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Toxicological Review of Ethyl Tertiary Butyl Ether

(CASRN 637-92-3)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2014

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ABBREVIATIONS

α2u-g	alpha2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CIIT	Chemical Industry Institute of Toxicology
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
JPEC	Japan Petroleum Energy Center
KO	Knockout
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MTD	maximum tolerated dose
MTBE	Methyl tertiary butyl ether
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erthyrocytes
PCNA	proliferating cell nuclear antigen
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RD	Relative Deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
TAME	methyl tertiary butyl ether
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertain factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
U.S.	United States of America
WT	wild type

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PREFACE

This Toxicological Review critically reviews the publicly available studies on ethyl tertiary butyl ether (ETBE) in order to identify its adverse health effects and to characterize exposure-response relationships. The assessment examined all effects by inhalation and oral routes of exposure and covers an oral noncancer Reference Dose (RfD), an inhalation noncancer Reference Concentration (RfC), a cancer weight of evidence descriptor, and a cancer dose-response assessment. It was prepared under the auspices of EPA's Integrated Risk Information System (IRIS) program.

This assessment updates a previous IRIS draft assessment of ETBE that was peer reviewed in 2010. The previous assessment was suspended pending completion of several studies that were identified during the peer review and are now included in this document. The Toxicological Reviews for ETBE and tert-butyl alcohol (*tert*-butanol) were developed simultaneously because they have a number of overlapping scientific issues:

- *tert*-Butanol is a metabolite of ETBE, thus some of the toxicological effects of ETBE may be attributable to *tert*-butanol. Therefore, data on *tert*-butanol may inform the hazard identification and dose-response assessment of ETBE, and vice versa.
- The scientific literature for chemicals include data on α_{2u}-globulin-related nephropathy; therefore, a common approach was employed to evaluate those data as they relate to the mode of action for kidney effects.
- A combined PBPK model for ETBE and *tert*-butanol in rats was developed to support the dose-response assessments for these chemicals.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to IRIS Toxicological Reviews. The findings of this assessment and draft materials produced during its development are available on the IRIS Web site (http://www.epa.gov/iris). Appendices for chemical and physical properties, toxicokinetic information, and summaries of toxicity studies and other information are provided as Supplemental Information to this assessment.

A public meeting was held in December 2013 to obtain input on preliminary materials for ETBE, including draft literature searches and associated search strategies, evidence tables, and exposure-response arrays prior to the development of the IRIS assessment. All public comments provided were taken into consideration in developing the draft assessment. The complete set of

public comments are available on the docket at http://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2009-0229).

In April 2011, the National Research Council (NRC) released its *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde*. In addition to offering comments specifically about EPA's draft formaldehyde assessment, the NRC made several recommendations to EPA for improving the development of IRIS assessments. EPA agreed with the recommendations and is implementing them consistent with the Panel's "Roadmap for Revision," which viewed the full implementation of their recommendations by the IRIS Program as a multi-year process.

In response to the NRC's 2011 recommendations, the IRIS Program has made changes to streamline the assessment development process, improve transparency, and create efficiencies in the Program. The NRC has acknowledged EPA's successes in this area. In May 2014, the NRC released their report *Review of EPA's Integrated Risk Information System Process* reviewing the IRIS assessment development process and found that EPA has made substantial improvements to the IRIS Program in a short amount of time.

The draft ETBE assessment represents a significant advancement in implementing the NRC recommendations. This assessment is streamlined, and uses tables, figures, and appendices to increase transparency and clarity. It is structured to have distinct sections for the literature search and screening strategy, study selection and evaluation, hazard identification, and dose-response assessment. The assessment includes a comprehensive, systematic, and documented literature search and screening approach, provides the database search strategy in a table (databases, keywords), visually represents the inclusion and exclusion of studies in a flow diagram, and all of the references are integrated within the Health and Environmental Research Online (HERO) database. A study evaluation section provides a systematic review of methodological aspects of epidemiology and experimental animal studies, including study design, conduct, and reporting, that was subsequently taken into consideration in the evaluation and synthesis of data from these studies. The evidence is presented in standardized evidence tables, and exposure-response arrays. The hazard identification and dose-response sections include subsections based on organ/system-specific effects in which the evidence is synthesized within and integrated across all evidence for each target organ/systems.

In the draft ETBE assessment, the IRIS Program has attempted to transparently and uniformly identify strengths and limitations that would affect interpretation of results. All animal studies of ETBE that were considered to be of acceptable quality, whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to ETBE. These studies were evaluated for aspects of design, conduct, and reporting that could affect the interpretation of results and the overall contribution to the synthesis of evidence for determination of human hazard potential using the study quality considerations outlined in the Preamble. A brief summary of the evaluation is included in the section on methods

for study selection and evaluation. Information on study features related to this evaluation is reported in evidence tables and documented in the synthesis of evidence. Discussion of study strengths and limitations (that ultimately supported preferences for the studies and data relied upon) were included in the text where relevant.

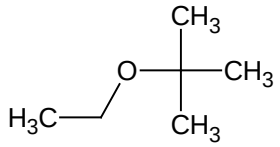
In this assessment, the IRIS Program is using existing guidelines to systematically approach the integration of noncancer human, animal, and mechanistic evidence. In conducting this analysis and developing the synthesis, the IRIS Program evaluates the data for the: strength of the relationship between the exposure and response and the presence of a dose-response relationship; specificity of the response to chemical exposure and whether the exposure precedes the effect; consistency of the association between the chemical exposure and response; and biological plausibility of the response or effect and its relevance to humans. The IRIS Program uses this weight-of-evidence approach to identify the potential human hazards associated with chemical exposure.

The IRIS ETBE assessment provides a streamlined presentation of information, integrated hazard identification of all toxic effects, and derivation of organ/system-specific reference values. Additionally, consistent with the goal that assessments should provide a scientifically sound and transparent evaluation of the relevant scientific literature and presentation of the analyses performed, this assessment contains an expanded discussion of study selection and evaluation, as well as increased documentation of key assessment decisions.

For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), 202-566-1749 (fax), or hotline.iris@epa.gov.

Chemical Properties and Uses

ETBE is volatile, relatively water soluble, stable under most conditions in soil and water, and relatively short-lived in the atmosphere. It does not bind strongly to soil and has a low potential to bioconcentrate in aquatic systems. ETBE does not occur naturally in the environment.[1]



Ethyl Tertiary-Butyl Ether

[1] http://www.epa.gov/oust/oxygenat/index.htm

($C_6H_{14}0$; CAS # 637-92-3)

ETBE has been used as a fuel oxygenate in the U.S. to improve combustion efficiency and reduce pollutants in exhaust. From approximately 1990 to 2006, ETBE was periodically added to gasoline at levels up to approximately 20%, but methyl tert-butyl ether (MTBE) and other oxygenates were more commonly used. In 2006, use of ETBE and other ether fuel additives ceased in the U.S., and the use of ethanol dramatically increased (Weaver et al., 2010).[2] ETBE is still registered with EPA for use as a fuel additive, but its current use has not been documented. The use of ether fuel additives has been banned or limited by several states, largely in response to groundwater contamination concerns.

The U.S. is a major exporter of ETBE, producing 25% of the world's ETBE in 2012. Worldwide consumption of ETBE is concentrated in Western Europe (~70%). Use in Eastern Europe and Japan is also relatively high. Japan's use increased dramatically in 2010 in order to fulfill its 2010 Kyoto Accord obligations (USDA, 2012).[3]

While it was used in the U.S., ETBE was released to the environment by gasoline leaks, evaporation, spills, and other releases. ETBE degrades slowly in the environment and can move with water in soil. Monitoring studies targeting groundwater near areas where petroleum contamination likely occurred commonly detect ETBE. For instance, a survey of states reported an average detection rate of 18% for ETBE in groundwater samples associated with gasoline contamination.[4] Non-targeted studies, such as a 2006 U.S. Geological Survey (USGS) study[5] measuring VOCs in general, have lower detection rates. The 2006 USGS study showed detections of ETBE above 0.2 µg/L in five samples from two public drinking water wells, corresponding to a 0.0013 rate of detection. The USGS study measured several VOCs and was not targeted to sites that would be most vulnerable to ETBE contamination.

Fuel contamination cleanup is largely done by states, and information on the number of private contaminated drinking water wells is not consistently available. The State of California

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[2] Gasoline Composition Regulations Affecting LUST Sites. EPA/600/R-10/001. January 2010.

[3] USDA Foreign Agricultural Service Global Agricultural Information Network. Japan Biofuels Annual: Japan Focuses on Next Generation Biofuels. 6/29/2012.

