene).

13
14 The SAB agrees that the evidence is minimal with regard to tBA for identifying vulnerable populations
15 and life stages. The draft tBA assessment states that there is no identified susceptible population.
16 However, it is unclear as a metabolite of ETBE why other populations mentioned in the ETBE draft
17 assessment were not considered by EPA. It is also unclear why vulnerable life stages are highlighted for
18 tBA and not for ETBE.

19
20 Finally, the SAB disagrees with certain findings presented by the EPA in Table 1-12 of the draft tBA
21 assessment. The actual body weight for the treated group was not double that of the control group, as
22 implied by Table 1-12. Rather, treated dams gained twice as much weight as the dams in the control
23 group during a specific interval. This difference in body weight gain is reasonable given other
24 characteristics noted within the EPA’s report and the EPA’s final assessment does not need to include
25 additional reasoning on this topic.

26 27 **Executive Summary**

28 The SAB finds the Executive Summaries in both draft assessments to be clear and include the major
29 conclusions from the draft assessments. As changes are made to the body of the draft assessments for
30 both ETBE and tBA, the Executive Summary of each document will need to be changed accordingly.
31 While the Executive Summaries include statements regarding the questions considered and summarize
32 the findings and approaches selected by the Agency, the sections on “Key Issues” should include
33 additional discussion that highlights the consequences of alternative choices for the final assessments
34 because the interpretation and relevance of key toxicity endpoints driving the analysis have been
35 contested by members of the public. How these issues are resolved is important for the assessments –
36 and for the Executive Summaries. The SAB suggests that the final conclusions on decisions to accept or
37 not accept debated arguments needs to be clearly explained, and simply not being certain about the
38 irrelevance of an endpoint to human risk does not result in certainty of relevance.

2. INTRODUCTION

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the EPA's *Draft IRIS Toxicological Review of Ethyl Tertiary Butyl Ether (ETBE)* and *Toxicological Review of tert-Butyl Alcohol (tert-Butanol; tBA)* (U.S. EPA 2017a and 2017c). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants.

The draft ETBE assessment reviewed the publicly available studies to identify its adverse health effects and to characterize dose-response relationships. The assessment examined all effects by oral and inhalation routes of exposure and included an oral noncancer reference dose (RfD), an inhalation noncancer reference concentration (RfC), a cancer weight of evidence descriptor, and a cancer dose-response assessment.

The draft tBA assessment reviewed the publicly available studies to identify its adverse health effects and to characterize exposure-response relationships. The assessment examined all effects by oral and inhalation routes of exposure and included an oral RfD, an inhalation RfC, a cancer weight of evidence descriptor, and a cancer dose-response assessment.

Disposition of the public comments received on earlier assessment drafts are available in the Supplemental Information to the draft ETBE (U.S. EPA 2017b) and tBA (U.S. EPA 2017d) assessments.

The draft assessments for ETBE and tBA were developed simultaneously by the agency because they have overlapping scientific aspects:

- *tert*-Butanol and acetaldehyde are the primary metabolites of ETBE, and some of the toxicological effects of ETBE are attributed to *tert*-butanol. Therefore, the Agency decided that certain data on *tert*-butanol are considered informative for the hazard identification and dose-response assessment of ETBE, and vice versa.
- The scientific literature for the two chemicals includes data on α_2 -globulin-related nephropathy; therefore, the Agency chose to use a common approach to evaluate the data as they relate to the mode of action for kidney effects.
- The Agency applied a combined physiologically based pharmacokinetic (PBPK) model for ETBE and *tert*-butanol in rats (Borghoff et al. 2016) to support the dose-response assessments for these chemicals.

In response to the EPA's request, the SAB convened an expert panel consisting of members of the Chemical Assessment Advisory Committee augmented with subject matter experts to conduct the review. The SAB panel held a teleconference on July 11, 2017 to discuss EPA's charge questions (see Appendix A), and a face-to-face meeting on August 15 - 17, 2017 to discuss responses to charge questions and consider public comments. The SAB panel also held teleconferences to discuss their draft report on [insert date]. The Chartered SAB conducted a quality review of this document on [insert date]. Oral and written public comments have been considered throughout the advisory process.

1 This SAB report is organized to follow the order of the charge questions addressing ETBE and tBA. The
2 presented recommendations are prioritized to indicate relative importance during EPA's revisions. As
3 noted within the EPA's charge for both ETBE and tBA the following recommendation tiers are defined:
4

- 5 • Tier 1: Recommended Revisions – Key recommendations that are necessary in order to improve
6 the critical scientific concepts, issues and/or narrative within the assessment.
7
- 8 • Tier 2: Suggestions – Recommendations that are encouraged for EPA to adopt in order to
9 strengthen the scientific concepts, issues and/or narrative within the assessment, but other factors
10 (e.g., EPA need) should be considered by EPA before undertaking these revisions.
11
- 12 • Tier 3: Future Considerations – Useful and informative scientific exploration that may inform
13 future evaluations of key science issues and/or the development of future assessments. These
14 recommendations are likely outside the immediate scope and/or needs of the current assessment
15 under review.

3. RESPONSES TO EPA'S CHARGE QUESTIONS

3.1. Literature Search/Study Selection and Evaluation - Systematic Review Methods

3.1.1. ETBE

Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

In general, the SAB finds that the structure and strategy for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods are clearly presented and appropriate with a few exceptions. The SAB also finds that the included reference tables are lucid and illustrative. In particular, the SAB is pleased with EPA's decision to perform additional statistical analysis when required and also to have the IRIS Program perform peer-reviews of some of the key studies published as part of reports. Of note, the strategy for the literature search for the draft ETBE assessment did not follow all the recommendations as outlined by the NRC (2011), except for some aspects of the selection of the studies to be included in evidence tables.

The approach to the study search and identification is illustrated clearly in Figure LS-1 and can be followed in the HERO database for ETBE. The general search strategy consisted of starting with four scientific literature databases (i.e., PubMed, Web of Science, Toxline, and TSCATS2) and other sources, followed by screenings for identifying and selecting the studies relevant to the human health risk assessment. Consistent with the broad search strategy, and as depicted in Table LS-1, initial searches were not topic-limited. Searches in scientific literature indexes were updated continually through November 2015 or December 01, 2016 (for the TSCATS2 database). Clarification is needed to address: 1) why these four databases are the only ones used; 2) why the set of search keywords did not include all synonyms for ETBE, and 3) why the searches were not updated through December 2016, across-the-board for all four databases.

The strategy for the ETBE literature search did not follow all the recommendations as outlined by the NRC (2011), except for some aspects of the selection of the studies included by the Agency in the evidence tables. Consistent with systematic review and the recommendations of NRC (2011), a literature search with updates that use the same search keywords is not necessarily comprehensive or sufficient since new questions may arise during the process of study review and toxicology assessment. For example, new inquiries have the potential to consider non-mammalian studies, or more in-depth evaluation of the development of genotoxic-related kidney disease in humans. It is not apparent, however, that this continuing and evolving literature search process occurred as extensively as needed in the draft ETBE assessment and, as a result, the hazard evaluation of ETBE may have been too limited. The SAB Panel was informed by staff of the EPA's IRIS program that EPA is developing IRIS-specific guidelines for literature reviews. The SAB supports this effort and encourages the IRIS program to give strong consideration to the NRC (2011) recommendations.

The SAB finds it appropriate that an additional literature search include two review articles, and a communication from the Japan Petroleum Energy Center (Table L-S2). However, the Agency should clarify (1) why citations were searched manually only in two review articles and no other peer-reviewed

1 publications, and (2) the searches in sources of citations other than peer-reviewed publications (e.g.,
2 overviews and assessments by other national and international agencies).

3
4 Inclusion/exclusion screening criteria are summarized in Table LS-3 and the SAB finds the criteria to be
5 generally appropriate. Identified studies are included or excluded for relevance to population, exposure,
6 and outcome and systematically included in HERO according to these criteria. The SAB finds that the
7 exclusion criteria should be better justified. For example, the exclusion of ecological and non-
8 mammalian species requires further clarification because this exclusion can result in constraining the
9 hazard evaluation.

10
11 The criteria for the quality evaluation of studies included in the evidence tables are well described and
12 appropriate. However, the SAB finds it difficult to ascertain the reasoning behind disposition of
13 individual studies and their classification into one or another category. Additionally, some information
14 pertaining to the evaluation of quality and impacts on interpretation of the sources listed under
15 “Database Evaluations” is presented within the main text. Unfortunately, this scattering and partial
16 reporting of information does not easily permit readers to follow or comment upon, if appropriate, the
17 treatment of individual issues with specific studies. Such discussion of any one study is not easily
18 located (other than by searching the entire draft ETBE assessment) and readers are limited to the aspects
19 that the EPA chooses to discuss. In addition, the Supplemental Information does not appear to include
20 information pertaining to the evaluation of quality or impacts on interpretation.

21
22 The SAB agrees that there are no relevant human chronic studies of the health effects of ETBE and that
23 the sources for health effects data are all animal studies, and that EPA identified the critical set of
24 experimental rat, mouse, or rabbit studies for potential inclusion in evidence tables. One of these studies
25 (Dorman et al. 1997) was excluded because studies of neurotoxic outcomes were found to be
26 inconsistent. The SAB recommends that the agency clarify a discrepancy in the number of studies
27 identified as potential sources of health effects data between Table LS-1 (33 studies identified with 32
28 left after removing the Dorman et al. 1997 study), and the 30 studies mentioned in the narrative and
29 listed in Table LS-5. This apparent discrepancy needs to be clarified.

30
31 The SAB is not able to provide advice on whether the EPA’s evaluation of study methods and quality
32 are applied objectively because of the lack of documentation within the draft assessment. There is no
33 clear documentation on the comparative quality evaluation for each of the studies (e.g., by providing the
34 information for each study/quality criterion in the HERO database). On the other hand, the SAB found
35 no evidence that quality evaluation criteria were applied in a non-objective manner.

36
37 *The following recommendations are noted:*

38
39 **Tier 1:**

- 40 • Address whether the whole range of available literature databases were considered.
- 41 • Address why not all synonyms for ETBE were used.
- 42 • Address why not all databases were updated through December 2016.
- 43 • Provide a rationale for not performing citation searches beyond sources listed in Table LS-2.
- 44 • Provide a rationale for what appears to be a limited search for additional citations.
- 45 • Address the discrepancy in the number of health effects studies reported in Table LS-1.

- Clarify why ecological/non-mammalian studies were apparently excluded from any consideration (despite the footnote in Table LS-3), which is inconsistent with the process of systematic review and the NRC (2011) recommendations.

Tier 2:

- EPA should provide more transparent documentation on the process for application of inclusion and exclusion criteria and the quality evaluation of studies, in order to support decision making by the Agency. This could be done through the HERO database.

Tier 3:

- None.

3.1.2. tBA

Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

Overall, the EPA's search strategies, criteria for study inclusion/exclusion and evaluation, and criteria for evaluating study quality are described clearly within the EPA's *tBA Toxicological Review (2017)* document. Both the narrative and the tables presented are informative, clear, and illustrative of the literature search, and study screening criteria and disposition. Several points of clarification/correction are necessary within this section of the assessment. The SAB has not reached consensus on the objectivity with which the strategies are applied.

The SAB finds that the EPA's overall strategy of starting from a broad, chemical-specific search of the scientific literature and other sources followed by successive screenings to identify key studies as appropriate within the draft tBA assessment. The approach followed the general recommendations of NRC (2011) in terms of the search and screening process. The strategy followed by EPA maximized the identification of publications potentially relevant to the Toxicological Review. The search was continually updated over time with a stated ending date of December 2016. The database was consistently maintained and updated over time in HERO.

Results from the process of data source identification and study screening and disposition are summarized clearly in Figure LS-1. As illustrated in Table LS-1, data sources included four relevant scientific literature databases (PubMed, Toxline, Web of Science, and TSCATS2). The four indexed scientific databases for the chemical-specific search are appropriate and likely to include most of the tBA-relevant published, peer-reviewed articles. The narrative does not indicate whether other databases are considered. Search keywords are appropriate consisting of typically-used synonyms for tBA and the CAS number. Given the range of names for this compound, the Agency should provide a rationale for why the Agency selected these specific names from the universe of those available for tBA.

The indexed literature searches are not topic-limited except for Web of Science and Toxline. EPA is not clear about why limits were applied in the very early phase of the scientific literature search of these two databases. The EPA's exclusion of reports in PubMed from the Toxline database search appears a reasonable approach for avoiding excessive duplication, but only if it is assumed that both databases (i.e., PubMed and Toxline) are completely error-free. While the EPA's approach to using *a priori* limits

1 for the search in Web of Science to specific research areas helps exclude potential non-relevant
2 publications, this approach appears to be inconsistent with the intended chemical-specific broad search
3 of the strategy and could potentially result in missing information. The agency should provide a brief
4 justification to clarify both these issues.

5
6 Additional research strategies included manual search of citations in review articles, public comments,
7 and reviews performed by other federal and international agencies (OSHA and IPCS, respectively), as
8 summarized in Table LS-2. This is appropriate. However, some SAB members agree that there should
9 be clarifications, including: 1) why the manual search for additional citations is restricted to review
10 articles and not done for other publications (including peer-review articles); 2) if there was a search for
11 all federal and international agencies that may have performed assessments for tBA (for example,
12 ATSDR), and why only IPCS (1987) and OSHA (1992) are included.

13
14 Study inclusion and exclusion criteria are described clearly and summarized succinctly in Table LS-3.
15 The SAB finds that the inclusion and exclusion criteria are generally appropriate, but several of the
16 exclusion criteria require revision and/or further clarification.

17
18 The SAB disagrees with the categorization of dermal exposure as a “Not Relevant Exposure Paradigm”
19 (Figure LS-1). tBA is used in perfumes, cosmetics, and personal hygiene products. EPA should review
20 available dermal exposure studies for tBA and present that information as part of the hazard
21 identification. Additional dermal studies that should be included are Edwards (1982) for contact
22 dermatitis and Hoshino et al. (1970) for squamous cell carcinoma.

23
24 As indicated in the footnote of Table LS-3, studies of ecological and non-mammalian species (e.g.
25 zebrafish) are “...not considered a source of health effects data or supplementary data...but were
26 considered as sources of contextual information”. However, there is no evidence that such studies are
27 considered for any purpose. The SAB notes that this exclusion yields a hazard identification which is not
28 sufficiently comprehensive. In addition, the lack of discussion on studies with non-mammalian species
29 may be a reflection of insufficient adherence to systematic review principles and the NRC (2011)
30 recommendations.

31
32 The general approach to database evaluation and the criteria for study quality are described in the
33 narrative and summarized in Table LS-4 for the experimental animal studies. The SAB agrees that the
34 quality evaluation criteria are appropriate, that these criteria follow EPA guidelines, and that their
35 application is illustrated in the draft tBA assessment and the Supplemental Information as individual
36 studies are discussed. However, the SAB notes that it is difficult to ascertain EPA’s reasoning behind the
37 disposition of individual identified literature items. That is, it is not possible for the reader to examine
38 the scoring of individual studies for the named criteria or to see clearly the reasons for which individual
39 studies are placed in one or another category. This renders critical comment on study selection and
40 disposition difficult. Issues about evaluation of quality and impacts on interpretation listed under
41 “Database Evaluation” (pp. xxxii-xxxiii of the Toxicological Review of tert-Butyl Alcohol) are said to
42 be discussed in the text, as the studies are described. However, scattering of the information and partial
43 reporting does not allow the reader to follow (and comment upon, if appropriate) the treatment of
44 specific issues on individual studies. There is no easy way to find such discussion of any one study
45 (other than by searching the entire draft tBA assessment) and the reader is limited to those aspects
46 selected by EPA to discuss. Supplemental Information does not appear to include this information either.

1 There is no evidence in the EPA's draft tBA assessment that the criteria were not applied objectively.
2 However, with little documentation on the detailed review and application of quality criteria for each
3 study, it is difficult to affirm that the quality criteria were applied objectively across the board. It would
4 be useful to have a way to capture the evaluations on specific criteria and to see the reasons for
5 disposition of each study, perhaps as part of the HERO database, such that the application of the
6 evaluation criteria in Tables LS-3 and LS-4 are more transparent.

7
8 Regarding the Preamble or from Literature Search Strategy/Study Selection and Evaluation section, the
9 Agency should clarify whether EPA intended to comply fully with the NRC (2011) recommendations
10 regarding search strategies and study selection and evaluation protocols. Also, the Agency should clarify
11 why EPA adhered to these recommendations in some aspects of the literature review and disposition of
12 studies, but not others. The SAB was informed that the EPA IRIS program is developing guidelines for
13 literature searches and encourages the EPA to adhere as much as possible to the NRC (2011)
14 recommendations.

15
16 There is limited information as to whether the search strategy evolved throughout the development of
17 the draft tBA assessment. For example, in the performance of a systematic critical review, specific
18 questions likely will arise and these frequently require additional searching of the literature with
19 keywords other than those employed in the initial search. The Agency should clarify if this process
20 occurred (or the extent that it did) in the draft tBA assessment.

21
22 ***The following recommendations are noted:***

23
24 **Tier 1:**

- 25 • Provide clarification for the rationale of the selection of some synonyms of tBA as search
- 26 keywords and not others.
- 27 • Provide clarification of the rationale for imposing limitations in the first stage of the scientific
- 28 literature search (i.e., PubMed, Web of Science).
- 29 • Provide clarification of the rationale for limiting the search for additional citations to only some
- 30 of the publications available in peer-reviewed literature and secondary sources, but not others.
- 31 • Provide a rationale for the exclusion of studies of dermal contact as a relevant route of exposure
- 32 in light of the occurrence of tBA in many consumer products such as perfumes and cosmetics.
- 33 • Provide a justification for the complete exclusion of studies with non-mammalian species, which
- 34 affects the completeness of the hazard identification.

35
36 **Tier 2:**

- 37 • EPA should provide more transparent documentation of the process of application of inclusion
- 38 and exclusion criteria and the quality evaluation of studies, in order to support decision making
- 39 by the EPA. This could be done through the HERO database.