[4] Summary Report on a Survey of State Experiences with MTBE and Other Oxygenate Contamination at LUST Sites. New England Interstate Water Pollution Control Commission. 2003 http://www.neiwpcc.org/neiwpcc_docs/2003mtbesum.pdf

[5] http://water.usgs.gov/nawqa/vocs/national_assessment/

maintains an online database of measurements from contaminated sites[6]. From 2010 to 2013, ETBE has been detected in California at 607 and 73 sites in groundwater and air, respectively. Most of the contamination is attributed to leaking underground storage tanks, and some contamination is associated with refineries and petroleum transportation. The contamination was noted in approximately 48 counties, with higher population counties (e.g., Los Angeles and Orange) having more contaminated sites.

The occurrence of ETBE in other states was found in fewer and less standardized data. Presently, only 13 states routinely analyze for ETBE at fuel contaminated sites[7]. Monitoring data associated with leaking storage tanks in Maryland show contamination in groundwater affecting multiple properties[8]. A review from Georgia noted that ETBE was detected at 6% of petroleum cleanup sites and that it was the least-frequently detected ether oxygenate. New Hampshire has noted two contaminated fuel sites with measured groundwater concentrations up to 190 ppb.

Assessments by Other National and International Health Agencies

Toxicity information on ETBE has been evaluated by the National Institute for Public Health and the Environment (Bilthoven, The Netherlands) (Tiesjema and Baars, 2009) and the American Conference of Governmental Industrial Hygienists (ACGIH, 2001). ETBE has not been evaluated by the International Agency for Research on Cancer (IARC). The results of these assessments are presented in Appendix A of the Supplemental Information. It is important to recognize that these assessments may have been prepared for different purposes and may utilize different methods, and that newer studies may be included in the IRIS assessment.

[6] http://geotracker.waterboards.ca.gov/

[7] Summary Report on a Survey of State Experiences with MTBE and Other Oxygenate Contamination at LUST Sites. New England Interstate Water Pollution Control Commission. 2003 http://www.neiwpcc.org/neiwpcc_docs/2003mtbesum.pdf

[8] http://www.mde.state.md.us/programs/Land/OilControl/RemediationSites/Pages/Programs/LandPrograms/Oil_Control/RemediationSites/index.aspx

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

Soon after the EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in decisions to protect human health and the environment. The Clean Air Act, for example, mandates that the EPA provide "an ample margin of safety to protect public health"; the Safe Drinking Water Act, that "no adverse effects on the health of persons may reasonably be anticipated to occur, allowing an adequate margin of safety." Accordingly, the EPA uses information on the adverse effects of chemicals and on exposure levels below which these effects are not anticipated to occur.

IRIS assessments critically review the publicly available studies to identify adverse health effects from exposure to chemicals and to characterize exposure-response relationships. In terms set forth by the National Research Council (NRC, 1983), IRIS assessments cover the hazard identification and dose-response assessment steps of risk assessment, not the exposure assessment or risk characterization steps that are conducted by the EPA's program and regional offices and by other federal, state, and local health agencies that evaluate risk in specific populations and exposure scenarios. IRIS assessments are distinct from and do not address political, economic, and technical considerations that influence the design and selection of risk management alternatives.

An IRIS assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture. These agents may be found in air, water, soil, or sediment. Exceptions are chemicals currently used exclusively as pesticides, ionizing and non-ionizing radiation, and criteria air pollutants listed under Section 108 of the Clean Air Act (carbon monoxide, lead, nitrogen oxides, ozone, particulate matter, and sulfur oxides).

Periodically, the IRIS Program asks other EPA programs and regions, other federal agencies, state health agencies, and the general public to nominate chemicals and mixtures for future assessment or reassessment. Agents may be considered for reassessment as significant new studies are published. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. Other agents may also be assessed in response to an urgent public health need.

2. Process for developing and peer-reviewing IRIS assessments

The process for developing IRIS assessments (revised in May 2009 and enhanced in July 2013) involves critical analysis of the pertinent studies, opportunities for public input, and multiple levels of scientific review. The EPA revises draft assessments after each review, and external drafts and comments become part of the public record (U.S. EPA, 2009).

Before beginning an assessment, the IRIS Program discusses the scope with other EPA programs and regions to ensure that the assessment will meet their needs. Then a public meeting on problem formulation invites discussion of the key issues and the

studies and analytical approaches that might contribute to their resolution.

Step 1. Development of a draft Toxicological Review. The draft assessment considers all pertinent publicly available studies and applies consistent criteria to evaluate study quality, identify health effects, identify mechanistic events and pathways, integrate the evidence of causation for each effect, and derive toxicity values. A public meeting prior to the integration of evidence and derivation of toxicity values promotes public discussion of the literature search, evidence, and key issues.

Step 2. Internal review by scientists in EPA programs and regions. The draft assessment is revised to address the comments from within the EPA.

Step 3. Interagency science consultation with other federal agencies and the Executive Offices of the President. The draft assessment is revised to address the interagency comments. The science consultation draft, interagency comments, and the EPA's response to major comments become part of the public record.

Step 4. Public review and comment, followed by external peer review. The EPA releases the draft assessment for public review and comment. A public meeting provides an opportunity to discuss the assessment prior to peer review. Then the EPA releases a draft for external peer review. The peer review meeting is open to the public and includes time for oral public comments. The peer reviewers assess whether the evidence has been assembled and evaluated according to guidelines and whether the conclusions are justified by the evidence. The peer review draft, written public comments, and peer review report become part of the public record.

Step 5. Revision of draft Toxicological Review and development of draft IRIS summary. The draft assessment is revised to reflect the peer review comments, public comments, and newly published studies that are critical to the conclusions of the assessment. The disposition of peer review comments and public comments becomes part of the public record.

Step 6. Final EPA review and interagency science discussion with other federal agencies and the Executive Offices of the President The draft assessment and summary are revised to address the EPA and interagency comments. The science discussion draft, written interagency comments, and EPA's response to major comments become part of the public record.

Step 7. Completion and posting. The Toxicological Review and IRIS summary are posted on the IRIS website (http://www.epa.gov/iris/).

The remainder of this Preamble addresses step 1, the development of a draft Toxicological Review. IRIS assessments follow standard practices of evidence evaluation and peer review, many of which are discussed in EPA guidelines (U.S. EPA, 2005a, b, 2000b, 1998, 1996, 1991b, 1986a, b) and other methods (U.S. EPA, 2012a, b, 2011, 2006a, b, 2002, 1994). Transparent application of scientific judgment is of paramount importance. To provide a harmonized approach across IRIS assessments, this Preamble summarizes concepts from these guidelines and emphasizes principles of general applicability.

3. Identifying and selecting pertinent studies

3.1. Identifying studies

Before beginning an assessment, the EPA conducts a comprehensive search of the primary scientific literature. The literature search follows standard practices and includes the PubMed and ToxNet databases of the National Library of Medicine, Web of Science, and other databases listed in the EPA's HERO system (Health and Environmental Research Online, http://hero.epa.gov/). Searches for information on mechanisms of toxicity are inherently specialized and may include studies on other agents that act through related mechanisms.

Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. The EPA posts the results of the literature search on the IRIS web site and requests information from the public on additional studies and ongoing research.

The EPA also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act. Material submitted as Confidential Business Information is considered only if it includes health and safety data that can be publicly released. If a study that may be critical to the conclusions of the assessment has not been peer-reviewed, the EPA will have it peer-reviewed.

The EPA also examines the toxicokinetics of the agent to identify other chemicals (for example, major metabolites of the agent) to include in the assessment if adequate information is available, in order to more fully explain the toxicity of the agent and to suggest dose metrics for subsequent modeling.

In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components.

The literature search seeks, in decreasing order of preference (U.S. EPA, 2000b, §2.2; 1986b, §2.1)]:

- Studies of the mixture being assessed.
- Studies of a sufficiently similar mixture. In evaluating similarity, the assessment considers the alteration of mixtures in the environment through partitioning and transformation.
- Studies of individual chemical components of the mixture, if there are not adequate studies of sufficiently similar mixtures.

3.2. Selecting pertinent epidemiologic studies

Study design is the key consideration for selecting pertinent epidemiologic studies from the results of the literature search.

- Cohort studies, case-control studies, and some population-based surveys (for example, NHANES) provide the strongest epidemiologic evidence, especially if they collect information about individual exposures and effects.
- Ecological studies (geographic correlation studies) relate exposures and effects by geographic area. They can provide strong evidence if there are large exposure contrasts between geographic areas, relatively little exposure variation within study areas, and population migration is limited.

- Case reports of high or accidental exposure lack definition of the population at risk and the expected number of cases. They can provide information about a rare effect or about the relevance of analogous results in animals.

The assessment briefly reviews ecological studies and case reports but reports details only if they suggest effects not identified by other studies.