40
41 **Tier 3:**

- 42 • None

43

1 **3.2. Hazard Identification - Chemical Properties and Toxicokinetics**
2

3 **3.2.1. Chemical properties.**
4

5 **3.2.1.1. ETBE**

6 *Is the information on chemical properties accurate?*
7

8 The SAB notes several inconsistencies between the chemical properties data provided in Table 1-1 and
9 values actually reported in the cited source or in other sources. Also as multiple values for a given
10 parameter (e.g., water solubility) could be found in the literature, data included in the assessment should
11 come from reliable sources that are accurately documented.
12

13 *The following recommendations are noted:*
14

15 **Tier 1:**

- 16 • The table entries for odor recognition or detection in different media need corrections/
17 clarifications.
- 18 • EPA should have a template and focus on listing only properties relevant to the compound and
19 the specific assessment.
- 20 • References used should be checked for quality control to ensure the accurate citations are
21 presented and that the data in tables match those in the reference provided.
- 22 • EPA should use primary data sources, not reviews or other government documents.
- 23 • If more than one value is found in primary peer reviewed sources, EPA should provide a
24 rationale for the choice of the one presented.
- 25 • If secondary sources or reviews are used, it is suggested that EPA cross check values with the
26 original citation provided in the secondary sources.
- 27 • If results from published studies are used, the quality of the experiment should be reviewed to
28 see if the design/data are sound.
- 29 • If values presented are estimates, that fact should be identified.
- 30 • Units and conditions under which the data were generated should be identified (e.g., solubility in
31 water at what temperature, etc.).
32

33 **Tier 2:**

- 34 • None.
35

36 **Tier 3:**

- 37 • None.
38

39 **3.2.1.2. tBA**

40 *Is the information on chemical properties accurate?*
41

42 As with ETBE, the SAB observed several inconsistencies among the chemical properties data provided
43 in Table 1-1.
44

1 ***The following recommendations are noted:***
2

3 **Tier 1:**

- 4 • EPA should have a template and focus on listing only properties relevant to the compound and
5 the specific assessment.
- 6 • References used should be checked for quality control to ensure the accurate citations are
7 presented and that the data in tables match those in the reference provided.
- 8 • EPA should use primary data sources, not reviews or other government documents.
- 9 • If more than one value is found in primary peer reviewed sources, EPA should provide a
10 rationale for the choice of the one presented.
- 11 • If secondary sources or reviews are used, EPA should cross check values with the original
12 citation provided in the secondary sources.
- 13 • If results from published studies are used, the quality of the experiment should be reviewed to
14 see if the design/data are sound.
- 15 • If values presented are estimates, that fact should be identified.
- 16 • Units and conditions under which the data were generated should be identified (e.g., solubility in
17 water at what temperature, etc.).

18
19 **Tier 2:**

- 20 • None.

21
22 **Tier 3:**

- 23 • None.

24
25 **3.2.2. Toxicokinetic modeling.**

26
27 **3.2.2.1. ETBE**

28 *Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification*
29 *of a physiologically-based toxicokinetic model of ETBE in rats (Borghoff et al. 2016). Is use of the*
30 *model appropriate and clearly described, including assumptions and uncertainties? Are there additional*
31 *peer-reviewed studies that should be considered for modeling?*
32

33 The SAB strongly supports EPA's application of the PBPK model for ETBE, including the tBA
34 submodel, in dose-response characterization of ETBE as an appropriate way to incorporate science using
35 state-of-the-art methods. However, a few concerns are noted by the SAB.
36

37 The overall presentation of the PBPK modeling should be more cohesive, clear, and transparent.
38 Providing essential information, assumptions, results and conclusions would be most helpful. Section
39 B.1.5 provides only a very brief description of the PBPK model used in this assessment. Model code
40 was made available through the HERO database, which is appropriate though it provides limited
41 information especially to those without access to the modeling software. The approach for model
42 evaluation is very clearly described in the U.S. EPA (2017) document "PK/PBPK Model Evaluation for
43 the IRIS Assessments of Ethyl Tertiary Butyl Ether and tert-Butyl Alcohol" cited in Section B.1.5 of
44 Appendix B. This evaluation appears to have been objective and thorough, with a detailed discussion of
45 uncertainties, assumptions and required modifications. The SAB identifies several concerns regarding

1 the assessment's text on the PBPK model documentation below. No peer reviewed animal or human
2 studies beyond those cited in the assessment had been identified.

3
4 Instead of using a default method to calculate the human equivalent dose (HED), the SAB encourages
5 the agency to create an ETBE and tBA model parameterization for humans using the published human
6 PBPK model of Nihlen and Johanson (1999) and data in Amberg et al. (2000). In Nihlen and Johanson
7 (1999) serum time course and exhaled breath samples were taken for 5, 25, and 50 ppm, 2-hour
8 inhalation exposures. Although, as stated in EPA (2017), the Nihlen and Johansen (1999) data and
9 modeling approach are not "conventional", they are useful for application in the PBPK modeling effort.
10 Similar experimental and PBPK modeling (Johanson et al. 1986; Corley et al. 1994) were used in the
11 Toxicological Review of 2-butoxyethanol. The oral route can be described in the model in the same
12 way that it is described in the PBPK models of vinyl chloride (Clewell et al. 1999) and 2-butoxyethanol
13 (Corley et al. 1994), which were used in the development of the RfDs and RfCs for these two chemicals,
14 as well as in both the oral and inhalation cancer assessments, despite the lack of human toxicokinetic
15 data for the oral route. For vinyl chloride, as with ETBE, the dose metric is rate of metabolism of the
16 parent chemical in the liver. For 2-butoxyethanol the dose metric is the concentration of the active
17 metabolite in the blood; for tBA the dose metric is the concentration of tBA in the blood.

18
19 EPA should give further consideration to maximizing the potential benefit for dose-response and
20 mechanistic analyses using appropriately selected dose metrics. Some noncancer kidney effects
21 observed following exposures to ETBE appear to show a consistent relationship to average daily
22 concentration of tBA at periodicity. Evaluation of dose metrics (e.g., average daily rates of metabolism
23 or concentrations) in relation to cancer or noncancer endpoints can be valuable in characterizing the
24 dose response relationship and the involvement of ETBE parent or its metabolites tBA and acetaldehyde
25 in toxicity.

26
27 The SAB recommends that the Agency consider dosimetry in both positive and negative studies (e.g.,
28 liver cancer in rats following ETBE exposures by inhalation and oral routes and tBA exposures by oral
29 administration), since this can also be informative about potential mechanisms and exposure-response
30 relationships. However, the Agency should also consider that the response may arise from combined
31 contributions of more than one compound (i.e., parent and metabolites).

32
33 On page B-23, the statement ("Finally, because induction is expected to have an equal impact on oral
34 and inhalation exposures—and only in the case that tert-butanol levels or metabolism are used as a dose-
35 metric—induction's potential impact on risk evaluation for ETBE is considered minimal") regarding
36 induction over time of tBA following ETBE administration seems to implicitly depend on the absence of
37 a first-pass effect of the liver with ingestion (versus inhalation) exposure. This should be addressed and
38 the description and underlying scientific logic of the handling of induction clarified.

39
40 Review of the acslX model code obtained from the HERO database found total concentration in liver
41 (CL), and not concentration in venous blood (CVL) representing free concentration in the liver, was
42 used. Since the metabolic rates are fitted to data changing the model to describe metabolism as a
43 function of the free liver concentration, CVL will alter estimated Michaelis constant (Km) values but
44 should not otherwise impact model results. None-the-less, this technical error needs to be corrected.

1 The model simulations shown in the draft ETBE assessment and the original peer-reviewed publication
2 generally provide good consistency with the experimental data in blood, exhaled breath, and urine. As
3 is frequently the case when modeling multiple data sets with two compounds (i.e., ETBE, tBA) from
4 multiple laboratories with different measures of compound (e.g., concentrations in blood, exhaled
5 breath, urine), some data are less well simulated than others.

6
7 Future modeling efforts could assess whether fits to data, particularly exhaled ETBE, which appear to
8 underestimate ETBE metabolism (i.e., exhaled concentrations of ETBE are higher in simulations than
9 data), would benefit from consideration of capacity-limited blood binding of ETBE or sex differences in
10 metabolism. EPA should consider assessing the following four topics and consider adjusting future
11 modeling efforts in light of the noted findings indicated in the literature:

12
13 1) Capacity-limited blood protein binding other than or in addition to $\alpha_2\mu$ -globulin:

14 Previous studies of methyl tertiary butyl ether (MTBE) metabolism indicate blood protein binding
15 and/or renal tubular reabsorption of tBA. Johanson et al. (1995) and Nihlen et al. (1998, 1998b)
16 reported toxicokinetics and acute effects of inhalation exposure of 10 male human subjects to MTBE
17 vapor at 5, 25, and 50 ppm for 2 hours during light physical exercise. Authors noted some exposure
18 dependence for the urinary half-life with shorter values seen at the highest exposure level (50 ppm for
19 2 hours). A low renal clearance for tBA (0.6 to 0.7 mL/hour/kg) suggests extensive blood protein
20 binding or renal tubular reabsorption of tBA.

21
22 The current Borghoff et al. (2016) model incorporates protein binding of tBA to $\alpha_2\mu$ -globulin
23 (Williams and Borghoff, 2001) and renal tubular reabsorption of this protein as observed by Neuhaus
24 (1986) in male rats. However, disproportionately less radiolabeled tBA (tBAc) was found in feces
25 after inhaling 100 ppm versus 1000 ppm for 6 hours in male rats (2.7 and 1%, respectively; Cruzan
26 and Kirkpatrick, 2006) which indicates that protein binding of acetates and esters that metabolize to
27 tBA may also be saturating at higher levels of exposure. Over-predictions of ETBE and tBA levels in
28 blood following ETBE inhalation observed in Figure 6 of Borghoff et al. (2016), along with evidence
29 of low renal clearance of tBA in humans, suggests that capacity-limited blood protein binding may be
30 occurring in male rats in addition to the $\alpha_2\mu$ -globulin-binding mechanism in male rats.

31
32 2) A greater rate of tBA metabolism is observed in male versus female rats.

33
34 3) In the current PBPK model, the impact of the omission of this gender-specific effect from repeated
35 doses is to under-represent the rate of urinary clearance and over-represent the rate of clearance of
36 tBA by exhaled breath.

37
38 4) Adjustment of the ETBE model to predict less loss through exhaled breath and capacity limited
39 protein binding might result in the model predicting lower amounts of parent compound (ETBE)
40 being metabolized to tBA, especially at higher doses.

1 ***The following recommendations are noted:***
2

3 **Tier 1:**

- 4 • Revise model code to describe metabolism as a function of the free liver concentration, CVL,
5 and re-estimate metabolic parameters (e.g., Km or first order rate constants). Metabolism based
6 upon total liver concentration, CL, is not scientifically correct.
- 7 • The overall presentation of the PBPK modeling should be cohesive, clear, and transparent, and
8 should provide essential information, assumptions, results and conclusions. It is misleading to
9 say “A more detailed summary of the toxicokinetic models is provided in Appendix B.1.5 (EPA
10 2017)” (page 1.3 lines 11-12). The text of the draft report (EPA 2017) could be included in the
11 Supplement, in which case it would benefit from adding a conclusions section.

12
13 **Tier 2:**

- 14 • EPA should give further consideration to modifying the model of Nihlen and Johanson (1999) in
15 a similar fashion to the way in which Corley et al. (1994) modified the model of Johanson et al.
16 (1986) to support cross-species extrapolations for both inhalation and oral routes of exposure.

17
18 **Tier 3:**

- 19 • For purposes of using PBPK models for the draft ETBE assessments, EPA needs to establish a
20 consistent practice for documentation of both the model itself and the review of the model (and
21 any modifications made by EPA to implement it). It is not desirable for EPA to write long
22 descriptions of the model it is using that would repeat much of what is in published literature, but
23 on the other hand providing clear summary information is desirable. Future model evaluations
24 would benefit from using the organizational structure captured in the IPCS PBPK (2010);
25 variants of this organizational structure were published by EPA in Clark et al. (2004) and Chiu et
26 al. (2007).
- 27
28 • Future modeling efforts could assess whether fits to data, particularly exhaled ETBE, which
29 appear to underestimate ETBE metabolism (i.e., exhaled concentrations of ETBE are higher in
30 simulations than data), would benefit from consideration of capacity-limited blood binding of
31 ETBE or sex differences in metabolism.

32
33 **3.2.2.2. tBA**

34 *Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification*
35 *of a physiologically-based toxicokinetic model of tert-butanol in rats (Borghoff et al., 2016). Is use of the*
36 *model appropriate and clearly described, including assumptions and uncertainties? Are there additional*
37 *peer-reviewed studies that should be considered for modeling?*
38

39 The SAB strongly supports EPA’s application of the PBPK model for tBA in dose-response
40 characterization of tBA as an appropriate way to incorporate science using state-of-the-art methods.

41
42 The overall presentation of the PBPK modeling should be more cohesive, clear, and transparent.
43 Providing essential information, assumptions, results and conclusions would be most helpful. Section
44 B.1.5 provides only a very brief description of the PBPK model used in this assessment. Model code is
45 made available through the HERO database, which is appropriate though it provides limited information
46 especially to those without access to the modeling software. The approach for model evaluation is very

1 clearly described in the U.S. EPA (2017) document “PK/PBPK Model Evaluation for the IRIS
2 Assessments of Ethyl Tertiary Butyl Ether and tert-Butyl Alcohol” cited in Section B.1.5 of Appendix
3 B. This evaluation appears to have been objective and thorough, with a detailed discussion of
4 uncertainties, assumptions and required modifications. The SAB identifies several concerns regarding
5 the assessment text on the PBPK model documentation below. No additional peer-reviewed studies
6 were identified.

7
8 Text in the draft tBA assessment, section 1.1.3 (page 1-3) needs to be revised so as not to be misleading
9 and to be consistent with the Supplement. While no models of tBA have been created independently of
10 other chemicals from which it arises as a metabolite (e.g., MTBE, ETBE), the tBA model has “been
11 developed specifically for administration of tert-butanol”. Pharmacokinetic studies with tBA exposures
12 are how the tBA model was parameterized, so the text needs rewording.

13
14 Instead of using a default method to calculate HED, EPA should give further consideration to creating a
15 tBA model parameterization for humans using the published human PBPK model of Nihlen and
16 Johanson (1999).

17
18 ***The following recommendations are noted:***

19
20 **Tier 1:**

- 21 • Revise model code to describe metabolism as a function of the free liver concentration, CVL,
22 and re-estimate metabolic parameters (e.g., Km or first order rate constants). Metabolism based
23 upon total liver concentration, CL, is not scientifically correct.
- 24 • Evaluation of tBA dose metrics for kidney toxicity should be compared for ETBE and tBA
25 exposures (similar to Figure 6 in Salazar et al., 2015).
- 26 • The overall presentation of the PBPK modeling should be cohesive, clear, and transparent, and
27 should provide essential information, assumptions, results and conclusions. Reword text in
28 Section 1.1.3 of the draft tBA assessment and text in Appendix. The SAB suggests that the
29 material in EPA (2017) be included in Appendix B or as a separate appendix and a conclusion
30 section added to it.

31
32 **Tier 2:**

- 33 • EPA should give further consideration to modifying the model of Nihlen and Johanson (1999) in
34 a similar fashion to the way in which Corley et al. (1994) modified the model of Johanson et al.
35 (1986) to support cross-species extrapolations for both inhalation and oral routes of exposure.

36
37 **Tier 3:**

- 38 • None

39
40 **3.2.3. Choice of dose metric.**

41 *Is the rate of ETBE metabolism an appropriate choice for the dose metric?*

42
43 **3.2.3.1. ETBE**

44 The EPA’s use of rate of metabolism of ETBE as the dose metric is a reasonable choice, but the SAB
45 does not recommend its use in extrapolation from inhalation to oral routes of administration of ETBE.
46 There is no “consistent dose-response relationship” for this dose metric dose (page B-27), when

1 combining oral and inhalation studies to assess liver tumors. This greatly weakens the case for route
2 extrapolation. The SAB considered but has not identified any other dose metrics that would work better
3 for route extrapolation of the liver cancer endpoint for ETBE than the dose metric chosen by the agency.
4 The SAB notes that where EPA's analysis question; is ETBE in blood versus in liver? The preference
5 for liver is appropriate when the toxicity in question happens in the liver. In practice, these will be
6 proportional to the extent that saturation of metabolism does not occur.

7
8 ***The following recommendations are noted:***

9
10 **Tier 1:**

- 11 • The SAB recommends that route extrapolation not be implemented for the oral cancer dose-
12 response analysis for ETBE. Therefore, the Agency does not need to select a dose metric.

13
14 **Tier 2:**

- 15 • EPA should give further consideration to undertaking a more thorough analysis of dose metrics
16 that examines animals dosed with both ETBE and *tert*-butanol. Since *tert*-butanol is a primary
17 metabolite of ETBE, a series of simulations to compare common dose metrics (*tert*-butanol AUC
18 (Area Under the Curve) and Cmax (Maximum plasma levels) across animals dosed with both
19 chemicals may provide insights into better utilization of the toxicology data. Additionally, ETBE
20 dose metrics, including AUC and Cmax, need to be evaluated as an alternative hypothesis, and
21 should not be excluded. The liver tumors at a high inhalation dose are probably not due to tBA
22 as a metabolite, since higher internal tBA doses did not produce them in tBA bioassays. This
23 implicates either parent ETBE or (maybe) acetaldehyde as a first metabolite as a cause. The
24 SAB has concerns regarding human relevance owing to constitutive androstane
25 receptor/pregnane X receptor/peroxisome proliferator-activated receptor MOA
26 (CAR/PXR/PPAR), but if liver tumors are considered by the agency, concentration of parent
27 ETBE may be a relevant dose metric. In general, as dose metrics are considered, the Agency
28 should consider how the choice enables comparison of effects of tBA dosed directly (in tBA
29 bioassays) and tBA as the principal metabolite of ETBE (in ETBE bioassays).
- 30 • The EPA should focus on the relationship between ETBE concentration in liver and its rate of
31 metabolism in liver, since this corresponds to the choice between ETBE as the direct actor
32 (through CAR/PXR/PPAR, presumably) and acetaldehyde as the direct actor. The two
33 alternatives will give similar results as long as metabolism is not saturated, as is noted in the
34 EPA's assessment, but the SAB recommends that the agency further examine this aspect. If
35 ETBE liver concentration is chosen, EPA should be prompted to investigate whether the mode of
36 action is CAR/PXR/PPAR or something else – and if CAR/PXR/PPAR, whether this is indeed
37 irrelevant to humans.
- 38 • It is difficult paging through the draft tBA assessment trying to link toxicity/cancer outcomes
39 with the modeling efforts. To be more transparent and succinct, the SAB encourages the Agency
40 to place the information on outcomes and the information on modeling near each other.

41
42 If EPA proceeds with this dose metric for the route extrapolation the SAB encourages the EPA to make
43 the following corrections:

- 44 • The text in the supplemental section of the PBPK model (page B-24 and B-27) needs to be
45 reworded to clarify the units of this dose metric (average daily rate of ETBE metabolized per day

1 at periodicity). Figure B-3 on ETBE page B-26, the X axis needs to be specified with correct
2 units.

3
4 **Tier 3:**

- 5 • None

6
7 **3.2.3.2. tBA**

8 *Is the average concentration of tert-butanol in blood an appropriate choice for the dose metric?*

9
10 The SAB agrees with the EPA's approach as presented for the dose metric. The SAB notes that while
11 the effects occur in kidney, use of blood concentration is reasonable given that it is related to kidney
12 concentration by the partition coefficient in female rats. Since the analysis only used effects in females,
13 any issues of modeling male rat specific protein binding do not impact these analyses.

14
15 ***The following recommendations are noted:***

16
17 **Tier 1:**

- 18 • The average concentration of tBA in blood is an appropriate choice for the dose metric because
19 there is a dose-response relationship for this dose metric and a kidney noncancer endpoint. Thus
20 the SAB recommends use of an oral to inhalation extrapolation for tBA. The SAB recommends
21 that the EPA state in the draft tBA assessment how the average concentration was calculated for
22 tBA.

23
24 **Tier 2:**

- 25 • It is difficult paging through the draft tBA assessment trying to link toxicity outcomes with
26 modeling efforts. To be more transparent and succinct, the SAB encourages the Agency to place
27 these pieces of information near each other.

28
29 **Tier 3:**

- 30 • None

31
32 **3.3. Hazard Identification and Dose-Response Assessment: Noncancer**

33
34 **3.3.1. Noncancer kidney toxicity.**

35
36 **3.3.1.1. ETBE**

37
38 *The draft assessment (sections 1.2.1, 1.3.1) identifies kidney effects as a potential human hazard of*
39 *ETBE. EPA evaluated the evidence, including the role of $\alpha_2\mu$ -globulin and chronic progressive*
40 *nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this*
41 *conclusion is scientifically supported and clearly described.*

42
43 Although both absolute and relative kidney weights are increased after ETBE exposure, the EPA's draft
44 assessment document clearly explains why absolute organ weight is a more reliable reflection of specific
45 effects on the kidneys in that body weight changes will impact relative organ weights and potentially
46 obscure effects of the chemical exposure.

1
2 The SAB finds that the EPA thoroughly and systematically considered $\alpha_2\mu$ -globulin effects and their
3 role in explaining renal tumors in male rats as presented in the EPA's draft ETBE assessment. The draft
4 ETBE assessment reviews the specific criteria established by EPA regarding $\alpha_2\mu$ -globulin as a male rat-
5 specific mode of action (MOA) and concludes that ETBE does not fulfill all the established criteria for
6 having an $\alpha_2\mu$ -globulin MOA. In recognition of gaps and uncertainties in the database, EPA concluded
7 that the database is insufficient to conclude that ETBE is an inducer of $\alpha_2\mu$ -globulin nephropathy in
8 male rats, which is a MOA that is not relevant to humans. Importantly, however, the EPA further
9 concluded that other MOAs operating may be relevant to human hazard assessment.

10
11 The draft ETBE assessment also discusses the role of CPN as an underlying MOA for renal dysfunction
12 in male and female rats. EPA noted that CPN is not a specific diagnosis on its own but an aggregate
13 term describing a spectrum of effects. These individual lesions or processes may well occur in the
14 human kidney, and the fact they happen to occur as a group in the aged rat kidney does not guarantee
15 that the individual lesions are rat-specific. Importantly, these effects are exacerbated by ETBE exposure
16 despite being common in aging male and female rats (primarily of the Fischer 344 and Sprague-Dawley
17 strains). Thus, the EPA concludes in the assessment that exacerbation of increased absolute kidney
18 weight, urothelial hyperplasia, and serum biomarkers by ETBE exposure are not due to a rat-specific
19 CPN and, therefore, may be relevant to human kidney hazard assessment.

20
21 Members of the public presented comments, in writing and orally, suggesting that noncancer kidney
22 effects of ETBE should not be considered a hazard for humans. The commenters maintained that all of
23 the noncancer kidney effects of ETBE in male rats could be explained by the $\alpha_2\mu$ -globulin or CPN MOA
24 and those in female rats by the CPN MOA rendering these effects not relevant to humans. The SAB is
25 unable to reach consensus with respect to the EPA's interpretation of the ETBE database for noncancer
26 kidney effects. Some members agree with the overall interpretation and conclusion presented in the
27 assessment and some agree that there was little evidence to support the human relevance of noncancer
28 kidney effects in rats. It was also noted that the focus of the assessment should be on public health and
29 although the EPA should not arbitrarily conclude that a MOA is relevant to human hazard, in the
30 absence of compelling evidence to the contrary and in order to protect human health, considerations
31 should be conservative and the potential for human relevance should not be discounted.

32
33 There is also a concern about the use of urothelial hyperplasia as a surrogate for noncancer kidney
34 effects. The SAB notes that although urothelial hyperplasia encompasses effects in both the papillary
35 and bladder epithelium, there is no known mechanistic link between bladder effects such as urothelial
36 hyperplasia and the various types of kidney injury typically observed with chemicals similar to ETBE.
37 With that said, however, the SAB agrees that the conclusion in section 1.3.1 on page 1-109, lines 29-32
38 is appropriate: "Urothelial hyperplasia in male rats, increased severity of CPN, increased blood
39 biomarkers in male and female rats, and increased kidney weights in male and female rats are considered
40 the result of ETBE exposure, independent (of) $\alpha_2\mu$ -globulin, and relevant for assessing human health
41 hazard." While some members agree with the conclusion that noncancer kidney effects in rats are a
42 potential hazard for humans is correct and appropriate, the urothelial hyperplasia effect could be
43 considered separately from kidney effects. Furthermore, use of exacerbation of CPN and/or increased
44 blood (serum) biomarker levels would seem more appropriate to quantify the hazard for noncancer
45 kidney effects in humans.

1 ***The following recommendations are noted:***
2

3 **Tier 1:**

- 4 • The SAB is unable to reach consensus on whether noncancer kidney effects should be considered
5 a hazard relevant to humans based on the presented information in the Toxicological Review.
6 Justification for the EPA's choice to consider the hazard relevant to humans should be
7 strengthened.
8

9 **Tier 2:**

- 10 • Although consideration of the role of $\alpha_2\mu$ -globulin in ETBE-induced nephropathy in male rats is
11 thoroughly considered according to the 1991 criteria established by the EPA, the SAB
12 recommends that the agency apply the more detailed criteria published by IARC in 1999.
13 • The EPA should consider use of another parameter, such as increases in blood (serum)
14 biomarkers or exacerbation of nephropathy, besides urothelial hyperplasia, as a surrogate for
15 noncancer kidney effects.
16 • The EPA should consider urothelial hyperplasia as separate from noncancer kidney effects in
17 developing the human hazard assessment.
18

19 **Tier 3:**

- 20 • None.
21

22 **3.3.1.2. tBA**

23 *The draft assessment (sections 1.2.1, 1.3.1) identifies kidney effects as a potential human hazard of tert-*
24 *butanol. EPA evaluated the evidence, including the role of $\alpha_2\mu$ -globulin and chronic progressive*
25 *nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this*
26 *conclusion is scientifically supported and clearly described.*
27

28 The SAB agrees that the EPA's draft tBA assessment thoroughly evaluated the role of $\alpha_2\mu$ -globulin in
29 male rat kidney effects of tBA in accordance with the EPA's guidance policy (EPA 1991). Similarly, the
30 SAB finds that the EPA's review methodically discussed the issue of CPN and its potential role and
31 relevance to humans was also discussed methodically. The SAB is divided on whether the EPA's draft
32 tBA assessment is scientifically supported and clearly described. Some members find the EPA's
33 conclusion to be clear and supported, while others find that the Agency presents no clear evidence for
34 tBA noncancer kidney effects in rats that have any relevance for hazard assessment in humans.
35

36 The SAB notes the following per the draft Toxicological Review:
37

- 38 1) Kidney effects are identified as a potential human hazard of tert-butanol exposure based on
39 several endpoints in female rats, including suppurative inflammation, transitional epithelial
40 hyperplasia, severity and incidence of nephropathy, and increased kidney weights. These effects
41 are similar to the kidney effects observed with ETBE exposure (e.g., CPN and urothelial
42 hyperplasia) and MTBE (e.g., CPN and mineralization).
43
44 2) Any kidney effects associated with $\alpha_2\mu$ -globulin nephropathy are not considered relevant for
45 human hazard identification.
46

- 1 3) CPN played a role in the renal tubule nephropathy observed following tert-butanol exposure in
2 female rats. Because female rats were not affected by $\alpha_2\mu$ -globulin nephropathy and the
3 individual lesions associated with the spectrum of toxicities collectively described as CPN can
4 occur in the human kidney, exacerbation of one or more of these lesions might reflect a type of
5 injury relevant to the human kidney. Effects associated with such nephropathy are considered
6 relevant for human hazard identification and suitable for derivation of reference values.
7
- 8 4) Overall, the female rat kidney effects (i.e., suppurative inflammation, transitional epithelial
9 hyperplasia, increased severity of CPN, and increased kidney weights) are considered by the
10 Agency to be the result of tert-butanol exposure and relevant to human hazard characterization.
11 The Agency therefore considers these effects as suitable for consideration for dose-response
12 analysis and derivation of reference values.
13

14 The SAB identifies a few minor concerns within this portion of the EPA's draft tBA assessment. The
15 SAB finds that suppurative inflammation and transitional epithelial hyperplasia may both have multiple,
16 poorly defined etiologies and may not be mechanistically linked to nephropathy. The SAB also finds
17 that suppurative inflammation and transitional epithelial hyperplasia is not mechanistically linked to
18 nephropathy associated with proximal tubular cell injury. The SAB recommends that the EPA consider
19 providing additional discussion of this topic within the draft tBA assessment. The overall conclusion
20 that kidney effects are a potential human hazard associated with tBA exposure is appropriate and
21 scientifically supported. However, the SAB notes some members concluded that all the tBA noncancer
22 kidney effects in rats could be explained by either $\alpha_2\mu$ -globulin nephropathy or CPN and are, therefore,
23 not scientifically supported nor relevant for hazard assessment in humans.
24

25 *The following recommendations are noted:*

26
27 **Tier 1:**

- 28 • None.
29

30 **Tier 2:**

- 31 • The EPA should provide a more thorough explanation for considering the enhancement of CPN
32 as a kidney effect relevant to human hazard assessment.
33 • The EPA should consider other indicators besides suppurative inflammation and transitional
34 epithelial hyperplasia as indicators of kidney effects or provide better justification for their
35 choice.
36

37 **Tier 3:**

- 38 • None.
39

40 **3.3.2. Noncancer toxicity at other sites.**

41
42 **3.3.2.1. ETBE**

43 *The draft assessment (sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1) presents conclusions for noncancer*
44 *toxicity at other sites that were not used as the basis for deriving noncancer oral reference dose or*
45 *inhalation reference concentration purposes. Please comment on whether these conclusions are*
46 *scientifically supported and clearly described. If there are publicly available studies to associate other*

1 *health outcomes with ETBE exposure, please identify them and outline the rationale for including them*
2 *in the assessment.*

- 3 • *Liver effects: Suggestive evidence*
- 4 • *Developmental toxicity: Inadequate evidence*
- 5 • *Male and female reproductive toxicity: Inadequate evidence*

6
7 The SAB agrees with the EPA's conclusions within the draft assessment report that noncancer toxicity
8 at sites other than the kidney should not be used as the basis for deriving oral reference dose or
9 inhalation reference concentrations. The SAB agrees that nearly all of the possible effects at these sites
10 occurred at much higher exposure levels than did effects observed on the kidney, which occurred at 170
11 mg/kg-d or 6,000 mg/m³. The SAB's responses regarding the three targets that the Agency considered
12 (liver, developmental, and male and female reproductive toxicity) are discussed below.

13 14 Liver Toxicity:

15 The SAB reviewed the extensive database developed by the agency on liver toxicity. The SAB agrees
16 that there are liver weight increases in rats, but these were only significant at the highest oral (1,000
17 mg/kg-day) and inhalation (29,900 mg/m³) exposure levels. There are no consistent effects on liver
18 serum enzyme markers. Histological changes (basophilic foci, centrilobular hypertrophy) are observed
19 at the highest doses. The basophilic foci could be of toxicological significance if they are progenitors of
20 adenomas. The hypertrophy is consistent with the increase in total P450 (CYP) and the increase in
21 expression of mRNAs of several CYPs measured by Kakehashi et al. (2013). These researchers also
22 presented findings indicating that events in the male F344 rat liver are mediated through PPAR α -, CAR-
23 and PXR. Upon review of the EPA's draft ETBE assessment and database, the SAB agrees that the EPA
24 provides scientific support for their conclusion that these data are an inadequate basis to conclude that
25 ETBE causes liver tumors by these modes of action. The SAB agrees that the draft assessment report
26 provides scientific support for the EPA's conclusion that there is suggestive evidence for liver effects
27 contributing to noncancer toxicity

28 29 Developmental Toxicity:

30 The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion
31 that almost all assays show no significant developmental or neurodevelopmental toxicities with oral
32 gavage administration of ETBE to rats and rabbits at 1,000 mg/kg-d. Minor effects (skeletal variations,
33 postnatal deaths at a specific time point) were only observed at a high oral dose (1,000 mg/kg-d) in some
34 studies. Since some of these effects may be associated with maternal systemic toxicity and the
35 significance depended on the method of analysis, the SAB agrees that the draft tBA assessment provides
36 scientific support for the EPA's conclusion that these results should not be considered for hazard
37 assessment.

38 39 Male Reproductive Toxicity:

40 The SAB analyzed the data on male reproductive toxicity and in most cases agree that there was very
41 little toxicity in normal (non-mutant) animal models. No male reproductive toxicity (testis weight, sperm
42 number, morphology, and motility, histopathology, androgen dependent accessory organs, fertility) was
43 reported in rats after oral exposure, even at high dose (1,000 mg/kg-d). Only one study (Bond *et al.*,
44 1996b, *citation by* Medinsky, 1999) observed some increase in histological damage (tubule atrophy) in
45 rats exposed to inhalation doses of 7,000 and 21,000 mg/m³, but the increase was not statistically
46 significant, not found in another similar but longer term study (JPEC, 2010b; Saito *et al.*, 2013), and

1 must have been minor because testis weights were unaffected. In normal mice, there were no effects of
2 inhalation doses up to 21,000 mg/m³ on testis weight, sperm production, overall motility, and
3 histopathology; there was only a small effect on quality of motility at 21,000 mg/m³. More sensitive
4 assays of sperm DNA damage indicated effects in normal mice of inhalation doses of 7,000 and 21,000
5 mg/m³; however, it is difficult to evaluate magnitude of that damage and the consequences of this level
6 of damage. The only clearly significant toxic effects were observed with mice deficient in aldehyde
7 dehydrogenase 2 (ALDH2 knockout and heterozygotes). The effects included reductions in overall
8 motility and sperm production and increased levels of DNA damage. The results convincingly showed
9 that damage was produced by exposures as low as 2,100 mg/m³.

10 11 Female Reproductive Toxicity:

12 The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion
13 that there are no female reproductive toxicities observed in studies of ETBE, even despite some systemic
14 toxicities indicated by reduced weight gains of the dam. As adequately discussed within the draft
15 assessment report, these studies assessed ovarian and uterine weights, counts of primordial and growing
16 follicles, estrous cyclicity, pregnancy rates, embryo survival, and overall pup survival to weaning. These
17 parameters are unaffected by oral administration of up to 1000 mg/kg-d for up to 10 weeks plus during
18 development and pregnancy, or by inhalation of up to 5000 ppm (20,900 mg/m³) for up to 2 years. In
19 rabbits, fetal implantation, viability, development, and body weight are unaffected by ETBE
20 administered by gavage to rabbits up to 1000 mg/kg-d during pregnancy

21 22 *The following recommendations are noted:*

23 24 **Tier 1:**

- 25 • Male reproductive toxicity: The SAB suggests describing recommendation as "minimal effects at
26 otherwise toxic dose levels", rather than "inadequate evidence", since the SAB concludes there is
27 an adequate amount of evidence which show minimal effects.
- 28 • Male reproductive toxicity: Since there are male reproductive effects on genetically susceptible
29 mice, which mirror large human populations, at lower doses than other toxicities, the SAB
30 recommends giving more emphasis to these results.
- 31 • Female reproductive toxicity: The SAB suggests describing recommendation as "no effects even
32 at otherwise toxic dose levels", rather than "inadequate evidence", since the SAB concludes there
33 is an adequate amount of evidence, which show minimal effects.

34 35 **Tier 2:**

- 36 • None.

37 38 **Tier 3:**

- 39 • There have been no developmental toxicity studies with inhalation exposure; such studies would
40 be necessary for a complete characterization of the developmental toxicity, but in the absence of
41 indications from oral gavage exposure, these are not of high priority.
- 42 • Because of profound neurological impairment in PXR and CAR knockout mice (Boussadia et al.
43 2016; 2017) and indications that ETBE interferes with these receptors and it is unclear that the
44 most relevant neurological and behavioral endpoints were assessed in the older developmental
45 studies of ETBE, further assessment of developmental neurotoxicity of ETBE is warranted.
46 Studies that examine cognition, plasticity, and behavioral outcomes would be important.

- 1 • Further studies of reproductive effects of ETBE in animals with the specific ALDH2 mutant
2 allele that is present in major human populations are needed. In addition, the association of that
3 ALDH2 polymorphism in humans with cardiovascular and neurological disease (Zou and Wang,
4 2015) suggests that the effects of ETBE on these endpoints should be also analyzed in such an
5 animal model.
- 6 • Further studies should be conducted on the consequences of the DNA damage produced by
7 ETBE compared with those produced by known reproductive genotoxic agents (e.g., ionizing
8 radiation) that also act primarily by producing DNA strand breaks and 8-hydroxy-
9 deoxyguanosine.
- 10 • Further research on mechanisms of action of ETBE inducing liver toxicity, particularly those
11 involving receptor-mediated targets and nuclear signaling pathways, is warranted to better
12 understand its toxic effects and their applicability to human.

13 14 **3.3.2.2. tBA**

15 *The draft assessment (sections 1.2.3-6, and 1.3.1) finds inadequate information to assess developmental,*
16 *neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are*
17 *scientifically supported and clearly described. If there are publicly available studies to associate other*
18 *health outcomes with tert-butanol exposure, please identify them and outline the rationale for including*
19 *them in the assessment.*

20
21 The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion
22 that noncancer toxicity at sites other than the kidney should not be used as the basis for deriving an oral
23 reference dose. Nearly all of the possible effects at these sites occurred at much higher exposure levels
24 than did effects on the rat kidney, which occurred at 180 mg/kg-d. However, the EPA's calculation of an
25 equivalent inhalation dose by PBPK modeling, which yielded 472 mg/m³, is open to question as no
26 significant increases in severity of nephropathy were reported at doses of 6,400 mg/m³ for 13-weeks and
27 the LOAEL for increased kidney weight is 3,300 mg/m³. The EPA's discussion of other sites within the
28 draft assessment report is warranted as there are some questions regarding the applicability of the kidney
29 endpoint to humans.

30 31 **Developmental Toxicity:**

32 The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion
33 that almost all assays show no significant developmental toxicity from tBA at oral doses of <1000
34 mg/kg-day or inhalation exposures of <10,600 mg/m³. Reductions in rat and mouse fetal and pup
35 survival and pup body weight were observed at oral tBA doses of ≥1,000 mg/kg-d or inhalation doses of
36 ≥10,600 mg/m³, but indirect effects related to maternal toxicities are probable.

37
38 Regarding neurodevelopmental toxicity, the SAB agrees that the draft assessment report provides
39 scientific support for the EPA's conclusion that although several studies have reported
40 neurodevelopmental effects of exposure to tBA, because of limitations of these studies, these studies
41 should not be used in calculating reference values. The SAB also agrees that the draft assessment report
42 provides scientific support for the EPA's conclusion that effects have only been observed at high
43 exposure levels. Behavioral deficiencies were observed in mice exposed to 5,000 but not 3,300 mg/kg-d
44 during gestation. Changes in brain neurotransmitters were observed in offspring of mice exposed to
45 6,000 mg/m³ during gestation.