3.3. Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental animal studies or human clinical studies.

- Studies of oral, inhalation, or dermal exposure involve passage through an absorption barrier and are considered most pertinent to human environmental exposure.
- Injection or implantation studies are often considered less pertinent but may provide valuable toxicokinetic or mechanistic information. They also may be useful for identifying effects in animals if deposition or absorption is problematic (for example, for particles and fibers).

Exposure duration is also a key design consideration for selecting pertinent experimental animal studies.

- Studies of effects from chronic exposure are most pertinent to lifetime human exposure.
- Studies of effects from less-than-chronic exposure are pertinent but less preferred for identifying effects from lifetime human exposure. Such studies may be indicative of effects from less-than-lifetime human exposure.

Short-duration studies involving animals or humans may provide toxicokinetic or mechanistic information.

For developmental toxicity and reproductive toxicity, irreversible effects may result from a brief exposure during a critical period of development. Accordingly, specialized study designs are used for these effects (U.S. EPA, 2006b, 1998, 1996, 1991b).

4. Evaluating the quality of individual studies

After the subsets of pertinent epidemiologic and experimental studies have been selected from the literature searches, the assessment evaluates the quality of each individual study. This evaluation considers the design, methods, conduct, and documentation of each study, but not whether the results are positive, negative, or null. The objective is to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

4.1. Evaluating the quality of epidemiologic studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each epidemiologic study in the overall evaluation (U.S. EPA, 2005a, 1998, 1996, 1994, 1991b):

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study group and comparison group.
- Ascertainment of exposure to the chemical or mixture.
- Ascertainment of disease or health effect.

- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects.
- Characterization of exposure during critical periods.
- Sample size and statistical power to detect anticipated effects.
- Participation rates and potential for selection bias as a result of the achieved participation rates.
- Measurement error (can lead to misclassification of exposure, health outcomes, and other factors) and other types of information bias.
- Potential confounding and other sources of bias addressed in the study design or in the analysis of results. The basis for consideration of confounding is a reasonable expectation that the confounder is related to both exposure and outcome and is sufficiently prevalent to result in bias.

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating epidemiologic studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991b).

4.2. Evaluating the quality of experimental studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each experimental animal study, in-vitro study, or human clinical study (U.S. EPA, 2005a, 1998, 1996, 1991b). Research involving human subjects is considered only if conducted according to ethical principles.

- Documentation of study design, animals or study population, methods, basic data, and results.
- Nature of the assay and validity for its intended purpose.
- Characterization of the nature and extent of impurities and contaminants of the administered chemical or mixture.
- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects.
- Sample sizes and statistical power to detect dose-related differences or trends.
- Ascertainment of survival, vital signs, disease or effects, and cause of death.
- Control of other variables that could influence the occurrence of effects.

The assessment uses statistical tests to evaluate whether the observations may be due to chance. The standard for determining statistical significance of a response is a trend test or comparison of outcomes in the exposed groups against those of concurrent controls. In some situations, examination of historical control data from the same laboratory within a few years of the study may improve the analysis. For an uncommon effect that is not statistically significant compared with concurrent controls, historical controls may show that the effect is unlikely to be due to chance. For a response that appears significant against a concurrent control response that is unusual, historical controls may offer a different interpretation (U.S. EPA, 2005a, §2.2.2.1.3).

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991b). In multi-generation studies, agents that produce developmental effects at doses that are not toxic to the maternal animal are of special concern. Effects that occur at doses

associated with mild maternal toxicity are not assumed to result only from maternal toxicity. Moreover, maternal effects may be reversible, while effects on the offspring may be permanent (U.S. EPA, 1998, §3.1.2.4.5.4; 1991b, §3.1.1.4),.

4.3. Reporting study results

The assessment uses evidence tables to present the design and key results of pertinent studies. There may be separate tables for each site of toxicity or type of study.

If a large number of studies observe the same effect, the assessment considers the study quality characteristics in this section to identify the strongest studies or types of study. The tables present details from these studies, and the assessment explains the reasons for not reporting details of other studies or groups of studies that do not add new information. Supplemental information provides references to all studies considered, including those not summarized in the tables.

The assessment discusses strengths and limitations that affect the interpretation of each study. If the interpretation of a study in the assessment differs from that of the study authors, the assessment discusses the basis for the difference.

As a check on the selection and evaluation of pertinent studies, the EPA asks peer reviewers to identify studies that were not adequately considered.

5. Evaluating the overall evidence of each effect

5.1. Concepts of causal inference

For each health effect, the assessment evaluates the evidence as a whole to determine whether it is reasonable to infer a causal association between exposure to the agent and the occurrence of the effect. This inference is based on information from pertinent human studies, animal studies, and mechanistic studies of adequate quality. Positive, negative, and null results are given weight according to study quality.

Causal inference involves scientific judgment, and the considerations are nuanced and complex. Several health agencies have developed frameworks for causal inference, among them the U.S. Surgeon General (CDC, 2004; HEW, 1964), the International Agency for Research on Cancer (IARC, 2006), the Institute of Medicine (IOM, 2008), and the EPA (2010, §1.6; 2005a, §2.5). Although developed for different purposes, the frameworks are similar in nature and provide an established structure and language for causal inference. Each considers aspects of an association that suggest causation, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998), and U.S. EPA (2005a, §2.2.1.7; 1994, Appendix C).

Strength of association: The finding of a large relative risk with narrow confidence intervals strongly suggests that an association is not due to chance, bias, or other factors. Modest relative risks, however, may reflect a small range of exposures, an agent of low potency, an increase in an effect that is common, exposure misclassification, or other sources of bias.

Consistency of association: An inference of causation is strengthened if elevated risks are observed in independent studies of different populations and exposure scenarios. Reproducibility of findings constitutes one of the strongest arguments for causation. Discordant results sometimes reflect differences in study design, exposure, or confounding factors.

Specificity of association: As originally intended, this refers to one cause associated with one effect. Current understanding that many agents cause multiple effects and many effects have multiple causes make this a less

informative aspect of causation, unless the effect is rare or unlikely to have multiple causes.

Temporal relationship: A causal interpretation requires that exposure precede development of the effect.

Biologic gradient (exposure-response relationship): Exposure-response relationships strongly suggest causation. A monotonic increase is not the only pattern consistent with causation. The presence of an exposure-response gradient also weighs against bias and confounding as the source of an association.

Biologic plausibility: An inference of causation is strengthened by data demonstrating plausible biologic mechanisms, if available. Plausibility may reflect subjective prior beliefs if there is insufficient understanding of the biologic process involved.

Coherence: An inference of causation is strengthened by supportive results from animal experiments, toxicokinetic studies, and short-term tests. Coherence may also be found in other lines of evidence, such as changing disease patterns in the population.

"Natural experiments": A change in exposure that brings about a change in disease frequency provides strong evidence, as it tests the hypothesis of causation. An example would be an intervention to reduce exposure in the workplace or environment that is followed by a reduction of an adverse effect.

Analogy: Information on structural analogues or on chemicals that induce similar mechanistic events can provide insight into causation.

These considerations are consistent with guidelines for systematic reviews that evaluate the quality and weight of evidence. Confidence is increased if the magnitude of effect is large, if there is evidence of an exposure-response relationship, or if an association was observed and the plausible biases would tend to decrease the magnitude of the reported effect. Confidence is decreased for study limitations, inconsistency of results, indirectness of evidence, imprecision, or reporting bias (Guyatt et al., 2008b; Guyatt et al., 2008a).

5.2. Evaluating evidence in humans

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole. The objective is to determine whether a credible association has been observed and, if so, whether that association is consistent with causation. In doing this, the assessment explores alternative explanations (such as chance, bias, and confounding) and draws a conclusion about whether these alternatives can satisfactorily explain any observed association.

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may select a standard descriptor to characterize the epidemiologic evidence of association between exposure to the agent and occurrence of a health effect.

Sufficient epidemiologic evidence of an association consistent with causation: The evidence establishes a causal association for which alternative explanations such as chance, bias, and confounding can be ruled out with reasonable confidence.

Suggestive epidemiologic evidence of an association consistent with causation: The evidence suggests a causal association but chance, bias, or confounding cannot be ruled out as explaining the association.

Inadequate epidemiologic evidence to infer a causal association: The available studies do not permit a conclusion regarding the presence or absence of an association.

Epidemiologic evidence consistent with no causal association: Several adequate studies covering the full range of human exposures and considering susceptible populations, and for which alternative explanations such as bias and confounding can be ruled out, are mutually consistent in not finding an association.