1 Reproductive Toxicity:

2 The SAB agrees that the draft tBA assessment provides scientific support for the EPA's conclusion that
3 the studies performed did not find any evidence for reproductive effects of tBA. The only apparently
4 statistically significant changes in males are a decline in sperm motility in rats and a possible loss in
5 testis weight in mice. However, the decline in motility observed in rats at 1000 mg/kg-d is only
6 marginal, from 94% to 91% (Huntingdon Life Sciences, 2004), and within historical control ranges and
7 the possible (see Comments below) testis weight loss (NTP, 1995) at a dose of 3900 mg/kg-d could be
8 an indirect effect as there is 60% mortality at this dose. The only reported significant effect in females
9 in the draft tBA assessment was an extension of the mouse estrous cycle from 4 to 5 days after oral
10 exposure to 11,600 mg/kg-d of tBA for 13 weeks. However, as indicated in the recommendations below,
11 EPA should also have analyzed the percentages of females that did not show clear estrous cycles. Then,
12 as none of the rats exposed to 3,620 mg/kg-d showed clear estrous cycles, a significant effect in the rat
13 would have been shown. Nevertheless, the SAB agrees that the draft tBA assessment provides scientific
14 support for the EPA's conclusion that neither of these effects should be considered to be a result of
15 direct action on the reproductive system as animal weight gains were significantly reduced and mortality
16 was 40% and 60% in the rats and mice, respectively.

17
18 *The following recommendations are noted:*

19
20 **Tier 1:**

- 21 • The SAB recommends that the Agency include contact dermatitis (Edwards, 1982) in hazard
22 identification as dermal exposure is a relevant route of exposure.
- 23 • Maternal toxicity has effects on offspring development, particularly on neural and behavioral
24 development, and female reproductive performance. Therefore, the LOAEL for lethargy and
25 ataxia should be considered in the reference dose analysis. More specific information on
26 metabolic and sedative actions of tBA on the exposed dam is needed since it impacts
27 reproductive function and development of the offspring. Therefore, the LOAEL for lethargy and
28 ataxia should be considered in the reference dose analysis.
- 29 • The SAB suggests changing the description to "minimal effects at otherwise toxic dose levels",
30 rather than "inadequate information to assess", since the SAB believes there is an adequate
31 amount of information, and only minimal effects have been shown, even at toxic dose levels.

32
33 **Tier 2:**

- 34 • None.

35
36 **Tier 3:**

- 37 • Because available studies, which were conducted more than 20 years ago, have detected effects
38 but those studies are limited, further studies with more modern methods are warranted. Detailed
39 behavioral testing in the cognitive, social, anxiety, and hyperexcitability domains are needed.
- 40 • Research on non-mammalian systems (e.g., zebrafish) to determine whether or not there are
41 developmental targets of tBA should be pursued.

1 **3.3.3. Oral reference dose for noncancer outcomes.**
2

3 **3.3.3.1. ETBE**

4 *Section 2.1 presents an oral reference dose of 5×10^{-1} mg/kg-day, based on urothelial hyperplasia in*
5 *male rats (Suzuki et al. 2012). Please comment on whether this value is scientifically supported and its*
6 *derivation clearly described. If an alternative data set or approach would be more appropriate, please*
7 *outline how such data might be used or how the approach might be developed.*
8

9 The responses to question 3c. are premised on overall acceptance of the support of kidney effects of
10 ETBE as an appropriate endpoint. The SAB has not reached consensus to support acceptance of kidney
11 effects. The differing views are based on the extent of confidence in a CPN-based mechanism for these
12 effects. However, if urothelial hyperplasia in male rats (Suzuki et al., 2012) is used for risk assessment,
13 then the derivation of an oral reference dose of 5×10^{-1} mg/kg-day is considered to be scientifically
14 supported and its derivation clearly described in the text. Several recommendations emerged from the
15 SAB's deliberations.

16
17 *The following recommendations are noted:*
18

19 **Tier 1:**

- 20 • The EPA should carefully examine the question of the validity and applicability of the endpoints
21 chosen and analyzed for the oral RfD, including the potential for CPN to serve as the mechanism
22 of the kidney effects.
- 23 • The tables within this section need to include units for completeness and interpretability.
- 24 • The EPA should consider a more integrated presentation of the current text, tables and graph; as
25 is, it is difficult to track information and the text often requires much page-flipping.

26
27 **Tier 2:**
28

- 29 • If urothelial hyperplasia is deemed an inappropriate endpoint for derivation of the oral reference
30 dose, then the SAB encourages the Agency to consider use of liver hypertrophy instead as the
31 basis of the oral reference dose for ETBE.
- 32 • Because the assessment report notes that ETBE and tBA appear to have caused a similar set of
33 kidney responses, and because tBA as a metabolite of ETBE is implicated as a cause, the SAB
34 encourages the Agency to examine the degree to which patterns and response levels are similar
35 across the two chemicals, and whether a common response to tBA (either as a metabolite of
36 dosed ETBE or as the tested material itself) can be discerned.

37
38 **Tier 3:**

- 39 • Lack of consensus on the role of CPN as a mechanism of kidney effects included disagreement
40 as to whether CPN constituted a set of manifestations or whether individual components seen in
41 CPN could also occur separate from CPN. An updated assessment of CPN including criteria for
42 its definition, manifestation as a group of outcomes vs. individual outcomes would be helpful. It
43 would be informative for EPA to include outcomes of statistical analyses and their rationale in
44 study selection choice.

- EPA should consider evaluation of rat-human differences in ETBE metabolic activity so as to assess rat to human extrapolation that could then be used to assess effects relative to internal dose of tBA as they appear in both profiles.

3.3.3.2. tBA

Section 2.1 presents an oral reference dose of 4×10^{-1} mg/kg-day, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

The SAB's response to this question is premised on overall acceptance of the support of nephropathy effects in female rats as an appropriate endpoint for the oral reference dose for tBA. However, there is not a consensus among members as to the appropriateness of the selection of nephropathy effects. The differing views are based on the extent of confidence in CPN- and/or $\alpha_2\mu$ -globulin-based mechanisms for these effects as a mechanism of tBA effects. However, if the EPA's selection of increases in severity of nephropathy in female rats in response to drinking water tBA administration remains the basis of the oral reference dose, then the SAB considers the derivation of the oral reference dose of 4×10^{-1} mg/kg-day to be scientifically supported and its derivation clearly described.

The following recommendations are noted:

Tier 1:

- The validity and applicability of the endpoints chosen and analyzed for the oral RfD for tBA should be carefully reexamined, including the potential for CPN and/or $\alpha_2\mu$ -globulin to serve as mechanism(s) of the kidney effects of tBA, in light of SAB advice regarding consideration of the criteria for definition of CPN.

Tier 2:

- If nephropathy in females is ultimately deemed to be an inappropriate endpoint for derivation of the oral reference dose for tBA, then the SAB encourages the EPA to use liver hypertrophy for the endpoint derivation.

Tier 3:

- The role of CPN as a mechanism of kidney effects include disagreement as to whether CPN constituted a set of manifestations or whether individual components seen in CPN could also occur separate from CPN. An updated assessment of CPN including, e.g., criteria for its definition, manifestation as a group of outcomes vs. individual outcomes would clarify these issues.
- The outcomes of statistical analyses and their rationale in study selection choice should be included in the EPA's draft tBA assessment.
- The units need to be added to the tables in this section for completeness and interpretability.
- It would be useful to attempt a more integrated presentation of the current text, tables and graphs. As currently laid out, the reader is forced to engage in a lot of page flipping in order to read the draft tBA assessment, making it difficult to track information.

- The Agency should consider evaluation of rat-human differences in ETBE metabolic activity so as to assess rat to human extrapolation that could then be used to assess effects relative to internal dose of tBA as they appear in both profiles.

3.3.4. Inhalation reference concentration for noncancer outcomes.

3.3.4.1. ETBE

Section 2.2 presents an inhalation reference concentration of $9 \times 10^0 \text{ mg/m}^3$, based on urothelial hyperplasia in male rats (Saito et al. 2013). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

The SAB concludes that the derivation of all RfC candidate values is described clearly. The SAB also agrees that there is human relevance regarding the increased urothelial hyperplasia in the male rat kidneys (Saito et al, 2013), and that the EPA's derivation of the RfC of $9 \times 10^0 \text{ mg/m}^3$ is scientifically supported. But the SAB also raises concerns regarding the human relevance of the kidney endpoints reported in the rat. Beyond this issue, some members suggested alternative approaches to derivation of the RfC, including selection of alternative endpoints.

As is the case with previous charge questions, there was concern expressed about the limited critical discussion of the comparative pathology between the rat and the human kidney for the specific lesions/injuries selected for deriving candidate reference values for noncancer effects. EPA argues that individual lesions, rather than the cluster of lesions (not necessarily all present simultaneously) that conforms the CPN pathology in the rat, may occur in the human kidney, and that exacerbation of any one of these lesions is likely to reflect cell injury relevant to humans even if CPN is not. This is not an unreasonable argument; however, this perspective should be more strongly supported by additional, explicit discussions of the comparative nephropathology in both species that can justify the use of these injuries for derivation of reference values. Otherwise, the argument remains more of an assumption than an evidence-based decision.

For noncancer outcomes, evidence found to be consistent relied upon kidney effects of organ weight changes, and histopathology (urothelial hyperplasia). Organ weight changes for chronic and sub-chronic exposures by the inhalation route were clearly reproducible across studies in the rat. For urothelial hyperplasia, chronic 2-year studies using inhalation exposure established that this response increased with treatment in male rats. The urothelial hyperplasia data were the only endpoint from chronic exposure (2-year) studies, and organ weight changes were the only endpoint from sub-chronic (13-week) studies that were considered for dose-response analysis (Saito et al., 2013; JPEC, 2010b). The SAB agrees that the Saito et al. (2013) study, upon which the RfC is based, was well conducted, and adhered strictly to GLP guidelines, including evaluations of sufficient numbers of animals per group (both sexes), and a broad collection and assessment of appropriate kidney tissue samples, and relevant outcome measures (organ weight, histopathology).

The SAB agrees that the draft assessment report provides scientific support for the EPA's conclusion that urothelial hyperplasia is, in principle, a more specific indicator of kidney toxicity than the relatively nonspecific endpoint of kidney weight change. However, the SAB notes that data of urothelial hyperplasia from the 2-year male rat study by Saito et al. (2013) and increased absolute kidney weight in

1 male rats from the 13-week study by JPEC (2008) suggest that increased absolute kidney weight
2 appeared as a more sensitive index of kidney toxicity (Table 2-5, Pg. 2-16). If the EPA utilized increases
3 in absolute kidney weight in male rats as the candidate value for the RfC, a more health-protective RfC
4 value would be determined. Further it appears that what the EPA refers to as urothelial hyperplasia is
5 actually renal pelvis transitional hyperplasia, which is part of CPN. Thus, the SAB recommends that the
6 Agency describe this endpoint as “increases in severity of nephropathy” to more appropriately
7 correspond with the characterization in the draft tBA assessment.
8

9 The SAB also notes that the absence of ETBE effects on most reproductive and developmental
10 endpoints at doses of 21,000 mg/m³ [compared to a LOEL of 1500 ppm (6270 mg/m³) for kidney
11 endpoints] indicates such effects are not important for setting an RfC. However, sperm DNA damage
12 (DNA breaks, 8-oxo-deoxyguanine) and minor histopathological changes in B6 mice were observed at
13 similar exposure levels to those reported in rat kidneys and, therefore, could also be considered in the
14 setting of an RfC.
15

16 Also of note, the Agency should consider utilizing the exacerbation of CPN in female rats as a toxic
17 endpoint. As described previously, exacerbation of CPN, which is apparently augmented by $\alpha_2\mu$ -
18 globulin in male rat kidneys, is not relevant to humans.
19

20 The SAB largely agrees that benchmark dose (BMD) modeling for kidney effects was performed
21 appropriately by the EPA. The EPA adjusted intermittent concentrations, human equivalent
22 concentration derivation and benchmark modeling or extrapolation for deriving points of departure
23 (PODs) in accordance with EPA guidelines. It was noted that the use of PBPK modeling be reconsidered
24 for cross-species extrapolation to replace the body weight ³/₄ default.
25

26 Also in accordance with EPA guidelines, the Agency applied uncertainty factors to the PODs and the
27 SAB finds that this application is reasonable and consistent with EPA guidelines. Page 2-18, line 8 of
28 the assessment notes the range among candidate values for an inhalation RfC varied over a 100-fold
29 range. The SAB’s examination of Table 2-5 suggests that much of this range is actually a consequence
30 of differing choices of uncertainty factors (UFs) [depending on what is done for UFs] as well as of the
31 male-female differences. The PODs themselves are rather similar (for shorter and longer durations)
32 within the same sex, though somewhat different between males and females.
33

34 The SAB finds that the assessment clearly describes the limitations of the estimates. All the candidate
35 values except for the estimated RfCs based on increased CPN severity in male and female rats in the
36 chronic study, and on increased absolute kidney weight in male rats in the 13-week study, are within the
37 same order of magnitude. The RfC based on increased CPN severity in both male and female rats was
38 approximately 4 times higher than the selected RfC. Therefore, the draft assessment report provides
39 scientific support that there is relative consistency in the various estimates.
40

41 The kidney responses in the rat are similar for inhalation and oral exposures to ETBE and are,
42 presumably, attributable to metabolic generation of tBA. Considering that the exposure to tBA is
43 systemic from metabolism occurring elsewhere (the liver), the SAB recommends that the Agency assess
44 how similar the apparent potencies are for inhalation and oral exposure if rendered in terms of projected
45 tissue concentrations for tBA.
46

1 Although there is copious tabulation of experimental results, it is difficult to trace the information on
2 any one study from its discussion in the draft assessment to the tabulations in the Supplemental
3 Information, and to the dose-response analysis elsewhere in the Supplemental Information. A
4 complicating factor is that there are different analyses of the same data (e.g., absolute and relative organ
5 weights), different durations of exposure for otherwise similar experiments, and lack of uniqueness of
6 “author (date)” designations, making it challenging to be sure that one is examining corresponding data
7 in the different places where the data are discussed or presented.
8

9 Critically, there does not seem to be any reporting of statistical analysis of individual studies (trend tests
10 or pair wise significance tests, and other statistical tests determined to be appropriate), and this omission
11 hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role
12 of such statistical analysis in identifying results to report and the decisions to include or exclude them
13 from analysis is not and should be clearly stated within the assessment report.
14

15 ***The following recommendations are noted:***

16
17 **Tier 1:**

- 18 • None.

19
20 **Tier 2:**

- 21 • The SAB suggests the following alternatives for deriving the RfC, including:
 - 22 ○ DNA damage (DNA breaks, 8-oxo-deoxyguanine) and minor histopathological changes in
 - 23 B6 mice.
 - 24 ○ Exacerbation of CPN in female rats.
 - 25 ○ Basing the RfC on increased absolute kidney weights in male rats in the 13-week study
 - 26 because this endpoint appears to be more sensitive than urothelial hyperplasia.
 - 27 ○ Assess specific potencies and cross-route evaluation by comparing estimated tissue doses of
 - 28 the metabolite tBA.
- 29 • EPA should provide statistical analysis to help elucidate differences in response based on sex and
- 30 to make clear the rationale for including or excluding studies.
- 31 • Sex differences in response appear more marked for inhalation than for oral exposures. An
- 32 evaluation of possible reasons for this (including mere statistical fluctuation which, if responsible
- 33 would suggest averaging endpoint values across sexes) would be informative.

34
35 **Tier 3:**

- 36 • None.

37
38 **3.3.4.2. tBA**

39 *Section 2.2 presents an inhalation reference concentration of 5×10^0 mg/m³, based on increases in*
40 *severity of nephropathy in female rats via drinking water (NTP, 1995), converted for inhalation*
41 *exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is*
42 *scientifically supported and its derivation clearly described. If an alternative data set or approach*
43 *would be more appropriate, please outline how such data might be used or the approach might be*
44 *developed.*
45

1 The SAB agrees that if the agency accepts that the severity of tBA-induced nephropathy in the female
2 rat is relevant to humans, the estimated 5×10^0 mg/m³ RfC is scientifically defensible. The derivation of
3 the RfC is mostly described clearly, except for issues that require further clarification as described
4 below.

5
6 As with ETBE, the SAB expresses concern that EPA did not provide a clear summary description of
7 histological changes observed in the development of nephropathies in humans as compared to the
8 changes observed in rats, both age-related-only and consequent to chemical exposure. The stated
9 assumption that some of the individual tissue outcomes observed in the rodent studies (associated with
10 CPN) could also be relevant to humans even if CPN is not relevant is, in principle, reasonable, but the
11 draft tBA assessment does not and should provide the line of scientific evidence of comparative
12 histological changes in rodent and human nephropathies to support this statement.

13
14 The SAB agrees that the EPA's evaluation of the literature for noncancer effects supports the selection
15 of kidney effects for establishing the overall RfC. The SAB also agrees with EPA's rationale for
16 selecting dose-response data from female rats as a more appropriate basis for calculating BMDs for
17 kidney effects than dose-response data from male rats.

18
19 The SAB agrees that the tBA draft assessment provides scientific support for the EPA's conclusion that
20 no route-specific chronic studies are available for derivation of candidate RfC values for tBA. EPA
21 derived PODs from the female rat dose-response data from the NTP (1995) 2-year oral study with route-
22 to-route extrapolation, and dose-response data for the same strain of rat as the oral study from the 13-
23 week NTP (1997) study adjusted for exposure duration. The SAB considers that the use of route-to-route
24 extrapolation based on the Borghoff et al. (2016) PBPK model as modified by U.S.EPA (2017) is
25 reasonable. However, the use of route-to-route extrapolation for deriving RfC's is a deviation from
26 typical practice. Route-to-route extrapolation leads to considerable added uncertainty and the
27 contingency of the estimates on the validity of the chosen extrapolation method. Given the availability
28 of some inhalation data (though not for full lifetime), the discussion to use route-to-route extrapolation
29 ought to explain in more detail why preference was given to an RfC derived from the extrapolated oral
30 doses over inhalation results. More specific details need to be provided about the application of the
31 PBPK model for route-to-route extrapolation, which are not presented in sufficient detail on page 2-12
32 of the draft tBA assessment or in Appendix B of the Supplemental Information. In particular, the choice
33 of the dose metric to represent "internal dose" (page 2-12, line of the draft tBA assessment) requires
34 further explanation and additional justification. The caveats introduced by the uncertainty inherent in the
35 use of the PBPK model for route-to-route extrapolation should be more explicitly stated when
36 summarizing the findings in this section and in the Executive Summary.

37
38 Aside from issues of route-to-route extrapolation, derivation of PODs from the NTP (1997) 13- week
39 inhalation study (endpoint: absolute kidney weight), and from the NTP (1995) oral study with route-to-
40 route extrapolation (endpoints: increased absolute kidney weight, kidney inflammation, kidney
41 transitional epithelial hyperplasia, and increases in severity of nephropathy) are performed following
42 straightforward methods according to EPA guidelines. The PODs for candidate RfC values are adjusted
43 by uncertainty factors also according to established guidelines. The SAB agrees that the range of
44 candidate values for the RfC is not excessively large (i.e., within a factor of 7), which adds some
45 measure of reliability to the derivation of candidate values. In particular, the difference in the candidate

1 RfC derived from the subchronic inhalation study based on increases in kidney weight is approximately
2 only 20% lower than the selected RfC.

3
4 There are additional areas that require clarification by EPA (similar to concerns in Questions 3c and 3d
5 for ETBE, and Question 3c for tBA). Although there is extensive tabulation of experimental results, it is
6 difficult to trace the information provided for any one study from its discussion in the text to the
7 tabulations in the Supplemental Information, and to the dose-response analysis elsewhere in the
8 Supplemental Information. This is complicated by the multiple different analyses of the same primary
9 data (e.g., absolute and relative organ weights), different durations of exposure for otherwise similar
10 experiments, and lack of uniqueness of “author (date)” designations, making it challenging to the reader
11 to be certain that data under examination correspond as presented and discussed in different locations of
12 the draft tBA and ETBE assessments.