5.3. Evaluating evidence in animals

For each effect, the assessment evaluates the evidence from the animal experiments as a whole to determine the extent to which they indicate a potential for effects in humans. Consistent results across various species and strains increase confidence that similar results would occur in humans. Several concepts discussed by Hill (1965) are pertinent to the weight of experimental results: consistency of response, dose-response relationships, strength of response, biologic plausibility, and coherence (U.S. EPA, 2005a, §2.2.1.7; 1994, Appendix C).

In weighing evidence from multiple experiments, U.S. EPA (2005a, §2.5) distinguishes:

Conflicting evidence (that is, mixed positive and negative results in the same sex and strain using a similar study protocol) from

Differing results (that is, positive results and negative results are in different sexes or strains or use different study protocols).

Negative or null results do not invalidate positive results in a different experimental system. The EPA regards all as valid observations and looks to explain differing results using mechanistic information (for example, physiologic or metabolic differences across test systems) or methodological differences (for example, relative sensitivity of the tests, differences in dose levels, insufficient sample size, or timing of dosing or data collection).

It is well established that there are critical periods for some developmental and reproductive effects (U.S. EPA, 2006b, 2005a, b, 1998, 1996, 1991b). Accordingly, the assessment determines whether critical periods have been adequately investigated. Similarly, the assessment determines whether the database is adequate to evaluate other critical sites and effects.

In evaluating evidence of genetic toxicity:

- Demonstration of gene mutations, chromosome aberrations, or aneuploidy in humans or experimental mammals (*in vivo*) provides the strongest evidence.
- This is followed by positive results in lower organisms or in cultured cells (*in vitro*) or for other genetic events.
- Negative results carry less weight, partly because they cannot exclude the possibility of effects in other tissues (IARC, 2006).

For germ-cell mutagenicity, The EPA has defined categories of evidence, ranging from positive results of human germ-cell mutagenicity to negative results for all effects of concern (U.S. EPA, 1986a, §2.3).

5.4. Evaluating mechanistic data

Mechanistic data can be useful in answering several questions.

- The biologic plausibility of a causal interpretation of human studies.
- The generalizability of animal studies to humans.
- The susceptibility of particular populations or lifestages.

The focus of the analysis is to describe, if possible, mechanistic pathways that lead to a health effect. These pathways encompass:

- *Toxicokinetic processes* of absorption, distribution, metabolism, and elimination that lead to the formation of an active agent and its presence at the site of initial biologic interaction.
- *Toxicodynamic processes* that lead to a health effect at this or another site (also known as a *mode of action*).

For each effect, the assessment discusses the available information on its *modes of action* and associated *key events* (*key events* being empirically observable, necessary precursor steps or biologic markers of such steps; *mode of action* being a series of key events involving interaction with cells, operational and anatomic changes, and resulting in disease). Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms. Information on mode of action is not required for a conclusion that the agent is causally related to an effect (U.S. EPA, 2005a, §2.5).

The assessment addresses several questions about each hypothesized mode of action(U.S. EPA, 2005a, §2.4.3.4).

1) **Is the hypothesized mode of action sufficiently supported in test animals?** Strong support for a key event being necessary to a mode of action can come from experimental challenge to the hypothesized mode of action, in which studies that suppress a key event observe suppression of the effect. Support for a mode of action is meaningfully strengthened by consistent results in different experimental models, much more so than by replicate experiments in the same model. The assessment may consider various aspects of causation in addressing this question.

2) **Is the hypothesized mode of action relevant to humans?** The assessment reviews the key events to identify critical similarities and differences between the test animals and humans. Site concordance is not assumed between animals and humans, though it may hold for certain effects or modes of action. Information suggesting quantitative differences in doses where effects would occur in animals or humans is considered in the dose-response analysis. Current levels of human exposure are not used to rule out human relevance, as IRIS assessments may be used in evaluating new or unforeseen circumstances that may entail higher exposures.

3) **Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?** The assessment reviews the key events to identify populations and lifestages that might be susceptible to their occurrence. Quantitative differences may result in separate toxicity values for susceptible populations or lifestages.

The assessment discusses the likelihood that an agent operates through multiple modes of action. An uneven level of support for different modes of action can reflect disproportionate resources spent investigating them (U.S. EPA, 2005a, §2.4.3.3). It should be noted that in clinical reviews, the credibility of a series of studies is reduced if evidence is limited to studies funded by one interested sector (Guyatt et al., 2008a).

For cancer, the assessment evaluates evidence of a mutagenic mode of action to guide extrapolation to lower doses and consideration of susceptible lifestages. Key data include the ability of the agent or a metabolite to react with or bind to DNA, positive results in multiple test systems, or similar properties and structure-activity

relationships to mutagenic carcinogens (U.S. EPA, 2005a ,§2.3.5).

5.5. Characterizing the overall weight of the evidence

After evaluating the human, animal, and mechanistic evidence pertinent to an effect, the assessment answers the question: Does the agent cause the adverse effect? (NRC, 2009, 1983). In doing this, the assessment develops a narrative that integrates the evidence pertinent to causation. To provide clarity and consistency, the narrative includes a standard hazard descriptor. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (U.S. EPA, 2005a, §2.5).

Carcinogenic to humans: There is convincing epidemiologic evidence of a causal association (that is, there is reasonable confidence that the association cannot be fully explained by chance, bias, or confounding); or there is strong human evidence of cancer or its precursors, extensive animal evidence, identification of key precursor events in animals, and strong evidence that they are anticipated to occur in humans.

Likely to be carcinogenic to humans: The evidence demonstrates a potential hazard to humans but does not meet the criteria for *carcinogenic*. There may be a plausible association in humans, multiple positive results in animals, or a combination of human, animal, or other experimental evidence.

Suggestive evidence of carcinogenic potential: The evidence raises concern for effects in humans but is not sufficient for a stronger conclusion. This descriptor covers a range of evidence, from a positive result in the only available study to a single positive result in an extensive database that includes negative results in other species.

Inadequate information to assess carcinogenic potential: No other descriptors apply. *Conflicting evidence* can be classified as *inadequate information* if all positive results are opposed by negative studies of equal quality in the same sex and strain. *Differing results*, however, can be classified as *suggestive evidence* or as *likely to be carcinogenic*.

Not likely to be carcinogenic to humans: There is robust evidence for concluding that there is no basis for concern. There may be no effects in both sexes of at least two appropriate animal species; positive animal results and strong, consistent evidence that each mode of action in animals does not operate in humans; or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

Multiple descriptors may be used if there is evidence that carcinogenic effects differ by dose range or exposure route (U.S. EPA, 2005a, §2.5).

Another example of standard descriptors comes from the EPA's Integrated Science Assessments, which evaluate causation for the effects of the criteria pollutants in ambient air (U.S. EPA, 2010, §1.6).

Causal relationship: Sufficient evidence to conclude that there is a causal relationship. Observational studies cannot be explained by plausible alternatives, or they are supported by other lines of evidence, for example, animal studies or mechanistic information.

Likely to be a causal relationship: Sufficient evidence that a causal relationship is likely, but important uncertainties remain. For example, observational studies show an association but co-

exposures are difficult to address or other lines of evidence are limited or inconsistent; or multiple animal studies from different laboratories demonstrate effects and there are limited or no human data.

Suggestive of a causal relationship: At least one high-quality epidemiologic study shows an association but other studies are inconsistent.

Inadequate to infer a causal relationship: The studies do not permit a conclusion regarding the presence or absence of an association.

Not likely to be a causal relationship: Several adequate studies, covering the full range of human exposure and considering susceptible populations, are mutually consistent in not showing an effect at any level of exposure.

The EPA is investigating and may on a trial basis use these or other standard descriptors to characterize the overall weight of the evidence for effects other than cancer.

6. Selecting studies for derivation of toxicity values

For each effect where there is credible evidence of an association with the agent, the assessment derives toxicity values if there are suitable epidemiologic or experimental data. The decision to derive toxicity values may be linked to the hazard descriptor.

Dose-response analysis requires quantitative measures of dose and response. Then, other factors being equal:

- Epidemiologic studies are preferred over animal studies, if quantitative measures of exposure are available and effects can be attributed to the agent.
- Among experimental animal models, those that respond most like humans are preferred, if the comparability of response can be determined.
- Studies by a route of human environmental exposure are preferred, although a validated toxicokinetic model can be used to extrapolate across exposure routes.
- Studies of longer exposure duration and follow-up are preferred, to minimize uncertainty about whether effects are representative of lifetime exposure.
- Studies with multiple exposure levels are preferred for their ability to provide information about the shape of the exposure-response curve.
- Studies with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

Studies with non-monotonic exposure-response relationships are not necessarily excluded from the analysis. A diminished effect at higher exposure levels may be satisfactorily explained by factors such as competing toxicity, saturation of absorption or metabolism, exposure misclassification, or selection bias.

If a large number of studies are suitable for dose-response analysis, the assessment considers the study characteristics in this section to focus on the most informative data. The assessment explains the reasons for not analyzing other groups of studies. As a check on the selection of studies for dose-response analysis, the EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving toxicity values

7.1. General framework for dose-response analysis

The EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (U.S. EPA, 2005a, §3).

Within the observed range, the preferred approach is to use modeling to incorporate a wide range of data into the analysis. The modeling yields a *point of departure* (an exposure level near the lower end of the observed range, without significant extrapolation to lower doses) (Sections 7.2-7.3).

Extrapolation to lower doses considers what is known about the modes of action for each effect (Sections 7.4-7.5). If response estimates at lower doses are not required, an alternative is to derive *reference values*, which are calculated by applying factors to the point of departure in order to account for sources of uncertainty and variability (Section 7.6).

For a group of agents that induce an effect through a common mode of action, the dose-response analysis may derive a *relative potency factor* for each agent. A full dose-response analysis is conducted for one well-studied *index chemical* in the group, then the potencies of other members are expressed in relative terms based on relative toxic effects, relative absorption or metabolic rates, quantitative structure-activity relationships, or receptor binding characteristics (U.S. EPA, 2005a, §3.2.6; 2000b, §4.4).

Increasingly, the EPA is basing toxicity values on combined analyses of multiple data sets or multiple responses. The EPA also considers multiple dose-response approaches if they can be supported by robust data.

7.2. Modeling dose to sites of biologic effects

The preferred approach for analysis of dose is toxicokinetic modeling because of its ability to incorporate a wide range of data. The preferred dose metric would refer to the active agent at the site of its biologic effect or to a close, reliable surrogate measure. The active agent may be the administered chemical or a metabolite. Confidence in the use of a toxicokinetic model depends on the robustness of its validation process and on the results of sensitivity analyses (U.S. EPA, 2006a; 2005a, §3.1; 1994, §4.3).

Because toxicokinetic modeling can require many parameters and more data than are typically available, the EPA has developed standard approaches that can be applied to typical data sets. These standard approaches also facilitate comparison across exposure patterns and species.

- Intermittent study exposures are standardized to a daily average over the duration of exposure. For chronic effects, daily exposures are averaged over the lifespan. Exposures during a critical period, however, are not averaged over a longer duration (U.S. EPA, 2005a, §3.1.1; 1991b, §3.2).
- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.
- Oral doses are scaled allometrically using $mg/kg^{3/4}$-day as the equivalent dose metric across species. Allometric scaling pertains to equivalence across species, not across lifestages, and is not used to scale doses from adult humans or mature animals to infants or children (U.S. EPA, 2011; 2005a, §3.1.3).
- Inhalation exposures are scaled using dosimetry models that apply species-specific physiologic and anatomic factors and consider whether the

effect occurs at the site of first contact or after systemic circulation (U.S. EPA, 2012a; 1994, §3).

It can be informative to convert doses across exposure routes. If this is done, the assessment describes the underlying data, algorithms, and assumptions (U.S. EPA, 2005a, §3.1.4).

In the absence of study-specific data on, for example, intake rates or body weight, the EPA has developed recommended values for use in dose-response analysis (U.S. EPA, 1988).

7.3. Modeling response in the range of observation

Toxicodynamic ("biologically based") modeling can incorporate data on biologic processes leading to an effect. Such models require sufficient data to ascertain a mode of action and to quantitatively support model parameters associated with its key events. Because different models may provide equivalent fits to the observed data but diverge substantially at lower doses, critical biologic parameters should be measured from laboratory studies, not by model fitting. Confidence in the use of a toxicodynamic model depends on the robustness of its validation process and on the results of sensitivity analyses. Peer review of the scientific basis and performance of a model is essential (U.S. EPA, 2005a, §3.2.2).

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, the EPA has developed a standard set of empirical ("curve-fitting") models (http://www.epa.gov/ncea/bmds/) that can be applied to typical data sets, including those that are nonlinear. The EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (U.S. EPA, 2012b). Additional judgment or alternative analyses are used if the procedure fails to yield reliable results, for example, if the fit is poor, modeling may be restricted to the lower doses, especially if there is competing toxicity at higher doses (U.S. EPA, 2005a, §3.2.3).

Modeling is used to derive a point of departure (U.S. EPA, 2012b; 2005a, §3.2.4). (See Section 7.6 for alternatives if a point of departure cannot be derived by modeling.):

- If linear extrapolation is used, selection of a response level corresponding to the point of departure is not highly influential, so standard values near the low end of the observable range are generally used (for example, 10% extra risk for cancer bioassay data, 1% for epidemiologic data, lower for rare cancers).
- For nonlinear approaches, both statistical and biologic considerations are taken into account.
- For dichotomous data, a response level of 10% extra risk is generally used for minimally adverse effects, 5% or lower for more severe effects.
- For continuous data, a response level is ideally based on an established definition of biologic significance. In the absence of such definition, one control standard deviation from the control mean is often used for minimally adverse effects, one-half standard deviation for more severe effects.

The point of departure is the 95% lower bound on the dose associated with the selected response level.

7.4. Extrapolating to lower doses and response levels

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation, typically used for cancer data,

considers what is known about modes of action (U.S. EPA, 2005a, §3.3.1 and §3.3.2).

1) If a biologically based model has been developed and validated for the agent, extrapolation may use the fitted model below the observed range if significant model uncertainty can be ruled out with reasonable confidence.

2) Linear extrapolation is used if the dose-response curve is expected to have a linear component below the point of departure. This includes:

   - Agents or their metabolites that are DNA-reactive and have direct mutagenic activity.
   - Agents or their metabolites for which human exposures or body burdens are near doses associated with key events leading to an effect.

   Linear extrapolation is also used when data are insufficient to establish mode of action and when scientifically plausible.

   The result of linear extrapolation is described by an oral slope factor or an inhalation unit risk, which is the slope of the dose-response curve at lower doses or concentrations, respectively.

3) Nonlinear models are used for extrapolation if there are sufficient data to ascertain the mode of action and to conclude that it is not linear at lower doses, and the agent does not demonstrate mutagenic or other activity consistent with linearity at lower doses. Nonlinear approaches generally should not be used in cases where mode of action has not ascertained. If nonlinear extrapolation is appropriate but no model is developed, an alternative is to calculate reference values.

4) Both linear and nonlinear approaches may be used if there a multiple modes of action. For example, modeling to a low response level can be useful for estimating the response at doses where a high-dose mode of action would be less important.

If linear extrapolation is used, the assessment develops a candidate slope factor or unit risk for each suitable data set. These results are arrayed, using common dose metrics, to show the distribution of relative potency across various effects and experimental systems. The assessment then derives or selects an overall slope factor and an overall unit risk for the agent, considering the various dose-response analyses, the study preferences discussed in Section 6, and the possibility of basing a more robust result on multiple data sets.

7.5. Considering susceptible populations and lifestages

The assessment analyzes the available information on populations and lifestages that may be particularly susceptible to each effect. A tiered approach is used (U.S. EPA, 2005a, §3.5).

1) If an epidemiologic or experimental study reports quantitative results for a susceptible population or lifestage, these data are analyzed to derive separate toxicity values for susceptible individuals.

2) If data on risk-related parameters allow comparison of the general population and susceptible individuals, these data are used to adjust the general-population toxicity values for application to susceptible individuals.

3) In the absence of chemical-specific data, the EPA has developed *age-dependent adjustment factors* for early-life exposure to potential carcinogens that have a mutagenic mode of action. There is evidence of early-life susceptibility to various carcinogenic agents, but most epidemiologic studies and cancer bioassays do not include early-life

exposure. To address the potential for early-life susceptibility, the EPA recommends (U.S. EPA, 2005b, §5):

- 10-fold adjustment for exposures before age 2 years.
- 3-fold adjustment for exposures between ages 2 and 16 years.

7.6. Reference values and uncertainty factors

An *oral reference dose* or an *inhalation reference concentration* is an estimate of an exposure (including in susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002, §4.2). Reference values are typically calculated for effects other than cancer and for suspected carcinogens if a well characterized mode of action indicates that a necessary key event does not occur below a specific dose. Reference values provide no information about risks at higher exposure levels.

The assessment characterizes effects that form the basis for reference values as adverse, considered to be adverse, or a precursor to an adverse effect. For developmental toxicity, reproductive toxicity, and neurotoxicity there is guidance on adverse effects and their biologic markers (U.S. EPA, 1998, 1996, 1991b).

To account for uncertainty and variability in the derivation of a lifetime human exposure where adverse effects are not anticipated to occur, reference values are calculated by applying a series of *uncertainty factors* to the point of departure. If a point of departure cannot be derived by modeling, a no-observed-adverse-effect level or a lowest-observed-adverse-effect level is used instead. The assessment discusses scientific considerations involving several areas of variability or uncertainty.