13
14 Critically, there seems to be little reporting of statistical analysis of individual studies (trend tests or
15 pairwise significance tests, and other statistical tests determined to be appropriate) and this omission
16 hampers consideration of the appropriateness of inclusion and use of studies. Also importantly, the role
17 of such statistical analysis in identifying results to report and decisions to include or exclude them from
18 analysis are not very clearly stated.

19
20 There is no reporting of units for the responses (as opposed to the exposures) in the Supplemental
21 Information tables, and this leads to difficulty in interpretation.

22
23 Given that the kidney responses are similar for inhalation and oral exposure to ETBE, and are
24 presumably attributable to metabolic generation of tBA, and also considering that the exposure to tBA is
25 systemic from metabolism occurring elsewhere (i.e., the liver), it would be valuable to assess how
26 similar the apparent potencies are for inhalation and oral exposure if rendered in terms of projected
27 tissue concentrations for tBA. The sex differences in response appear more marked for inhalation
28 exposure than for oral, and an interpretation of possible reasons for this (including mere statistical
29 fluctuation which, if responsible, would suggest averaging across sexes) would be informative.

30
31 ***The following recommendations are noted:***

32
33 **Tier 1:**

- 34 • More detailed information should be provided about the specific application of the Borghoff et
35 al. (2106)/U.S.EPA (2017) PBPK model used for route-to-route extrapolation to derive the
36 inhalation RfC.

37
38 **Tier 2:**

- 39 • It would be valuable to provide estimates of route-specific potencies for inhalation and oral
40 exposure in terms of projected tissue concentrations for tBA, including estimates for tBA as the
41 principal metabolite in studies of exposures to ETBE.
- 42 • EPA should provide more reporting of statistical analysis of individual studies to help clarify the
43 appropriateness of inclusion/exclusion and use of studies.
- 44 • Additional statistical and cross-route analyses may help elucidate sex difference in response, and
45 could potentially permit averaging responses across sexes.

1 **Tier 3:**

- 2 • None.

3
4 **3.4. Hazard Identification and Dose-Response Assessment: Cancer**

5
6 **3.4.1. Cancer modes-of-action in the liver.**

7
8 **3.4.1.1. ETBE**

9 *As described in section 1.2.2, the draft assessment evaluated the roles of the receptor pathways PPAR α , PXR, and CAR in ETBE tumorigenesis in male rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005), considered the liver tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.*

13
14 The SAB finds that there is scientific support for the EPA's conclusion that liver tumors in male rats are relevant to human hazard identification. According to EPA Cancer Guidelines, a conclusion that carcinogenic effects in animals are not relevant to humans requires "convincing and extensive experimental evidence." (U.S. EPA, 2005). For example, for a PPAR α agonist, evidence must be sufficient to show that the liver tumors are the result of a PPAR α MOA, and other potential MOAs have been examined and found to be inoperative (U.S. EPA, 2003). The draft assessment examines in some detail evidence that male rat liver tumors from ETBE might be the result of a PPAR α , PXR, or CAR MOA, each of which has been postulated to produce liver tumors in a manner not relevant to humans. The draft ETBE assessment also considers other possible MOAs, including acetaldehyde-mediated liver effects, genotoxicity, and oxidative stress. A study by Takehashi et al. (2013) found evidence of PPAR-, PXR-, and CAR-mediated events in rats treated with ETBE, but activation of these receptors alone is insufficient to establish a MOA. The EPA found that data to support the existence of other key events necessary to establish one or more of these nuclear receptor-mediated MOAs are weak or absent. Further, the draft assessment points out that other plausible, potentially human-relevant MOAs cannot be ruled out, in particular one mediated through metabolism of ETBE to acetaldehyde. The SAB agrees that experimental evidence for a PPAR, PXR, or CAR MOA for ETBE does not rise to the "convincing and extensive" threshold as described in the EPA cancer guidelines. The SAB also agrees that the MOA for the rat liver tumors remains at this point undetermined. Under circumstances such as this, tumor responses in animals are assumed to be relevant to human hazard identification.

27
28
29
30
31
32
33 While supporting the EPA's decision regarding human relevance of the male rat liver tumors, the SAB finds that improvement is needed for aspects of the discussion of MOA for hepatic effects of ETBE in Section 1.2.2 of the draft assessment. Specifically:

- 34
35
36
37 1. The draft assessment lacks clarity on specific information needed to conclude that a PPAR α , CAR, or PXR MOA is operative. Key events for each of the MOAs are outlined, followed by a narrative regarding the nature and existence of data available regarding these key events. However, there is no articulation of a framework or set of criteria to determine what data would be sufficient to conclude that one of these MOAs is operative. In other words, the draft assessment provides no sense of where the bar is set for establishing a PPAR α , CAR, or PXR MOA for ETBE rat liver tumors. As a result, the MOA analysis for receptor-mediated events appears more subjective than it should. Further, some of the EPA criticisms of data regarding key events are seen as inconsequential or in error, which further detracted from this section.

1 2. Evidence for other, human-relevant, MOAs is not clearly presented. The draft assessment
2 provides a summary table (Table 1-13) with evidence regarding each of 10 potential carcinogen
3 mechanisms, or “key characteristics.” Examples, include genotoxicity; oxidative stress;
4 immunosuppression; altered cell proliferation, cell death, or nutrient supply. For half of these, the
5 evidence is summarized as “No positive studies identified.” Regarding this presentation, the Agency
6 should clarify whether data exist for these key characteristics but are negative, or that no evidence
7 for or against exists.
8

9 3. Evidence for an acetaldehyde MOA is not well developed. The draft assessment states (pg. 1-55)
10 “Evidence suggests that metabolism of ETBE to acetaldehyde could contribute to ETBE-induced
11 liver carcinogenesis.” This evidence is summarized on pages 1-53 and 1-54, and is based primarily
12 upon previously demonstrated carcinogenic effects of acetaldehyde, which is a metabolite of ETBE.
13 The EPA appears to consider this a strong candidate MOA, and it is the basis for most of the
14 discussion of Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes in Section
15 1.3.3. However, the SAB finds that to advance this as a potential MOA for ETBE rat liver tumors,
16 additional critical analysis of the literature is needed. This should include more detailed
17 comparisons with acetaldehyde tumor data, including dose (from ETBE versus acetaldehyde given
18 directly) and tumor site concordance.
19

20 Although the charge question asks about human relevance based upon MOA, the SAB raises concern
21 regarding the human relevance of the ETBE rat liver tumors because they were only observed at an
22 excessively high dose (as defined in the EPA Cancer Guidelines).

23
24 ***The following recommendations are noted:***

25
26 **Tier 1:**

- 27 • None.

28
29 **Tier 2:**

- 30 • EPA should clarify the evidence needed to conclude that a PPAR α , CAR, and/or PXR MOA is
31 operative and indicative that liver tumors may not be relevant to humans. Examples may be
32 helpful to illustrate the types of studies/information needed to satisfy each criterion.
- 33 • EPA should revisit the evaluation of information available for ETBE using these criteria. The
34 EPA may specifically want to reconsider statements about transient hypertrophy.
- 35 • EPA should revise Table 1-13 and accompanying narrative to be more descriptive regarding
36 availability of information for each MOA. Instead of saying “No positive studies identified”
37 indicate whether studies relevant to the MOA exist and where results are positive or negative.
- 38 • Acetaldehyde is proposed as a strong candidate MOA for male rat liver tumors, but the
39 plausibility of this MOA is not well explored. Evidence for this MOA should be developed and
40 presented more thoroughly; or, alternatively, the agency is encouraged to reduce emphasis on
41 this MOA in the final assessment.
42

1 **Tier 3:**

- 2 • None.

3
4 **3.4.1.2. tBA**

5 *Cancer modes-of-action in the kidney. As described in section 1.2.1, kidney tumors were observed in*
6 *male rats following tert-butanol exposure, and a mode-of-action involving $\alpha_2\mu$ -globulin and/or chronic*
7 *progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on*
8 *renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats*
9 *to be relevant to human hazard identification. Please comment on whether this conclusion is*
10 *scientifically supported.*

11
12 The SAB has not reached consensus regarding the EPA's conclusion that male rat kidney tumors are
13 relevant to human hazard identification and is scientifically supported. The draft assessment concludes
14 that evidence for a MOA involving $\alpha_2\mu$ -globulin or CPN is incomplete or not coherent, respectively.
15 While some tumors might be attributable to $\alpha_2\mu$ -globulin nephropathy augmented by CPN, others could
16 be due to other unspecified processes that are assumed to be relevant to humans.

17
18 The SAB has not reached consensus because some members agree with the assessment and some
19 members conclude that renal tumors could be explained by CPN, and are therefore not relevant to
20 humans.

21
22 ***The following recommendations are noted:***

23
24 **Tier 1:**

- 25 • None.

26
27 **Tier 2:**

- 28 • None.

29
30 **Tier 3:**

- 31 • None.

32
33 *Cancer modes-of-action in the thyroid. As described in section 1.2.2, thyroid tumors were observed in*
34 *male and female mice following tert-butanol exposure, and an anti-thyroid mode-of-action was*
35 *evaluated. The analysis, conducted in accordance with EPA's guidance on thyroid follicular cell tumors*
36 *in rodents (U.S. EPA, 1998), found the information inadequate to determine whether an anti-thyroid*
37 *mode-of-action was operating and considered the thyroid follicular cell tumors in male and female mice*
38 *to be relevant to humans. Please comment on whether this conclusion is scientifically supported.*

39
40 The SAB finds that there is scientific support for the EPA's conclusion that thyroid follicular cell tumors
41 in mice are relevant to humans for tBA. However, the SAB finds that there is uncertainty as to whether
42 an increase in thyroid follicular cell tumors is demonstrated in male mice.

1 ***The following recommendations are noted:***
2

3 **Tier 1:**

- 4 • None.
5

6 **Tier 2:**

- 7 • None.
8

9 **Tier 3:**

- 10 • None.
11

12 **3.4.2. Cancer characterization.**

13
14 **3.4.2.1. ETBE**

15 *As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in accordance with EPA’s cancer guidelines*
16 *(U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of carcinogenic*
17 *potential for ETBE by all routes of exposure, based on liver tumors in male F344 rats via inhalation and*
18 *on promotion of liver, colon, thyroid, forestomach, and urinary bladder tumors in male rats via oral*
19 *exposure. ~~Does the classification give appropriate weight to the results from initiation-promotion~~*
20 *~~studies? Please comment on whether this cancer descriptor is scientifically supported. If another cancer~~*
21 *~~descriptor should be selected, please outline how it might be supported.~~*

22 *Please comment on whether the decision to include 2-stage initiation-promotion studies in the human*
23 *cancer hazard characterization is sufficiently justified and if the amount of emphasis placed on the*
24 *initiation promotion data in the cancer hazard characterization is scientifically supported. Please*
25 *comment on whether the “suggestive evidence” cancer descriptor is scientifically supported for all*
26 *routes of exposure. If another cancer descriptor should be selected, please outline how it might be*
27 *supported.*
28

29 The SAB considered whether evidence for ETBE’s carcinogenic potential technically meets
30 requirements under EPA’s 2015 Guidelines for Carcinogen Risk Assessment for the descriptor
31 “Suggestive Evidence of Carcinogenic Potential”. There is concern that the quality of data supporting
32 this designation is weak, even for the relatively low threshold for evidence needed for that designation.
33 The SAB notes that there are conflicting cancer bioassay results (adenomas (+1 carcinoma) in 1 organ of
34 1 sex of 1 species in 1 of 3 bioassays, at an exceedingly high carcinogenic dose (5,000 ppm vapor = 4.2
35 g/kg/day), lack of genotoxicity (preponderance of data), and lack of concordance of effect with different
36 exposure routes. Nevertheless, the SAB agrees that the “Suggestive Evidence” descriptor should be
37 retained for inhaled ETBE, as evidence of the carcinogenic potential of ETBE met minimal criteria for
38 that designation as described in EPA’s 2015 Cancer Guidelines.
39

40 The SAB notes that inhaled ETBE has been found to produce liver tumors in male rats (Saito et al.
41 2013). Ingestion of ETBE, however, was neither carcinogenic to the liver of rats in two 2-year bioassays
42 (Maltoni et al. 1999; Suzuki et al., 2012) nor in a 23-week gavage study (Hagiwara et al. 2011). The
43 EPA Guidelines for Cancer Risk Assessment (2015) state “When tumors occur at a site other than the
44 point of initial contact, the descriptor ‘Suggestive Evidence’ generally applies to all exposure routes that
45 have not been adequately tested at sufficient doses.” ETBE, as noted above, has been consistently
46 negative in oral bioassays.

1 There were differences of opinion on the relevance, justification and scientific validity of the initiation-
2 promotion assay described in the Cancer Hazard Characterization section, and therefore the SAB does
3 not reach consensus in its response to this question. One opinion was that the inclusion of the assay
4 findings is warranted, because they could have relevance to ETBE's carcinogenic potential or provided
5 information about the possible risks of chemical interactions involving ETBE. Other members did not
6 attach scientific value to the assay's results because they felt that the assay was not relevant to humans
7 due to its use of high doses of multiple potent initiators, only reflected ETBE-induced metabolic
8 activation of the initiators, or believed the assay had no value in risk assessment.

9
10 The SAB also observed that initiation-promotion assays had been used in the past by the EPA as
11 supportive evidence for conclusions based on more traditional cancer bioassays, but felt that it was not
12 appropriate to use such as assay as key evidence to support a conclusion of carcinogenic potential for a
13 given route of exposure.

14
15 ***The following recommendations are noted:***

16
17 **Tier 1:**

- 18 • The SAB supports the use of the descriptor "Inadequate Information" for oral ETBE, and
19 "Suggestive Evidence" for inhaled ETBE.

20
21 **Tier 2:**

- 22 • The SAB maintains that EPA should explain within the assessment that the assigned cancer
23 classifications are an EPA Cancer Guidelines policy-based decision.

24
25 **Tier 3:**

- 26 • The SAB observes that it would be useful for the EPA in future Toxicological Reviews to devote
27 more attention to assessing assays' design, relevance, interpretation, limitations and utility in
28 characterizing chemicals' cancer potential.

29
30 **3.4.2.2. tBA**

31 *Cancer characterization. As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's*
32 *cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is suggestive evidence of*
33 *carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female*
34 *B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water.*
35 *Please comment on whether this cancer descriptor is scientifically supported. If another cancer*
36 *descriptor should be selected, please outline how it might be supported. Please comment on whether the*
37 *"suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another*
38 *cancer descriptor should be selected, please outline how it might be supported.*

39
40 The SAB agrees that there is scientific support for the EPA's decision to select "Suggestive Evidence of
41 Carcinogenic Potential" as the proper descriptor for tBA, because tBA was found to cause renal tubule
42 adenomas in male F344 rats and thyroid follicular adenomas in female B6C3F1 mice. This cancer
43 descriptor is scientifically supported for oral exposure, though there have apparently been no inhalation
44 bioassays of tBA. The EPA Guidelines for Cancer Risk Assessment (2015) state: "When tumors occur at
45 a site other than the point of initial contact, the descriptor generally applies to all exposure routes that
46 have not been adequately tested at sufficient doses." While there was a suggestion that the correct

1 descriptor for tBA is “Likely to be Carcinogenic to Humans”, since tBA produced tumors in two
2 species, the SAB, however, recommends that the “Suggestive Evidence” be applied to both oral and
3 inhalation tBA exposure.

4
5 The SAB has not reached consensus regarding the mode(s) of action by which tBA caused renal tubule
6 tumors in male rats. One opinion expressed was an agreement with the assessment in that it is difficult to
7 disentangle other potential mechanisms from $\alpha_2\mu$ -globulin and CPN. A second opinion expressed
8 centered on the fact that some of the manifestations of CPN could occur in the human kidney due to tBA
9 exposure. A third opinion expressed that pathological findings in the kidneys of tBA-dosed male rats
10 were consistent with $\alpha_2\mu$ -globulin and CPN acting as co-MOAs to cause the renal tubule adenomas.
11 EPA’s guidance on renal tumors in male rats (U.S. EPA, 1991a) states that unless the relative
12 contribution of $\alpha_2\mu$ -globulin nephropathy and other processes can be determined, dose-response analysis
13 should not be performed. The SAB, therefore, notes that the cancer risk assessment of tBA must be
14 based upon thyroid follicular tumors because renal tumors findings were less convincing.

15
16 ***The following recommendations are noted:***

17
18 **Tier 1:**

- 19 • None.

20
21 **Tier 2:**

- 22 • The SAB recommends that EPA expand the scope and breadth of its discussion of potential
23 modes and sites of action of tBA on the thyroid.

24
25 **Tier 3:**

- 26 • The SAB suggests that EPA consider organizing a scientific forum in the future to address
27 MOAs and relevance of chemically-induced rodent thyroid tumors to humans.

28
29 **3.4.3. Cancer toxicity values.**

30
31 **3.4.3.1. ETBE**

32 *Section 3 of EPA’s cancer guidelines (2005) states:*

33 *“When there is suggestive evidence, the Agency generally would not attempt a dose-response*
34 *assessment, as the data usually would not support one. However, when the evidence includes a well-*
35 *conducted study, quantitative analyses may be useful for some purposes, for example, providing a*
36 *sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting*
37 *research priorities. In each case, the rationale for the quantitative analysis is explained, considering*
38 *the uncertainty in the data and the suggestive nature of the weight of evidence.”*

39 *Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the*
40 *rationale for quantitative analysis, and whether the Saito et al. (2013) study is suitable for this purpose.*
41 *Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the*
42 *rationale for including a quantitative analysis given the “suggestive evidence” descriptor. Also*
43 *comment whether the Saito et al. (2013) study is a suitable basis for this quantitative analysis.*
44

45 The SAB has observed that there does not appear to be a rationale for performing a quantitative analysis
46 for ETBE liver cancer in Sections 2.3 or 2.4. These Sections simply refer to the Section 1.3.2, which

1 describes the basis for the selection of the “Suggestive Evidence” descriptor, and cite the EPA
2 Guidelines for Carcinogen Risk Assessment (2005) to support the fact that the EPA guidelines provide
3 the option of performing a quantitative analysis when this descriptor is selected. The EPA guidelines,
4 however, indicate that when such a determination is made, a rationale for the quantitative analysis
5 should explain how a quantitative analysis based on the available evidence “may be useful for some
6 purposes”, when “considering the uncertainty in the data and the suggestive nature of the weight of
7 evidence.” No such rationale is provided in the EPA’s draft ETBE assessment for the decision to
8 perform a quantitative analysis in the case of ETBE. A rationale based on potential worker and
9 consumer exposures was suggested. The Agency could clarify the rationale within the draft assessment.

10
11 The SAB concludes it is highly unlikely that performing a quantitative assessment of the data on ETBE
12 liver carcinogenicity would be useful for “providing a sense of the magnitude and uncertainty of
13 potential risks, ranking potential hazards, or setting research priorities” (*Section 3 of EPA’s cancer*
14 *guidelines; 2005*). In Section 1.3.2 (p. 1-112, lines 9-11), the EPA summarizes the limited evidence for
15 ETBE carcinogenicity, stating: “The results for ETBE raise a concern for cancer, but the effects are
16 limited primarily to one tissue (liver), at one dose (highest), and in one sex/species combination (male
17 rats), which were almost entirely benign.” There is also supporting evidence from initiation/promotion
18 studies and genotoxicity studies that ETBE is carcinogenic. Any quantitative analysis based on this
19 limited evidence would be highly uncertain and potentially misleading.

20
21 In addition, the SAB expresses concern about the ability of dose-response modeling (in this case,
22 benchmark dose modeling) to provide meaningful and useful information when there is a flat,
23 unresponsive dose response at all doses except the high dose. Because EPA’s policy (EPA 2005) is to
24 use only the multistage model for benchmark dose modeling of cancer dose-response, only a single
25 estimate of the benchmark dose lower confidence limit (BMDL) is produced. However, the rationale for
26 this policy is uncertain and had other models been investigated, it would have been seen that many
27 different models could adequately fit these data, and would likely yield widely divergent BMDL values.
28 This is because these data provide minimal meaningful information for dose-response analysis. Given
29 this, it is difficult to see how any, or all, possible models could provide realistic estimates of the true
30 cancer risk, or even relative risk compared to other carcinogens whose cancer potency was derived from
31 more robust data. Nevertheless, several members favored conducting a quantitative analysis to provide
32 some sense of the magnitude of potential risks.

33
34 The SAB agrees that the Saito et al. (2013) study is well-conducted and well-reported, but the data for
35 neoplastic liver lesions from inhalation exposure, by themselves, are not suitable for a quantitative
36 analysis because tumors were only observed at the highest concentration. The SAB noted that the
37 highest concentration is also where centrilobular hypertrophy, nuclear receptor activation, and induction
38 of metabolism may have contributed to the outcome. With a statistically significant increase in tumors
39 at the high dose only, the Saito et al. (2013) data are not sufficiently robust to provide a meaningful
40 quantitative estimate of human cancer risk for ETBE.

1 ***The following recommendations are noted:***
2

3 **Tier 1:**

- 4 • EPA should refrain from conducting a quantitative analysis for ETBE carcinogenicity or explain
5 the limitations of the analysis and clearly state the intended purpose is to simply provide some
6 sense of the magnitude of potential risks.
7

8 **Tier 2:**

- 9 • None.
10

11 **Tier 3:**

- 12 • EPA should reconsider its policy (EPA 2005) of limiting benchmark dose modeling of cancer
13 dose response to the multistage model as this model is not a biologically-based model and does
14 not provide a unique description of cancer dose-response.

15 **3.4.3.2. tBA**

16 *Section 3 of EPA's cancer guidelines (2005) states:*

17 *“When there is suggestive evidence, the Agency generally would not attempt a dose-response*
18 *assessment, as the data generally would not support one, however, when the evidence includes a*
19 *well-conducted study, quantitative analyses may be useful for some purposes, for example, providing*
20 *a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting*
21 *research priorities. In each case, the rationale for the quantitative analysis is explained, considering*
22 *the uncertainty in the data and the suggestive nature of the weight of evidence.”*

23 *~~Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for~~*
24 *~~quantitative analysis, and whether the NTP (1995) study is suitable for this purpose. Please comment~~*
25 *on whether Sections 2.3 of the draft assessment adequately explains the rationale for including a*
26 *quantitative analysis given the “suggestive evidence” descriptor. Also comment whether the NTP*
27 *(1995) study is a suitable basis for this quantitative analysis.*
28

29 The SAB has observed that there does not appear to be a rationale for performing a quantitative analysis
30 for tBA thyroid cancer in Sections 2.3. This Section simply refers to the Section 1.3.2, which describes
31 the basis for the selection of the “Suggestive Evidence” descriptor, and cites the EPA Guidelines for
32 Carcinogen Risk Assessment (2005) to support the fact that the EPA guidelines provide the option of
33 performing a quantitative analysis when this descriptor is selected. The EPA guidelines, however,
34 indicate that when such a determination is made, a rationale for the quantitative analysis should explain
35 how a quantitative analysis based on the available evidence “may be useful for some purposes”, when
36 “considering the uncertainty in the data and the suggestive nature of the weight of evidence.” No such
37 rationale is provided in the draft tBA assessment for the decision to perform a quantitative analysis in
38 the case of tBA. One rationale to consider potential worker and consumer exposures was suggested.
39

40 The SAB deems it highly unlikely that performing a quantitative assessment of the data on tBA thyroid
41 carcinogenicity would be useful for “providing a sense of the magnitude and uncertainty of potential
42 risks, ranking potential hazards, or setting research priorities.” In Section 1.3.2 (p. 1-112, lines 9-11),
43 the EPA summarizes the limited evidence for tBA carcinogenicity, stating: “In B6C3F1 mice,
44 administration of tert-butanol in drinking water increased the incidence of thyroid follicular cell
45 adenomas in females and adenomas or carcinomas (only one carcinoma observed) in males (NTP,

1 1995), as discussed in Section 1.2.2”. In addition, the SAB concludes that there is serious concern about
2 the ability of dose-response modeling (in this case, benchmark dose modeling) to provide meaningful
3 and useful information when there is a flat, unresponsive dose response at all doses except the high dose.
4 Because EPA’s policy (EPA 2005) is to use only the multistage model for benchmark dose modeling of
5 cancer dose-response, only a single estimate of the BMDL is produced. However, had other models
6 been investigated, it would have been seen that many different models could adequately fit these data,
7 and would likely yield widely divergent BMDL values. This is because these data provide minimal
8 meaningful information for dose-response analysis. Given this, it is difficult to see how any, or all
9 possible models could provide realistic estimates of the true cancer risk, or even relative risk compared
10 to other carcinogens whose cancer potency was derived from more robust data. Thus, any quantitative
11 analysis based on this limited evidence would entail significant uncertainty and have the potential to be
12 misleading. Nevertheless, several members favor conducting a quantitative analysis to provide some
13 sense of the magnitude of potential risks.

14
15 The SAB agrees that the NTP 1995 study is well-conducted and well-reported, but the data for
16 neoplastic thyroid lesions from drinking water exposure, by themselves, are not a suitable basis for a
17 quantitative analysis because a tumor response was only observed at the highest concentration. With a
18 statistically significant increase in tumors at the high dose only, and evidence from other studies
19 supporting a potentially nonlinear mode of action, the NTP 1995 data are not sufficiently robust to
20 provide a meaningful quantitative estimate of human cancer risk for tBA.

21
22 ***The following recommendations are noted:***

23
24 **Tier 1:**

- 25 • EPA could refrain from conducting a quantitative analysis for tBA carcinogenicity or explain the
26 limitations of the analysis and clearly state the intended purpose is to simply provide some sense
27 of the magnitude of potential risks.

28
29 **Tier 2:**

- 30 • None.

31
32 **Tier 3:**

- 33 • EPA should reconsider its policy of limiting benchmark dose modeling of cancer dose response
34 to the multistage model as this model is not a biologically based model and does not provide a
35 unique description of cancer dose-response.

36
37 **3.4.4. Oral slope factor for cancer.**

38
39 **3.4.4.1. ETBE**

40 *Section 2.3 presents an oral slope factor of 1×10^{-3} per mg/kg–day, based on liver tumors in male rats*
41 *by inhalation (Saito et al. 2013), converted for oral exposure using a toxicokinetic model (Borghoff et*
42 *al. 2016). Please comment on whether this value is scientifically supported and its derivation clearly*
43 *described. If an alternative approach would be more appropriate, please outline how it might be*
44 *developed.*

1 The Saito et al. (2013) study used three concentrations of ETBE and observed significant increases in
2 liver tumors in male but not female rats at the highest inhaled concentration only. The oral cancer slope
3 factor was obtained by converting the inhalation point of departure to an oral dose using rate of ETBE
4 metabolism in the liver, which was derived from a PBPK model.

5
6 The SAB is concerned that the Saito et al. (2013) ETBE inhalation study is not suitable for developing
7 an oral cancer slope factor, due to the lack of biological relevance. The SAB notes the following flaws
8 associated with developing an oral cancer slope factor using the Saito et al. (2013) study:

- 9
10 • Well-conducted contemporary oral cancer studies up to the limits of solubility did not
11 demonstrate cancer. Therefore, route extrapolation could explain these negative findings by oral
12 exposure as well as the findings by inhalation exposure. The route differences could be due to
13 pharmacokinetic (addressed below) or toxicodynamic processes, but either would require
14 quantification to demonstrate cross-route consistency of the tumor observations.
- 15 • The ETBE inhalation concentration which induced liver tumors was excessively high.
- 16 • Having only one significantly elevated dose and two doses with response statistically
17 indistinguishable from the control response provides little useful information in the range of
18 interest for BMD/BMDL calculation (i.e., between the single significantly elevated dose and the
19 control response).
- 20 • As EPA's analyses indicated, combining oral and inhalation studies did not result in a consistent
21 dose response relationship using the dose metric of average daily rate of ETBE metabolism at
22 periodicity. This argues against route extrapolation using this dose metric; no other dose metric
23 was identified that provided consistent results between oral and inhalation exposures.

24
25 The SAB is somewhat concerned regarding EPA's policy to use the Multi Stage Cancer model as the
26 preferred cancer dose-response model. Many different models could fit these data with equally good
27 statistics of fit, but with widely different dose-response functions in the dose range of interest.
28 Therefore, EPA should consider a wider choice of models when performing cancer dose-response
29 analyses.

30
31 The SAB agrees that oral slope factor chosen is scientifically supported. The draft ETBE assessment
32 noted that 1) the high dose used in Saito *et al.* (1993) did not exceed the Maximum Tolerated Dose
33 (MTD), and 2) EPA policy permits the dose-response modeling of tumor data where only the high dose
34 induces a significant tumor increase. The SAB has no alternative approach suggestion for developing an
35 oral cancer slope factor, and noted that the oral slope factor derivation was well described. The SAB
36 also agrees that the modeling was performed correctly.

37
38 ***The following recommendations are noted:***

39
40 **Tier 1:**

- 41 • Since the Saito et al. (2013) ETBE inhalation study is not suitable for developing an oral cancer
42 slope factor, EPA should not derive an oral slope factor by route extrapolation absent
43 pharmacokinetic/ pharmacodynamics modeling that demonstrates consistency between the oral
44 and inhalation study results.

1 **Tier 2:**

- 2 • None.

3
4 **Tier 3:**

- 5 • None.

6
7 **3.4.4.2. tBA**

8 *Section 2.3 presents an oral slope factor of 5×10^{-4} per mg/kg-day, based on thyroid tumors in male or*
9 *female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically*
10 *supported and its derivation clearly described. If an alternative approach would be more appropriate,*
11 *please outline how it might be developed.*

12
13 The NTP (1995) tBA drinking water study used three doses of tBA and observed significant increases in
14 thyroid follicular cell tumors in female mice at the high dose only. The dose-metric for the dose-
15 response analysis used to develop the oral cancer slope factor was exposed dose.

16
17 The SAB agrees that the NTP (1995) tBA drinking water study was not suitable for developing an oral
18 cancer slope factor. The SAB was concerned about the lack of biological relevance due to the
19 magnitude of the high dose and the possibility of nonlinear metabolism kinetics at that dose.

20
21 The SAB is not comfortable with EPA's policy to permit the dose-response modeling of tumor data
22 where only the high study dose induces a significant tumor increase. In the case of the tBA-induced
23 female mouse thyroid follicular cell tumors, the SAB observed that having only one significantly
24 elevated dose and two doses with response statistically indistinguishable from the control response
25 provides little useful information in the range of interest for BMD/BMDL calculation (i.e., between the
26 single significantly elevated dose and the control response).

27
28 The SAB also suggests that EPA may want to rethink their policy to use the Multi Stage Cancer model
29 as the preferred cancer dose-response model. The SAB noted that many different models could fit these
30 data with equally good statistics of fit, but with widely different dose-response functions in the dose
31 range of interest. Therefore, EPA should consider a wider choice of models when performing cancer
32 dose-response analyses.

33
34 Some members conclude the EPA's choice for oral slope factor for tBA was scientifically supported.
35 Reasons supporting this position include:

- 36 • The lack of supporting data for a mouse anti-thyroid MOA, indicating that there is no reason to
37 conclude that the female mouse thyroid follicular cell tumor data are not relevant to human
38 cancer risk assessment.
- 39 • The tBA dose producing female mouse thyroid follicular cell tumors in the 1995 NTP study did
40 not cause excessive treatment-related mortality or otherwise exceed the Maximum Tolerated
41 Dose (MTD) in females although increased mortality is present in males at this dose.
- 42 • EPA policy permits the dose-response modeling of tumor data where only the high study dose
43 induces a significant tumor increase.

44
45 The SAB has no recommendations to alternative approaches for developing an oral cancer slope factor
46 and there are no comments to indicate that the oral slope factor derivation is poorly described.

1 ***The following recommendations are noted:***
2

3 **Tier 1:**

- 4 • None.
5

6 **Tier 2:**

- 7 • None.
8

9 **Tier 3:**

- 10 • None.
11

12 **3.4.5. Inhalation unit risk for cancer.**

13
14 **3.4.5.1. ETBE**

15 *Section 2.4 presents an inhalation unit risk of 8×10^{-5} per mg/m^3 , based on liver tumors in male rats by*
16 *inhalation (Saito et al. 2013). Please comment on whether this value is scientifically supported and its*
17 *derivation clearly described. If an alternative approach would be more appropriate, please outline how*
18 *it might be developed.*
19

20 The Saito et al. (2013) study used three concentrations of ETBE and observed significant increases in
21 liver tumors in male but not female rats at the high dose only. The dose-metric for the dose-response
22 analysis used to develop the oral cancer slope factor was exposure concentration, with continuous
23 exposure and Human Equivalent Concentration adjustments applied.
24

25 The SAB has not reached consensus on this question. Some members find that the Saito et al. (2013)
26 ETBE inhalation study is not suitable for developing a cancer inhalation unit risk (IUR), given a
27 potential lack of biological relevance. These members concluded that the ETBE concentration which
28 induced liver tumors to be excessively high and only one concentration significantly increased tumor
29 incidence in male rats, leading to questions among these members regarding whether modeling a single
30 positive concentration would produce a meaningful IUR.
31

32 Other members expressed that the Saito et al. (2013) study is appropriate for dose-response analysis, and
33 indicated that there is scientific support for the EPA's ETBE IUR derivation. Supporting reasons for
34 this position included the quality of the Saito et al. (2013) study, which is well designed, conducted and
35 reported, and also the liver metabolism of ETBE to acetaldehyde, a genotoxic carcinogen. These
36 members raised caveats regarding the biological relevance but did not alter their position that EPA
37 should develop an IUR for ETBE. These members also noted that EPA provides guidance for deriving
38 IURs in such cases and that this guidance is followed in the ETBE IUR derivation.
39

40 The SAB has no recommendations for alternative approaches to developing an IUR, and has no
41 comments indicating that the IUR derivation was done incorrectly or was poorly described. Some
42 members however did provide comments to indicate the modeling was performed correctly.

1 ***The following recommendations are noted:***
2

3 **Tier 1:**

- 4 • None.
5

6 **Tier 2:**

- 7 • None.
8

9 **Tier 3:**

- 10 • None.
11

12 **3.4.5.2. tBA**

13 *Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use*
14 *of the oral thyroid tumor data, and the inability to determine the relative contribution of $\alpha_2\mu$ -globulin*
15 *nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an*
16 *alternative approach would yield an inhalation unit risk estimate, please outline how it might be*
17 *developed.*
18

19 The NTP (1995) tBA drinking water study used three doses of tBA and observed significant increases in
20 thyroid follicular cell tumors in female mice at the high dose only. Based on that, the SAB concludes
21 that the NTP (1995) tBA drinking water study is not suitable for developing an inhalation cancer unit
22 risk. The SAB's concerns include the lack of biological relevance due to the magnitude of the high
23 dose, the lack of an available mouse tBA PBPK model in the literature, and the possibility of nonlinear
24 metabolism kinetics at that dose. The SAB also expresses concern that modeling a single positive
25 concentration may not produce a scientifically supportable oral slope factor.
26

27 One suggestion for an alternative approach in developing an inhalation cancer unit risk would be to
28 perform a route-to-route extrapolation from the oral cancer slope factor using default human body
29 weight and inspiration rate values.
30

31 ***The following recommendations are noted:***
32

33 **Tier 1:**

- 34 • None.
35

36 **Tier 2:**

- 37 • None.
38

39 **Tier 3:**

- 40 • None.
41

42 **3.5. Question on Susceptible Populations and Lifestages**
43

44 **3.5.1. ETBE**

45 *Section 1.3.3 identifies individuals with diminished ALDH2 activity as a susceptible population due to*
46 *an increased internal dose of acetaldehyde, a primary metabolite of ETBE. Please comment on whether*

1 *this conclusion is scientifically supported and clearly described. If there are publicly available studies to*
2 *identify other susceptible populations or lifestages, please identify them and outline their impact on the*
3 *conclusions.*

4
5 The SAB agrees with the position that EPA has taken regarding the “plausible evidence” for a
6 vulnerable subgroup. Specifically, EPA specifies that individuals with an inactive form of ALDH2*2
7 may be a susceptible population. In addition, the EPA states that there is inconclusive evidence for
8 CYP2A6 variants. The SAB finds this conclusion appropriate with revision as recommended below.

9
10 ***The following recommendations are noted:***

11
12 **Tier 1:**

- 13 • The draft ETBE assessment states “all routes of exposure” (which is completely plausible); yet
14 the only route of exposure that is mentioned or that appears to be cited (or evaluated in the
15 literature) is oral exposure as it relates to potentially susceptible populations. Since inhalation is
16 likely an important route of exposure, as might be other routes, it is important to note that the
17 literature is limited in this area and that oral exposure is likely to yield different responses than
18 an inhalation (or other types of) exposure route(s). The lack of information on this point raises
19 uncertainties about the degree of susceptibility from other routes of exposure. The SAB requests
20 that the Agency clearly describe these uncertainties in the ETBE assessment and provide relevant
21 positions with respect to differences in expected outcomes (i.e., gastrointestinal vs. nasal
22 endpoints).
- 23
24 • EPA suggests that individuals with inactive ALDH2*2 variants may be a potentially important
25 subpopulation that may have susceptibility to ETBE exposure. This statement is based on
26 literature documenting that metabolism of ETBE yields acetaldehyde, a documented genotoxic
27 compound, that in this subgroup could lead to prolonged exposure due to slow metabolism of
28 this compound. The EPA has importantly noted that an anticipated 50% of individuals of
29 Chinese, Japanese, and Korean descent are carriers of this variant. However, the EPA does not
30 note the possibility of other potentially vulnerable population subgroups, such as individuals that
31 have non-coding region variants in ALDH2. Individuals with these non-coding region variants
32 are noted in the literature. The SAB includes the following review, which includes articles by
33 Dickson et al. (2006) and others noting not only ALDH2 non-coding region variants, but also
34 other variants in alcohol metabolism that may be relevant to ETBE exposure in humans and
35 could be important for identification of susceptible populations. The SAB recommends the
36 incorporation of this point into the ETBE assessment to improve the scientific concepts of the
37 assessment.
- 38
39 • The ETBE assessment does not mention vulnerable life stages. This suggests that there may not
40 be any vulnerable life stage with respect to ETBE exposure. Yet, data exist showing that
41 pregnancy may be a sensitive time period for alcohol metabolism due to the presence of
42 estrogen, which inhibits ADH and ALDH activities. In addition to pregnant women, fetuses may
43 also be a vulnerable subpopulation, due to their limited ability to metabolize alcohol, despite the
44 presence of ADH. Specifically, the amount of ALDH2 expressed in the fetus is about half of that
45 expressed in adults. Information regarding life stages should be included in the assessment.