Human variation. The assessment accounts for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. A factor of 10 is generally used to account for this variation. This factor is reduced only if the point of departure is derived or adjusted specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991b, §3.4).

Animal-to-human extrapolation. If animal results are used to make inferences about humans, the assessment adjusts for cross-species differences. These may arise from differences in toxicokinetics or toxicodynamics. Accordingly, if the point of departure is standardized to equivalent human terms or is based on toxicokinetic or dosimetry modeling, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicokinetic and toxicodynamic differences. If a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species, this factor is not used. In most other cases, a factor of 10 is applied (U.S. EPA, 2011; 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991b, §3.4).

Adverse-effect level to no-observed-adverse-effect level. If a point of departure is based on a lowest-observed-adverse-effect level, the assessment must infer a dose where such effects are not expected. This can be a matter of great uncertainty, especially if there is no evidence available at lower doses. A factor of 10 is applied to account for the uncertainty in making this inference. A factor other than 10 may be used, depending on the magnitude and nature of the response and the shape of the dose-response curve (U.S. EPA, 2002, §4.4.5;

1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991b, §3.4).

Subchronic-to-chronic exposure. If a point of departure is based on subchronic studies, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. A factor of 10 is applied to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure. This factor may also be applied for developmental or reproductive effects if exposure covered less than the full critical period. A factor other than 10 may be used, depending on the duration of the studies and the nature of the response (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1994, §4.3.9.1).

Incomplete database. If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database uncertainty factor (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991b, §3.4). The size of the factor depends on the nature of the database deficiency. For example, the EPA typically follows the suggestion that a factor of 10 be applied if both a prenatal toxicity study and a two-generation reproduction study are missing and a factor of $10^{1/2}$ if either is missing (U.S. EPA, 2002, §4.4.5).

In this way, the assessment derives candidate values for each suitable data set and effect that is credibly associated with the agent. These results are arrayed, using common dose metrics, to show where effects occur across a range of exposures (U.S. EPA, 1994, §4.3.9).

The assessment derives or selects an *organ- or system-specific reference value* for each organ or system affected by the agent. The assessment explains the rationale for each organ/system-specific reference value (based on, for example, the highest quality studies, the most sensitive outcome, or a clustering of values). By providing these organ/system-specific reference values, IRIS assessments facilitate subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms (NRC, 2009).

The assessment then selects an overall reference dose and an overall reference concentration for the agent to represent lifetime human exposure levels where effects are not anticipated to occur. This is generally the most sensitive organ/system-specific reference value, though consideration of study quality and confidence in each value may lead to a different selection.

7.7. Confidence and uncertainty in the reference values

The assessment selects a standard descriptor to characterize the level of confidence in each reference value, based on the likelihood that the value would change with further testing. Confidence in reference values is based on quality of the studies used and completeness of the database, with more weight given to the latter. The level of confidence is increased for reference values based on human data supported by animal data (U.S. EPA, 1994, §4.3.9.2).

High confidence: The reference value is not likely to change with further testing, except for mechanistic studies that might affect the interpretation of prior test results.

Medium confidence: This is a matter of judgment, between high and low confidence.

Low confidence: The reference value is especially vulnerable to change with further testing.

These criteria are consistent with guidelines for systematic reviews that evaluate the quality of evidence. These also

focus on whether further research would be likely to change confidence in the estimate of effect (Guyatt et al., 2008b).

All assessments discuss the significant uncertainties encountered in the analysis. The EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a, §3.6). For example, the discussion distinguishes model uncertainty (lack of knowledge about the most appropriate experimental or analytic model) and parameter uncertainty (lack of knowledge about the parameters of a model). Assessments also discuss human variation (interpersonal differences in biologic susceptibility or in exposures that modify the effects of the agent).

August 2013

EXECUTIVE SUMMARY

Occurrence and Health Effects

Ethyl tert-butyl ether (ETBE) is an ether oxygenate primarily used as a gasoline additive. It was used until 2006 in the U.S., and continues to be used in Japan and the European Union. ETBE is released into the environment as a result of gasoline leaks, evaporation, and spills. Exposure to ETBE can occur by drinking contaminated groundwater or by inhaling volatiles containing ETBE. Dermal exposure is possible in occupational settings where the manufacture of ETBE occurs. The magnitude of human exposure to ETBE depends on factors such as the distribution of ETBE in groundwater and the extent of the contamination.

Animal studies demonstrate that exposure to ETBE is associated with kidney effects. Available animal studies have not demonstrated ETBE to be associated with reproductive or developmental effects. No epidemiological studies are available for ETBE. Studies in rats suggest that ETBE may be carcinogenic in the liver. There are no data in humans on carcinogenicity of ETBE. Studies in animals indicate that deficient clearance of acetaldehyde, a metabolite of ETBE, could increase susceptibility to ETBE toxicity or carcinogenicity.

Effects Other Than Cancer Observed Following Oral Exposure

EPA identified kidney effects as a human hazard of ETBE exposure, with increased kidney weight in male and female rats accompanied by increased chronic progressive nephropathy (CPN), urothelial hyperplasia (in males), and increased blood concentrations of total cholesterol, blood urea nitrogen (BUN), and creatinine. Changes in kidney parameters were consistently observed, but the magnitude of change was generally moderate, and males had greater severity of effects compared with females. Overall, there was consistency across multiple measures of potential kidney toxicity, including organ weight increases, exacerbated CPN, urothelial hyperplasia, and increases in serum markers of kidney function. Additionally, effects were consistently observed across routes of exposure, species, and sex; however, male rats appeared to be more sensitive to exposure than female rats, and rats seemed to be more sensitive to exposure than mice. Mechanistic data were insufficient to establish a mode of action; thus, kidney effects are considered relevant to humans.

Increased liver weight and centrilobular hypertrophy in male and female rats were consistently observed across studies. However, no additional histopathological findings were observed, and only one serum marker of liver toxicity [gamma-glutamyl transferase (GGT)] was elevated, while other markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)] were unchanged. The magnitude of change for these noncancer

effects was mild to moderate and, except for organ weight data, did not exhibit consistent dose-response relationships. Mechanistic data suggest that ETBE exposure leads to activation of several nuclear receptors, but a relationship between receptor activation and liver toxicity has not been established for ETBE. However, mechanistic data suggest possible susceptibility related to clearance of acetaldehyde, a metabolite of ETBE. Nonetheless, EPA concluded that the evidence does not support liver effects as a potential human hazard of ETBE exposure.

No other noncancer effects were identified as adverse or exposure related; thus, EPA concluded that the evidence does not support effects on the adrenals, the immune system, the reproductive system, development, or mortality as potential human hazards of ETBE exposure.

Oral Reference Dose (RfD) for Effects Other Than Cancer

The chronic study by (JPEC, 2010a) [selected data published as Suzuki et al. (2012)] and the observed increase incidences of urothelial hyperplasia were used to derive the RfD. The endpoint of increased incidences of urothelial hyperplasia was selected as the critical effect due to its specificity as an indicator of kidney toxicity, and the observed dose-response relationship of effects across dose groups. Benchmark dose (BMD) modeling was utilized to derive the $BMDL_{10\%}$ of 60.5 mg/kg-day. The BMDL was converted to a human equivalent dose of 14.5 mg/kg-day using body weight$^{3/4}$ scaling, and this value was used as the point of departure (POD) for RfD derivation (U.S. EPA, 2011).

The proposed overall RfD was calculated by dividing the POD for increased absolute kidney weight by a composite uncertainty factor (UF) of 30 to account for extrapolation from animals to humans ($10^{½}$) and interindividual differences in human susceptibility (10).

Table ES-1. Summary of reference dose (RfD) derivation

Effect	Basis	RfD (mg/kg-day)	Exposure description	Confidence
Kidney toxicity	Increased urothelial hyperplasia JPEC (2010b) [selected data published as Saito et al. (2013)]	5×10^{-1}	Chronic	HIGH
Proposed overall RfD	**Increased urothelial hyperplasia JPEC (2010b) [selected data published as Saito et al. (2013)]**	**5×10^{-1}**	**Chronic**	**HIGH**

Effects Other Than Cancer Observed Following Inhalation Exposure

EPA identified kidney effects as a human hazard of ETBE exposure. Studies in rats following inhalation exposure have shown increases in kidney weights, nephropathy, mineralization, urothelial hyperplasia, and increases in blood concentrations of cholesterol, BUN, and creatinine. There were no available human studies that evaluated the effects of ETBE inhalation exposure.

Mode-of-action analysis determined that kidney effects in male rats were not mediated by α_{2u}-globulin, and these effects were concluded to be relevant for human health hazard assessment.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

The chronic study by JPEC (2010b) [selected data published as Saito et al. (2013)] and the observed increase incidences of urothelial hyperplasia were used to derive the RfC. The endpoint of increased incidences of urothelial hyperplasia was selected as the critical effect due to its specificity as an indicator of kidney toxicity, and the observed dose-response relationship of effects across dose groups. Benchmark dose (BMD) modeling was utilized to derive the $BMCL_{10\%}$ of 1498 mg/m^3. The BMCL was adjusted to a continuous exposure and converted to a human equivalent concentration of 265 mg/m^3.

The RfC was calculated by dividing the POD by a composite UF of 30 to account for toxicodynamic differences between animals and humans (3) and interindividual differences in human susceptibility (10).

Table ES-2. Summary of reference concentration (RfC) derivation

Effect	Basis	RfC (mg/m^3)	Exposure Description	Confidence
Kidney toxicity	Increased urothelial hyperplasia Saito et al. (2013); JPEC (2010b)	9×10^0	Chronic	HIGH
Proposed overall RfC	Increased urothelial hyperplasia **Saito et al. (2013); JPEC (2010b)**	**9×10^0**	**Chronic**	**HIGH**

Evidence for Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "suggestive evidence of carcinogenic potential" for ETBE based on evidence in rats. The limited evidence includes three bioassays in rats exposed via inhalation, drinking water, or gavage, inadequate data in other experimental species or in humans, and limited mechanistic data. One 2-year inhalation rat study observed a statistically significant increase in hepatocellular adenomas and carcinomas in male rats at a single dose, but no other bioassay reported increased incidence of liver tumors. Mechanistic data were inadequate to establish a mode of action. Mechanistic studies reported that deficient enzyme function of aldehyde dehydrogenase 2 (ALDH2) enhanced ETBE-induced genotoxicity in hepatocytes and leukocytes, suggestive of genotoxicity being mediated by the ETBE metabolite acetaldehyde, which is directly genotoxic (IARC, 2012). Overall, because a statistically significant increase occurred at one dose only without a significant response at other doses and no overall trends, and because the mechanistic data only provide some evidence of

biological plausibility, ETBE is characterized as having "suggestive evidence of carcinogenic potential."

Quantitative Estimate of Carcinogenic Risk from Oral Exposure

The main evidence of ETBE carcinogenicity consisted of the increased incidence of liver tumors in male F344 rats following inhalation exposure (Saito et al., 2013; JPEC, 2010b). This study examined three exposure levels and controls, contained adequate numbers of animals per dose group (50/sex/group), treated animals for up to 2 years, and included detailed reporting methods and results (including individual animal data).

Although ETBE was considered to have "suggestive evidence of carcinogenic potential," EPA concluded that the main study was well-conducted and quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. A PBPK model in rats for ETBE and its metabolite, *tert*-butanol, was used for route-to-route extrapolation of the inhalation $BMCL_{10}$ (described below) to an oral equivalent $BMDL_{10}$, which was adjusted to a human equivalent $BMDL_{10}$ on the basis of $(body\ weight)^{3/4}$ scaling (U.S. EPA, 2011, 2005a). Using linear extrapolation from the $BMDL_{10}$, a human equivalent oral slope factor was derived (slope factor = $0.1/BMDL_{10}$). The oral slope factor is **9×10^{-4} per mg/kg-day** based on the liver tumor response in male rats (Saito et al., 2013; JPEC, 2010b).

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

Lifetime inhalation exposure to ETBE has been associated with increased liver adenomas and carcinomas in male F344 rats. This is the only evidence of carcinogenicity following inhalation exposure (Saito et al., 2013; JPEC, 2010b); however, the biological plausibility of these data are supported by mechanistic data on tumor promotion and genotoxicity in the absence of ALDH2, and are analogous to the human carcinogenicity of acetaldehyde after consumption of ethanol. This study examined three exposure levels and controls, contained adequate numbers of animals per dose group (50/sex/group), treated animals for up to 2 years, and included detailed reporting methods and results (including individual animal data).

Although ETBE was considered to have "suggestive evidence of carcinogenic potential," EPA concluded that the main study was well-conducted and quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. EPA used the multistage 1° model for the derivation of the $BMCL_{10}$, which was then adjusted to a human equivalent $BMCL_{10}$ on the basis of inhalation dosimetry (U.S. EPA, 1994). Using linear extrapolation (inhalation unit risk = $0.1/BMCL_{10}$), a human equivalent inhalation unit risk was derived. The inhalation unit risk is **8×10^{-5} per mg/m^3** based on the liver tumor response in F344 male rats (Saito et al., 2013; JPEC, 2010b).

Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

ETBE is metabolized to *tert*-butanol and acetaldehyde. There is suggestive evidence that genetic polymorphisms of aldehyde dehydrogenase (ALDH)—the enzyme that oxidizes acetaldehyde to acetic acid—may affect ETBE toxicity. The virtually inactive form, ALDH2*2, is found in about one-half of all East Asians. Thus, exposure to ETBE in individuals with the ALDH2*2 variant would increase the internal dose of acetaldehyde, and potentially increase risks associated with acetaldehyde produced by ETBE metabolism. Several in vivo and in vitro genotoxic assays in Aldh2 knockout (KO) mice reported that genotoxicity was significantly increased compared with wild type controls following ETBE exposure to similar doses associated with cancer and noncancer effects (Weng et al., 2014; Weng et al., 2013; Weng et al., 2012; Weng et al., 2011). Inhalation ETBE exposure increased blood concentrations of acetaldehyde in Aldh2 knockout mice compared with wild type. Altogether, these data present evidence that diminished ALDH2 activity could yield more severe health effect outcomes in sensitive human populations.

Key Issues Addressed in Assessment

Sufficient data were available to develop a PBPK model in rats for both oral and inhalation exposure that could be used to perform route-to-route extrapolation; therefore, rat studies from both routes of exposure were considered for dose-response analysis. Analysis of the noncancer endpoint available from the chronic inhalation and oral studies led to very similar PODs and candidate references values when extrapolated across routes, so the route-specific chronic data were used as the basis for the RfC and RfD. With respect to carcinogenic effects, the only available inhalation 2-year study had the most robust evidence of carcinogenicity and was selected for route-to-route extrapolation.

ETBE induced an increase in α_{2u}-globulin deposition and increased hyaline droplet accumulation in male rats; however, most of the subsequent steps in the pathological sequence were not observed despite identical study conditions and doses in a number of experiments over a 2-year exposure period. These data fail to provide sufficient evidence that the α_{2u}-globulin process is operative. EPA finds that the data are insufficient to demonstrate α_{2u}-globulin nephropathy due to ETBE exposure; thus, the male rat kidney data are relevant for humans.

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

A literature search and screening strategy was used to identify literature characterizing the health effects of ETBE. This strategy consisted of a broad search of online scientific databases and other sources in order to identify all potentially pertinent studies. In subsequent steps, references were screened to exclude papers not pertinent to an assessment of the health effects of ETBE, and remaining references were sorted into categories for further evaluation. This section describes the literature search and screening strategy in detail.

The chemical-specific search was conducted in four online scientific databases, including PubMed, Toxline, Web of Science, and TSCATS through March, 2014, using the keywords and limits described in Table LS-1. The overall literature search approach is shown graphically in Figure LS-1. Another 114 citations were obtained using additional search strategies described in Table LS-2. After electronically eliminating duplicates from the citations retrieved through these databases, 808 unique citations were identified.

The resulting 808 citations were screened into categories as presented in Figure LS-1 using the title, abstract, and/or full text for relevance in examining the health effects of ETBE exposure.

- 31 references were identified as potential "Sources of Health Effects Data" and were considered for data extraction to evidence tables and exposure-response arrays.

- 51 references were identified as "Supporting Studies." These included 20 studies describing physiologically-based pharmacokinetic (PBPK) models and other toxicokinetic information; 16 studies providing genotoxicity and other mechanistic information; 9 acute, short term, or preliminary toxicity studies; 1 human toxicokinetic study; and 5 direct administration (e.g., dermal) studies of ETBE. While still considered sources of health effects information, studies investigating the effects of acute and direct chemical exposures are generally less pertinent for characterizing health hazards associated with chronic oral and inhalation exposures. Therefore, information from these studies was not considered for extraction into evidence tables. Nevertheless, these studies were still evaluated as possible sources of supporting health effects information.

- 16 references were identified as secondary sources of health effects information (e.g., reviews and other agency assessments); these references were kept as additional resources for development of the Toxicological Review.

- 710 references were identified as not being pertinent to an evaluation of health effects for ETBE and were excluded from further consideration (see Figure LS-1 for exclusion categories).