1 **Tier 2:**

- 2 • It is unclear why the report is not fully utilizing available human data. The SAB encourages the
3 agency to provide more supportive evidence from human studies on acetaldehyde metabolism
4 throughout the report.
5
6 • The SAB encourages the agency to consider mentioning differences relevant to sex and possible
7 vulnerabilities that may be related to sex differences in exposures and outcomes.
8

9 **Tier 3:**

- 10 • The ETBE assessment should note that evidence pertaining to these inactive ALDH2*2 variants
11 is inconclusive and will be incorporated into future considerations.
12

13 **3.5.2. tBA**

14 *As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible*
15 *populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this*
16 *conclusion is scientifically supported and clearly described. If there are publicly available studies to*
17 *identify other susceptible populations or lifestages, please identify them and outline their impact on the*
18 *conclusions.*
19

20 The draft tBA assessment states that there is no identified susceptible population. The SAB finds that
21 this statement may be partly attributed to the implication, from in vitro studies, suggesting that CYP450
22 system may be involved in the metabolism of tBA, it is unknown which specific CYPs are involved in
23 the biotransformation. To this end, it is unclear as a metabolite of ETBE why other populations
24 mentioned in the previous draft ETBE assessment are not considered. Most of the studies mentioned
25 focused on non-human studies. However, there are human data to support altered metabolism with
26 respect to xenobiotic metabolism during pregnancy for the maternal-fetal unit.
27

28 It is unclear why vulnerable life stages are highlighted here and not in the draft ETBE assessment. That
29 said, the SAB agrees with the EPA that regarding tBA the evidence is minimal for identifying
30 vulnerable populations and life stages.
31

32 Finally, the SAB disagrees with certain findings presented by the Agency in Table 1-12 of the EPA's
33 draft tBA assessment. The actual body weight for the treated group was not double that of the control
34 group, as implied by Table 1-12. Rather, treated dams gained twice as much weight as the dams in the
35 control group during a specific interval. This difference in body weight gain is reasonable given other
36 characteristics noted within the EPA's report and the EPA's final draft tBA assessment does not need to
37 include additional reasoning on this topic.
38

39 ***The following recommendations are noted:***

40
41 **Tier 1:**

- 42 • The EPA should correct the actual body weight for the treated group in Table 1-12 of the EPA's
43 draft tBA assessment.
44

1 **Tier 2:**

- 2 • The SAB recommends adding some of this supporting evidence from ETBE to the argument
3 proposed, given the limited data for tBA and the fact that it is a metabolite of ETBE.
4

5 **Tier 3:**

- 6 • None.
7

8 **3.6. Question on the Executive Summary**

9
10 **3.6.1. ETBE**

11 *The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for*
12 *a broad range of audiences. Please comment on whether the executive summary clearly and*
13 *appropriately presents the major conclusions of the draft assessment.*
14

15 Generally, the Executive Summary is clear and presents the major conclusions of the draft assessment.
16 As changes are made to the body of the draft, the Executive Summary will need to be changed
17 accordingly. Comments here are therefore on overarching aspects of the draft Executive Summary and
18 the depth of details as they are presented.
19

20 The draft Executive Summary offers statements about the questions considered and summarizes the
21 findings that are in the end chosen. Little is said about the findings not selected even though they may
22 have some merit and provide important context for interpretation and application of the selected
23 findings. The section on “Key Issues” is helpful, but it should do more to highlight the consequences of
24 alternative choices for the final assessment. This is important because the interpretation and relevance
25 of key toxicity endpoints driving the analysis have been sharply contested (based on the history of public
26 comment on the draft assessment).
27

28 ***The following recommendations are noted:***
29

30 **Tier 1:**

- 31 • The “Key Issues” section of the Executive Summary should highlight the interpretation and
32 relevance of key toxicity endpoints. EPA should specify knowledge gaps in the decision-
33 making, and clearly describe uncertainties, assumptions, and the strength and weakness of the
34 decisions.
35
- 36 • The key issue of CPN should be explained with the universally known facts of renal senescence
37 in mammalian species, including both human and rats. The controversy over the species- and
38 breed-specific pathogenesis and the limitation of existing toxicological and pathological
39 information need to be addressed.
40
- 41 • The Executive Summary should acknowledge that the quantitative assessment of ETBE has a
42 high degree of uncertainty stemming from the relative lack of significant dose response at low
43 and intermediate doses. The weakness in the database, such as wide gaps between tested doses in
44 currently available studies, should also be addressed.
45

- 1 • For the broad audience, the Executive Summary needs to clearly describe why it is important to
2 examine the “key issues”. The Executive Summary should also describe the alternative findings
3 and explain how the resolutions/decisions on these key issues influence the outcome of the
4 toxicity assessment.
5
- 6 • All findings in the assessment should be summarized in the Executive Summary, including both
7 positive and negative findings in reproductive and developmental studies. As stated in the body
8 of the report, ETBE treatment results in increases in preimplantation loss and pup deaths during
9 PND 0-4. These effects should be stated in the Executive Summary. For the effects that appear to
10 be associated with maternal toxicity, they may be clarified as likely to be associated with
11 maternal toxicity, but not dismissed. Furthermore, the body of the report on reproductive and
12 developmental effects was incomplete; the body of the report missed several significant effects
13 observed in the reproductive/developmental studies used in this draft. If and when the
14 reproductive and developmental sections in the draft document are modified, the Executive
15 Summary should be modified accordingly.
16
- 17 • The connection between the assessment of ETBE and tBA needs to be explicitly discussed (in
18 the full draft ETBE assessment as well as in the Executive Summary). Since metabolic activation
19 of ETBE is invoked in its assessment, and since tBA is a main metabolite, the interpretation of
20 toxicity, dosimetry, and dose-response for the two compounds should inform one another, and
21 the consistency of interpretations needs to be more thoroughly addressed.
22
- 23 • The Executive Summary should provide more specifics about the chosen dose metrics,
24 explaining (in summary form) the basis for calculation, the role of metabolism in activation and
25 clearance, major assumptions or use of alternatives to defaults, and the basis for cross-species
26 dose equivalency. The dosimetry considerations applied to each endpoint should be clear (e.g.,
27 dose-metric definition, role of tissue specificity, etc.). The simple reference to “PBPK” being
28 used, or to the Category 3 gas approach, are not sufficiently specific.
29
- 30 • The Executive Summary should present all endpoints applied and values derived in dose-
31 response analyses before the selection of final toxicity values.
32

33 **Tier 2:**

- 34 • None.

35
36 **Tier 3:**

- 37 • Current understanding in neurobehavioral effects is insufficient. The needs for additional
38 investigation in this area and for other data gaps should be noted.
39

40 **3.6.2. tBA**

41 *The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for*
42 *a broad range of audiences. Please comment on whether the executive summary clearly and*
43 *appropriately presents the major conclusions of the draft assessment.*
44

45 Generally, the Executive Summary is clear and presents the major conclusions of the draft assessment.
46 As changes are made to the body of the draft, the Executive Summary will need to be changed

1 accordingly. Comments here are therefore on overarching aspects of the draft Executive Summary and
2 the depth of details as they are presented.

3
4 ***The following recommendations are noted:***

5
6 **Tier 1:**

- 7 • The Executive Summary should provide more specifics about the chosen dose metrics,
8 explaining (in summary form) the basis for calculation, the role of metabolism in clearance,
9 major assumptions or use of alternatives to defaults, and the basis for cross-species dose
10 equivalency. The dose metric considerations applied to each endpoint should be clearly stated
11 (e.g., dose-metric definition, role of tissue specificity, etc.).
- 12
13 • The key issue of CPN should be explained with the universally known facts of renal senescence
14 in mammalian species, including human and rats. The controversy over the species- and breed-
15 specific pathogenesis and the limitations of existing toxicological and pathological information
16 need to be addressed.
- 17
18 • The Executive Summary should acknowledge that the quantitative assessment of tBA has a high
19 degree of uncertainty stemming from the relative lack of significant dose response at low and
20 intermediate doses. The weakness in the database, such as wide gaps between tested doses in
21 currently available studies, should also be addressed.
- 22
23 • The Executive Summary needs to recognize that the conclusions drawn are critically dependent
24 on best scientific judgments about the relevance of observed toxicity endpoints to potential
25 human risks. The issues about how available animal results are interpreted for human health risk
26 assessment need to be summarized.
- 27
28 • All potential effects identified in Hazard Identification should be stated in the Executive
29 Summary, including reproductive, developmental and neurobehavioral effects. Maternal and
30 fetal effects should be recognized and reported, regardless whether the maternal and fetal effects
31 can be delineated. To place potential maternal-fetal interaction in context, fetal toxicity should be
32 noted in conjunction with maternal toxicity and the test dose.
- 33
34 • The Executive Summary should provide more specifics about the chosen dose metrics,
35 explaining (in summary form) the basis for calculation, the role of metabolism in activation and
36 clearance, major assumptions or use of alternatives to defaults, and the basis for cross-species
37 dose equivalency. The dose metric considerations applied to each endpoint should be clear (e.g.,
38 dose-metric definition, role of tissue specificity, etc.).
- 39
40 • The use of route-to-route extrapolation for inhalation noncancer effects is a notable deviation
41 from usual practice. Given that there are some inhalation data (though not for full lifetime), the
42 discussion of use of route-to-route extrapolation in the executive summary should include
43 additional discussion on why the Agency used the extrapolated oral results over inhalation
44 results (the existence of which, but not the shortcomings, is briefly noted in the report). Simply
45 saying that the extrapolation approach is “more specific and sensitive” does not sufficiently
46 explain the rationale.

- 1 • The Executive Summary should present all endpoints applied and values derived in dose-
2 response analyses before the selection of final toxicity values.
3
- 4 • P. xiii, Line 14: Reference HSDB (2007) is cited for t-butanol in human milk. In HSDB (2007),
5 two articles are cited for this claim. (1) Pellizzari ED et al; Bull Environ Contam Toxicol 28:
6 322-8 (1982) (2) Erickson MD et al; Acquisition and Chemical Analysis of Mothers Milk for
7 Selected Toxic Substances. U.S. EPA-560/13-80-029 (1980). These two articles do not provide
8 evidence for the presence of tBA in milk, although the presence of 1-butanol was demonstrated.
9 This statement needs clarification.

10
11 **Tier 2:**

- 12 • None.

13
14 **Tier 3:**

- 15 • Current understanding in neurobehavioral effects is insufficient. The needs for additional
16 investigation in this area and for other data gaps should be noted.

REFERENCES

The following materials are cited within this report.

Amberg, A; Rosner, E; Dekant, W. (2000). Biotransformation and kinetics of excretion of ethyl tert-butyl ether in rats and humans. *Toxicol Sci.* 53: 194-201.

IARC (International Agency for Research on Cancer). (1999). Methyl tert-butyl ether (group 3) (pp. 339-383). Lyon, France.

Nihlén, A; Johanson, G. 1999. Physiologically based toxicokinetic modeling of inhaled ethyl tertiary-butyl ether in humans. *Toxicol Sci.* 51: 184-194.

NRC (National Research Council). (2011). Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde (pp. 194). Washington, DC: National Academies Press.

NTP (National Toxicology Program). (1995). Toxicology and carcinogenesis studies of t-butyl alcohol (CAS no 75-65-0) in F344/N rats and B6C3F1 mice (Drinking water studies) (pp. 1-305). (NTPTR436). Research Triangle Park, NC.

Saito, A; Sasaki, T; Kasai, T; Katagiri, T; Nishizawa, T; Noguchi, T; Aiso, S; Nagano, K; Fukushima, S. 2013. Hepatotumorigenicity of ethyl tertiary-butyl ether with 2-year inhalation exposure in F344 rats. *Arch Toxicol.* 87: 905-914.

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-83). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S. EPA. 2017a. Toxicological Review of Ethyl Tertiary Butyl Ether) (CASRN 637-92-3).

U.S. EPA. 2017b. Toxicological Review of Ethyl Tertiary Butyl Ether) (CASRN 637-92-3). Supplemental Information.

U.S. EPA. 2017c. Toxicological Review of tert-Butyl Alcohol (tert-Butanol) (CASRN 75-65-0).

U.S. EPA. 2017d. Toxicological Review of tert-Butyl Alcohol (tert-Butanol) (CASRN 75-65-0). Supplemental Information.

ADDITIONAL PEER-REVIEWED STUDIES

The SAB recommends the following peer-reviewed studies for considered in the assessment of noncancer and cancer health effects of ETBE and tBA:

Boussadia, B., et al (2016) Lack of CAR impacts neuronal function and cerebrovascular integrity in vivo. *Experimental Neurology* 283, 39-48.

Boussadia, B., et al (2017) Pregnane X receptor deletion modifies recognition memory and electroencephalographic activity. *Neuroscience*, epub July 23, PMID:27240521.

Dickson PA, James MR, Heath AC, et al. Effects of variation at the ALDH2 locus on alcohol metabolism, sensitivity, consumption, and dependence in Europeans. *Alcoholism: Clinical and Experimental Research*. 2006;30(7):1093–1100.

Edenberg HJ. The Genetics of Alcohol Metabolism: Role of Alcohol Dehydrogenase and Aldehyde Dehydrogenase Variants. *Alcohol Res Health*. 2007; 30(1):5-13.

Edwards, EK. (1982) Allergic reaction to tertiary butyl alcohol in a sunscreen. *Cutis* 29(5): 476.

Hoshino, H; Chihara, G; Fukuoka, F. (1970) Detection of potential weak carcinogens and procarcinogens .2. Carcinogenicity of tertiary butyl hydroperoxide. *Gann* 61(2): 121.

Yoshida A, Shibuya A, Davé V, Nakayama M, Hayashi A. 1990. Developmental changes of aldehyde dehydrogenase isozymes in human livers: Mitochondrial ALDH2 isozyme is expressed in fetal livers. *Experientia* 46(7): 747-750.

Zhao Y, Wang, C. (2015) Glu504Lys Single Nucleotide Polymorphism of Aldehyde Dehydrogenase 2 Gene and the Risk of Human Diseases. *Biomed Res Int*. 2015: 174050.

APPENDIX A: EPA'S CHARGE QUESTIONS

Revised 7/26/17 by the CAAC-ETBE/tBA committee per discussion during the July 11, 2017, teleconference. Changes are presented as underlined text.

Charge to the Science Advisory Board for the IRIS Toxicological Review of Ethyl *tert*-Butyl Ether (ETBE) June 2017

Introduction

EPA thanks the expert scientists on the augmented SAB Chemical Assessment Advisory Committee for reviewing this draft assessment.

This draft assessment reviews publicly available studies on ETBE to identify adverse health outcomes and to characterize exposure–response relationships. Peer review is essential to the quality and integrity of IRIS assessments, which provide scientific information that supports EPA's actions to protect public health. The draft assessment was reviewed by scientists across EPA and other federal agencies. EPA also solicited public comment and convened a public science meeting to discuss major science issues. Experts identified by the National Academy of Sciences participated in the public discussions. Responses to major public comments appear as supplemental material to the draft assessment.

EPA is seeking SAB advice on the clarity and scientific underpinnings of the overall assessment. The peer review should consider whether the conclusions presented in the draft assessment are clearly presented and scientifically supported. Below, a set of charge questions for each major analysis are presented. The SAB is expected to consider questions and issues raised during public comment as part of its deliberations. The advice will be most useful when prioritized to indicate its relative importance during revision:

Tier 1: Recommended Revisions – Key recommendations that are necessary in order to improve the critical scientific concepts, issues and/or narrative within the assessment.

Tier 2: Suggestions – Recommendations that are encouraged for EPA to adopt in order to strengthen the scientific concepts, issues and/or narrative within the assessment, but other factors (e.g., Agency need) should be considered by EPA before undertaking these revisions.

Tier 3: Future Considerations – Useful and informative scientific exploration that may inform future evaluations of key science issues and/or the development of future assessments. These recommendations are likely outside the immediate scope and/or needs of the current assessment under review.

1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods

Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

Hazard Identification and Dose-Response Analysis

Chapter 1 (Hazard Identification) and the supplemental materials summarize the chemical properties, toxicokinetics, and health effects associated exposure to ETBE. Chapter 2 (Dose Response Analysis)

1 uses this information to derive an oral reference dose and inhalation reference concentration for
2 noncancer outcomes, in addition to an oral slope factor and inhalation unit risk for cancer.

3 4 **2. Chemical Properties and Toxicokinetics**

5 **2a. Chemical properties.** Is the information on chemical properties accurate?
6

7 **2b. Toxicokinetic modeling.** Section B.1.5 of Appendix B in the Supplemental Information
8 describes the application and modification of a physiologically-based toxicokinetic model of ETBE
9 in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including
10 assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered
11 for modeling?
12

13 **2c. Choice of dose metric.** Is the rate of ETBE metabolism an appropriate choice for the dose
14 metric?
15

16 Hazard Identification and Dose–Response Assessment.

17 Comment on EPA’s assessment of the toxicological studies and dose-response assessment, including
18 whether there are additional peer-reviewed studies that should be considered.
19

20 **3. Noncancer**

21
22 **3a. Noncancer kidney toxicity** (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects
23 as a potential human hazard of ETBE. EPA evaluated the evidence, including the role of $\alpha_2\mu$ -
24 globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991).
25 Please comment on whether this conclusion is scientifically supported and clearly described.
26

27 **3b. Noncancer toxicity at other sites** (Sections 1.2.2, 1.2.3, 1.2.4, 1.2.6, 1.3.1). The draft
28 assessment presents conclusions for noncancer toxicity at other sites that were not used as the basis
29 for deriving noncancer oral reference dose or inhalation reference concentration purposes. Please
30 comment on whether these conclusions are scientifically supported and clearly described. If there are
31 publicly available studies to associate other health outcomes with ETBE exposure, please identify
32 them and outline the rationale for including them in the assessment.
33

34 Liver effects: Suggestive evidence

35 Developmental toxicity: Inadequate evidence

36 Male and female reproductive toxicity: Inadequate evidence
37

38 **3c. Oral reference dose for noncancer outcomes.** Section 2.1 presents an oral reference dose of
39 5×10^{-1} mg/kg–day, based on urothelial hyperplasia in male rats (Suzuki et al., 2012). Please
40 comment on whether this value is scientifically supported and its derivation clearly described. If an
41 alternative data set or approach would be more appropriate, please outline how such data might be
42 used or how the approach might be developed.
43

44 **3d. Inhalation reference concentration for noncancer outcomes.** Section 2.2 presents an
45 inhalation reference concentration of 9×10^0 mg/m³, based on urothelial hyperplasia in male rats
46 (Saito et al., 2013). Please comment on whether this value is scientifically supported and its

1 derivation clearly described. If an alternative data set or approach would be more appropriate, please
2 outline how such data might be used or the approach might be developed.
3

4 **4. Cancer**

5
6 **4a. Cancer modes-of-action in the liver.** As described in section 1.2.2, the draft assessment
7 evaluated the roles of the receptor pathways PPAR α , PXR, and CAR in ETBE tumorigenesis in male
8 rats. The analysis, conducted in accordance with EPA's cancer guidelines (U.S. EPA, 2005),
9 considered the liver tumors in male rats to be relevant to human hazard identification. Please
10 comment on whether this conclusion is scientifically supported.
11

12 **4b. Cancer characterization.** As described in sections 1.2.1, 1.2.2, 1.2.5 and 1.3.2, and in
13 accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that
14 there is *suggestive evidence of carcinogenic potential* for ETBE by all routes of exposure, based on
15 liver tumors in male F344 rats via inhalation and on promotion of liver, colon, thyroid, forestomach,
16 and urinary bladder tumors in male rats via oral exposure. ~~Does the classification give appropriate
17 weight to the results from initiation-promotion studies? Please comment on whether this cancer
18 descriptor is scientifically supported. If another cancer descriptor should be selected, please outline
19 how it might be supported.~~

20 Please comment on whether the decision to include 2-stage initiation-promotion studies in the
21 human cancer hazard characterization is sufficiently justified and if the amount of emphasis placed
22 on the initiation promotion data in the cancer hazard characterization is scientifically supported.
23 Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported
24 for all routes of exposure. If another cancer descriptor should be selected, please outline how it
25 might be supported.
26

27 **4c. Cancer toxicity values.** Section 3 of EPA's cancer guidelines (2005) states:
28

29 "When there is suggestive evidence, the Agency generally would not attempt a dose-response
30 assessment, as the data usually would not support one. However, when the evidence includes a well-
31 conducted study, quantitative analyses may be useful for some purposes, for example, providing a
32 sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting
33 research priorities. In each case, the rationale for the quantitative analysis is explained, considering
34 the uncertainty in the data and the suggestive nature of the weight of evidence."
35

36 ~~Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately explain the~~
37 ~~rationale for quantitative analysis, and whether the Saito et al. (2013) study is suitable for this~~
38 ~~purpose. Please comment on whether Sections 2.3 and 2.4 of the draft assessment adequately~~
39 ~~explain the rationale for including a quantitative analysis given the "suggestive evidence"~~
40 ~~descriptor. Also comment whether the Saito et al. (2013) study is a suitable basis for this~~
41 ~~quantitative analysis.~~
42

43 **4d. Oral slope factor for cancer.** Section 2.3 presents an oral slope factor of 1×10^{-3} per mg/kg-
44 day, based on liver tumors in male rats by inhalation (Saito et al., 2013), converted for oral exposure
45 using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is

1 scientifically supported and its derivation clearly described. If an alternative approach would be
2 more appropriate, please outline how it might be developed.
3

4 **4e. Inhalation unit risk for cancer.** Section 2.4 presents an inhalation unit risk of 8×10^{-5} per
5 mg/m^3 , based on liver tumors in male rats by inhalation (Saito et al., 2013). Please comment on
6 whether this value is scientifically supported and its derivation clearly described. If an alternative
7 approach would be more appropriate, please outline how it might be developed.
8

9 **5. Question on Susceptible Populations and Lifestages**

10 Section 1.3.3 identifies individuals with diminished ALDH2 activity as a susceptible population due to
11 an increased internal dose of acetaldehyde, a primary metabolite of ETBE. Please comment on whether
12 this conclusion is scientifically supported and clearly described. If there are publicly available studies to
13 identify other susceptible populations or lifestages, please identify them and outline their impact on the
14 conclusions.
15

16 **6. Question on the Executive Summary**

17 The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions
18 for a broad range of audiences. Please comment on whether the executive summary clearly and
19 appropriately presents the major conclusions of the draft assessment.
20

1 *Revised 7/26/17 by the CAAC-ETBE/tBA committee per discussion during the July 11, 2017,*
2 *teleconference. Changes are presented as underlined text.*
3

4 **Charge to the Science Advisory Board for the IRIS Toxicological Review of *tert*-Butanol** 5 **June 2017**

7 Introduction

8 EPA thanks the expert scientists on the augmented SAB Chemical Assessment Advisory Committee for
9 reviewing this draft assessment.

10
11 This draft assessment reviews publicly available studies on *tert*-butanol to identify adverse health
12 outcomes and to characterize exposure–response relationships. Peer review is essential to the quality and
13 integrity of IRIS assessments, which provide scientific information that supports EPA’s actions to
14 protect public health. The draft assessment was reviewed by scientists across EPA and other federal
15 agencies. EPA also solicited public comment and convened a public science meeting to discuss major
16 science issues. Experts identified by the National Academy of Sciences participated in the public
17 discussions. Responses to major public comments appear as supplemental material to the draft
18 assessment.

19
20 EPA is seeking SAB advice on the clarity and scientific underpinnings of the overall assessment. The
21 peer review should consider whether the conclusions presented in the draft assessment are clearly
22 presented and scientifically supported. Below, a set of charge questions for each major analysis are
23 presented. The SAB is expected to consider questions and issues raised during public comment as part
24 of its deliberations. The advice will be most useful when prioritized to indicate its relative importance
25 during revision:

26
27 *Tier 1: Recommended Revisions* – Key recommendations that are necessary in order to improve the
28 critical scientific concepts, issues and/or narrative within the assessment.

29 *Tier 2: Suggestions* – Recommendations that are encouraged for EPA to adopt in order to strengthen the
30 scientific concepts, issues and/or narrative within the assessment, but other factors (e.g., Agency need)
31 should be considered by EPA before undertaking these revisions.

32 *Tier 3: Future Considerations* – Useful and informative scientific exploration that may inform future
33 evaluations of key science issues and/or the development of future assessments. These recommendations
34 are likely outside the immediate scope and/or needs of the current assessment under review.

35 36 **1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods**

37 Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and
38 evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection
39 and Evaluation section. Were the strategies clearly described and objectively applied?
40

41 **Hazard Identification and Dose-Response Analysis**

42 Chapter 1 (Hazard Identification) and the supplemental materials summarize the chemical properties,
43 toxicokinetics, and health effects associated exposure to *tert*-butanol. Chapter 2 (Dose Response
44 Analysis) uses this information to derive an oral reference dose and inhalation reference concentration
45 for noncancer outcomes, in addition to an oral slope factor for cancer.

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2. Chemical Properties and Toxicokinetics

2a. Chemical properties. Is the information on chemical properties accurate?

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of *tert*-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

2c. Choice of dose metric. Is the average concentration of *tert*-butanol in blood an appropriate choice for the dose metric?

Hazard Identification and Dose–Response Assessment

Comment on EPA’s assessment of the toxicological studies and dose-response assessment, including whether there are additional peer-reviewed studies that should be considered.

3. Noncancer

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of *tert*-butanol. EPA evaluated the evidence, including the role of $\alpha_2\mu$ -globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

3b. Noncancer toxicity at other sites. (Sections 1.2.3-6, and 1.3.1). The draft assessment finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with *tert*-butanol exposure, please identify them and outline the rationale for including them in the assessment.

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of 4×10^{-1} mg/kg–day, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of 5×10^0 mg/m³, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995), converted for inhalation exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

1 **4. Cancer**

2
3 **4a. Cancer modes-of-action.**

4
5 **(i) Cancer modes-of-action in the kidney.** As described in section 1.2.1, kidney tumors were
6 observed in male rats following *tert*-butanol exposure, and a mode-of-action involving $\alpha_2\mu$ -globulin
7 and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with
8 EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the
9 kidney tumors in male rats to be relevant to human hazard identification. Please comment on
10 whether this conclusion is scientifically supported.

11
12 **(ii) Cancer modes-of-action in the thyroid.** As described in section 1.2.2, thyroid tumors were
13 observed in male and female mice following *tert*-butanol exposure, and an anti-thyroid mode-of-
14 action was evaluated. The analysis, conducted in accordance with EPA's guidance on thyroid
15 follicular cell tumors in rodents (U.S. EPA, 1998), found the information inadequate to determine
16 whether an anti-thyroid mode-of-action was operating and considered the thyroid follicular cell
17 tumors in male and female mice to be relevant to humans. Please comment on whether this
18 conclusion is scientifically supported.

19
20 **4b. Cancer characterization.** As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance
21 with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is
22 *suggestive evidence of carcinogenic potential for tert-butanol*, based on thyroid follicular cell tumors
23 in male and female B6C3F₁ mice via drinking water and on renal tubule tumors in male F344 rats
24 via drinking water. ~~Please comment on whether this cancer descriptor is scientifically supported. If~~
25 ~~another cancer descriptor should be selected, please outline how it might be supported. Please~~
26 comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all
27 routes of exposure. If another cancer descriptor should be selected, please outline how it might be
28 supported.

29
30 **4c. Cancer toxicity values.** Section 3 of EPA's cancer guidelines (2005) states:

31
32 "When there is suggestive evidence, the Agency generally would not attempt a dose-response
33 assessment, as the data generally would not support one, however, when the evidence includes a
34 well-conducted study, quantitative analyses may be useful for some purposes, for example,
35 providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or
36 setting research priorities. In each case, the rationale for the quantitative analysis is explained,
37 considering the uncertainty in the data and the suggestive nature of the weight of evidence."

38
39 ~~Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for~~
40 ~~quantitative analysis, and whether the NTP (1995) study is suitable for this purpose. Please~~
41 comment on whether Sections 2.3 of the draft assessment adequately explains the rationale for
42 including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether
43 the NTP (1995) study is a suitable basis for this quantitative analysis.
44
45

1 **4d. Oral slope factor for cancer.** Section 2.3 presents an oral slope factor of 5×10^{-4} per mg/kg-
2 day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please
3 comment on whether this value is scientifically supported and its derivation clearly described. If an
4 alternative approach would be more appropriate, please outline how it might be developed.
5

6 **4e. Inhalation unit risk for cancer.** Section 2.4 presents no inhalation unit risk. The lack of a
7 toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to
8 determine the relative contribution of $\alpha_2\mu$ -globulin nephropathy and other processes precluded the
9 use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation
10 unit risk estimate, please outline how it might be developed.
11

12 **5. Susceptible Populations and Lifestages**

13 As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible
14 populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this
15 conclusion is scientifically supported and clearly described. If there are publicly available studies to
16 identify other susceptible populations or lifestages, please identify them and outline their impact on the
17 conclusions.
18

19 **6. Question on the Executive Summary**

20 The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions
21 for a broad range of audiences. Please comment on whether the executive summary clearly and
22 appropriately presents the major conclusions of the draft assessment.

APPENDIX B: EDITORIAL CORRECTIONS

ETBE:

1. On page 1-5, lines 35-36: It is stated that single mouse inhalation study showed “weak increases in kidney weight.” I think the text should be more specific as the descriptor “weak” is vague and not really informative.
2. Page 1-35, lines 20-21: Delete "renal" from the phrase "renal nephrotoxicity" as it is redundant.
3. Comments about presentation in draft ETBE assessment and Charges:
4. Delete the word "purposes" in the first sentence of Charge Question 3b.
5. Page 1-37, line 12. This sentence is unclear. Does “from adult exposure” mean that the fetuses were exposed from the parental oral exposure, or that the effects on the F1 liver weights were similar to those of exposed adults? It needs clarification.
6. Throughout male reproductive text; ALDH2+/- mice are referred to as "heterogeneous"; the correct term is "heterozygous".
7. Table 1-14; Page 1-60: The dose for inhalation studies is mistakenly given here as "mg/kg-d"; it should be "mg/m³".
8. Table 1-14; Figures 1-11, 1-12 (page 1-65 to 1-75): Absolute testis weight is a much better and more precise measure of toxicity. Testis weight does not vary with body weight changes. Wherever available, only absolute testis weights should be presented to avoid unnecessary confusion.
9. Table 1-14: This table says that the testis weight data were not shown in Bond et al. 1996b. The data on rat testis weights are found in Table 23; page 76.
10. Page 1-80, lines 32-33: The statement that the findings in the F1 adults were similar to the P (F0) adults should be modified to include the observation that in the F1, pituitary weights of males were significantly increased at the 1,000 mg/kg-d dose (Gaoua, 2004b).
11. Page 1-90, Line 23: Delete the words "or biological". Missing right atrioventricular valve is of biological significance.
12. Table 1-16, Page 1-95: Although there were no significant differences in postnatal day 21 weights in the F0 or F1 pups, Tables 25 and 26 of Gaoua (2004b) show significant increases in terminal body weights of these pups. That should be noted as a footnote in Table 1-16 of the draft ETBE assessment.

- 1 13. Throughout the male reproductive effects text; ALDH2+/- mice are referred to as
2 "heterogeneous"; the correct term is "heterozygous".
3
- 4 14. Table 1-14; Page 1-60: The dose for inhalation studies is mistakenly given here as "mg/kg-d", it
5 should be "mg/m³".
6
- 7 15. Figure 1-12 (page 1-75): Absolute testis weight is a much better and more precise measure of
8 toxicity. Testis weight does not vary with body weight change.
9
- 10 16. When reproductive and developmental endpoints are relevant to human health and when the data
11 are sufficient for reference dose derivation, reference doses should be developed to support the
12 statement on P.15, Line 21- 23, "The ETBE inhalation database... adequately covers all major
13 systemic effects, including reproductive, developmental, ...effects."
14
- 15 17. Recommended modification, P. 2-15, Line 23-24, "... the ETBE inhalation database...does not
16 suggest that additional studies would lead to identification of a more sensitive endpoint or a
17 lower POD": Please modify or delete this statement. This deviates from the logic of scientific
18 investigation: additional studies will always have the possibility of identifying a more sensitive
19 target tissue or a more sensitive species.
20
- 21 18. There is no reporting of units for the responses (as opposed to the exposures) in the
22 Supplemental Information tables, and this also leads to difficulty in interpretation. Units should
23 be added where appropriate.
24
- 25 19. Correct the following statements:
26 o p. 2-6, lines 9-10, "...does not suggest that additional studies would lead to identification
27 of a more sensitive endpoint or a lower POD"
28 o p. 2-10 line 3, "For ETBE, only kidney effects were identified as a hazard".
29
- 30 20. Electronic link to US EPA (2002) on p. R-10 does not lead to the correct draft ETBE assessment.
31
32

tBA

- 34
- 35 1. Tert-Butyl Alcohol is repeated twice in the list of passwords for the Toxline database in Table
36 LS-1.
37
- 38 2. The description of the dose metrics calculated with the model needs to clear and correct
39 throughout the draft tBA assessment. On page B-24, "daily amount of ETBE metabolized in
40 liver", presumably means "daily average rate of ETBE metabolized in liver" and similar changes
41 in language are needed for tBA. Figure B-3 the y-axes are indicated as "ETBE metabolized
42 (mg/hr)" and "tert-butanol metabolized (mg/hr)"; either y-axis legends or figure legend text
43 needs to clarify that this is "daily average rate metabolized". The SAB recommends that the
44 assessment should always use the complete description wherever the dose metric is defined, e.g.,
45 ETBE: "average rate of metabolism of ETBE in the liver after periodicity is achieved" tBA: "the
46 average concentration of tBA in the blood at steady state (for continuous inhalation) or after

- 1 periodicity is achieved (for oral exposure)”. Other modifications to text in response to comments
2 above would be desirable.
3
- 4 3. Descriptions of dose metrics in text and figures (e.g., Appendix B) should be corrected to reflect
5 the fact that the dose metric is average concentration of tBA in the blood after periodicity is
6 achieved. The SAB recommends that the assessment should always use the complete description
7 wherever the dose metric is defined, e.g., ETBE: “average rate of metabolism of ETBE in the
8 liver after periodicity is achieved” tBA: “the average concentration of tBA in the blood at steady
9 state (for continuous inhalation) or after periodicity is achieved (for oral exposure)”.
- 10
- 11 4. On page 2-16, lines 10-11: The draft tBA assessment describes, in this paragraph, the derivation
12 of an RfC value based on increased kidney weights and other kidney effects from a chronic oral
13 exposure study in female rats, and states that the effects occurred “spanning a range from $44 \times$
14 100 to 3×10^1 mg/m³, for an overall 7-fold range.” This is not a 7-fold range, but is only about
15 1.5-fold.
16
- 17 5. Table 1-14, Figure 1-13: The EPA should state that in the NTP (1995) publication there appeared
18 to be a significant loss of testis weight after 13 weeks’ exposure mice to tBA at 8210 mg/kg-d
19 from 0.115 mg to 0.096 mg (Table F3), but the draft tBA assessment was self-contradictory as in
20 Table H2 they indicate that this tBA exposure only decreased testis weights, non-significantly,
21 from 0.115 to 0.101 mg.
22
- 23 6. Table 1-14, Figure 1-13: The draft tBA assessment is incorrect when it indicated that the NTP
24 (1995) publication showed no effect of oral doses of tBA at 1,560 or 3,620 mg/kg-d on female
25 estrous cycle in rats (Page 1-59, line 16). While it is true that there was not any change in
26 estrous cycle length in rats with measurable estrous cycles, 2/10 and 4/4 surviving rats had cycles
27 that were longer than 7 days, were unclear, or showed no evidence of cyclicity (Table H1, NTP,
28 1995). This loss of clear cycles is a much more serious effect than a slight change in cycle
29 length in rats that showed somewhat normal cycles. It seems clear that there are significant
30 effects of high doses of tBA resulting in loss of cyclicity, and this endpoint should be analyzed in
31 the draft tBA assessment. The statistical significance of the slight increase in lack of cyclicity at
32 1,560 should be analyzed. Since the 1,560 mg/kg-d dose did not cause any lethality or effects on
33 body weight in the female rats, effects at this dose cannot be attributed to general toxicity.
34
- 35 7. Table 1-14, Figure 1-13, Page 1-59, lines 14-17: Data and results of percentages of mice showing
36 no measurable estrous cyclicity should be added since this is an endpoint that has more serious
37 consequences than the increase in estrous cycle length. There is a definite effect on this endpoint
38 at an oral dose of 8,210 mg/kg-d at which 4/6 mice failed to show clear evidence of estrous
39 cyclicity (Table H2, NTP, 1995). This addition does not change the conclusion since lethality
40 and effects on body weight were observed indicating that the loss of estrous cyclicity could have
41 been an indirect effect. In addition, the data on the incidence of mice showing no measurable
42 cyclicity after inhalation administration needs to be analyzed. In the control group 0/10 shown
43 no clear cyclicity, but in the various treatment groups 2/10, 1/10, and 3/10 showed loss of
44 measureable estrous cyclicity. Analysis of whether or not this is a significant effect needs to be
45 performed and if the effect is significant, changes in the draft tBA assessment need to be made.
46

- 1 8. Table 1-12 on page 1-50: The column of body weight gain PND1-21 as a percentage of the gain
2 in the control for the Huntingdon 2004 study should be deleted. It cannot be readily interpreted
3 and particularly the number of 100% gain during PND 1-21 in 1000 mg/kg-d females compared
4 to control has led to confusion in the SAB and was misinterpreted as suggesting a basic
5 metabolic alteration. This is not a surprising finding when the actual weights are examined.
6 During gestation (PND0-GD0) the treated dams only gained 27g compared to 50g in the control.
7 Lower weight gain is a typical effect of a toxicant. During lactation, the control dams gained 31g
8 but the treated dams gained 62g. That is readily explained by two factors: the treated dams were
9 only nursing 10.2 pups per litter while the control dams were nursing 15.2 pups and the treated
10 dams were making up for their reduced weight gain during gestation.
11
12 9. EPA should review p. 2-16, Line 10. "44" is likely a mistake, should it be "4"?