The complete list of references as sorted above can be found on the HERO website at http://hero.epa.gov/ETBE.

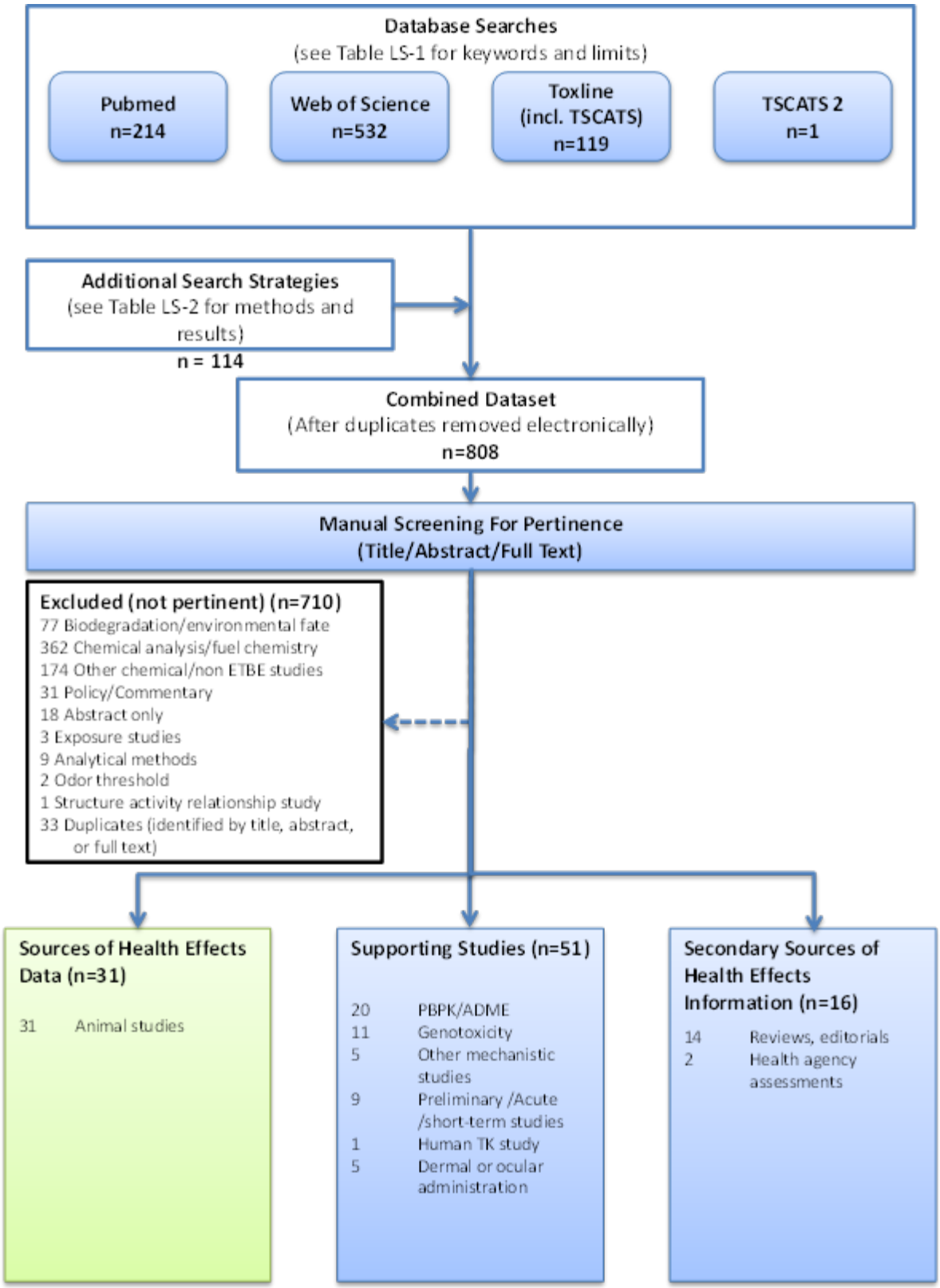


Figure LS-1. Literature search approach for ETBE

Table LS-1. Database search strategy for ETBE

Database (Search Date)	Keywords	Limits
PubMed (03/31/2014)	*"ETBE" OR "Ethyl tert-butyl ether" OR "2-ethoxy-2-methyl-propane" OR "ethyl tertiary butyl ether" OR "ethyl tert-butyl oxide" OR "tert-butyl ethyl ether" OR "ethyl t-butyl ether" OR "637-92-3"*	None
Web of Science (03/31/2014)	*"ETBE" OR "ethyl tert-butyl ether" OR "2-ethoxy-2-methyl-propane" OR "ethyl tertiary butyl ether" OR "ethyl tert-butyl oxide" OR "tert-butyl ethyl ether" OR "ethyl t-butyl ether" OR "637-92-3"*	Lemmatization on
Toxline (includes TSCATS) (03/31/2014)	*"ETBE" OR "Ethyl tert-butyl ether" OR "2-Ethoxy-2-methyl-propane" OR "ethyl tertiary butyl ether" OR "ethyl tert-butyl oxide" OR "tert-butyl ethyl ether" OR "ethyl t-butyl ether" OR "637-92-3"*	Not PubMed
TSCATS2 (3/31/2014)	637-92-3	01/01/2004 to 03/31/2014

Table LS-2. Summary of additional search strategies for ETBE

Approach used	Source(s)	Date performed	Number of additional references identified
Electronic backward search through Web of Science	Review article: McGregor (2007). "Ethyl tertiary-butyl ether: a toxicological review." Critical Reviews in Toxicology 37(4): 287−312	3/2014	68 references
	Review article: de Peyster (2010). "Ethyl t-butyl ether: Review of reproductive and developmental toxicity." Birth Defects Research, Part B: Developmental and Reproductive Toxicology 89(3): 239−263	3/2014	26 references
Personal communication	Japanese Petroleum Energy Center	3/2014	20 references

Selection of Critical Studies for Inclusion in Evidence Tables

Each study retained after the literature search and screen was evaluated for aspects of its design or conduct per the Preamble that could affect the interpretation of results and overall contribution to the evidence for determination of hazard potential. Much of the key information for conducting this evaluation can be determined based on study methods and how the study results were reported. Importantly, the evaluation at this stage does not consider the direction or magnitude of any reported effects.

To facilitate this evaluation, evidence tables were constructed that systematically summarized the important information from each study in a standardized tabular format as recommended by the NRC (2011). Thirty-one studies identified as "Sources of Health Effects" were considered for extraction into evidence tables for hazard identification in Chapter 1. Initial review of studies examining neurotoxic endpoints did not find consistent effects to warrant a comprehensive hazard evaluation; thus, the one subchronic study (Dorman et al., 1997) that examined neurotoxic endpoints only was not included in evidence tables. Data from the remaining 30 studies were extracted into evidence tables.

Supporting studies that contain pertinent information for the toxicological review and augment hazard identification conclusions—such as genotoxic and mechanistic studies, studies describing the kinetics and disposition of ETBE absorption and metabolism, pilot studies, and short-term or acute studies—were not included in the evidence tables. Such supporting studies may be discussed in the narrative sections of Chapter 1 or presented in Appendices if they provide additional or corroborating information.

Database Evaluation

The database for ETBE is comprised of animal toxicity studies containing three 2-year bioassays that employ oral and inhalation exposures in rats, and several studies with oral and inhalation exposures of ≥90 days in rats and mice. EPA externally peer-reviewed six unpublished technical reports prior to their subsequent publication: JPEC (2010a), JPEC, 2010b, JPEC, 2008a, JPEC, 2008c, and the pharmacokinetic studies JPEC (2008e) and JPEC (2008d). Several acute and short-term studies using oral and inhalation exposures were performed in rats but were grouped as supporting studies because the database of chronic and subchronic rat studies was considered most relevant for characterizing chronic health effects. No cohort studies, case reports, or ecological studies were found in the published literature. Health effect studies of gasoline and ETBE mixtures were not considered pertinent to the assessment because the separate effects of gasoline components could not be determined; thus, these studies were excluded during the manual screen. One controlled human exposure toxicokinetic study was identified, and this is discussed in Appendix B.2 (Toxicokinetics).

Some general questions that were considered in evaluating experimental animal studies are presented in Table LS-3. The "Sources of Health Effects Data" was comprised entirely of studies performed in rats, mice, and rabbits associated with drinking water, oral gavage, or inhalation exposures to ETBE. A large proportion of these 31 studies were conducted according to OECD Good Laboratory Practice (GLP) guidelines, presented extensive histopathological data, and provided clear presentation of the methodology; thus, these are considered high quality. Preliminary, acute, and short term studies contained information that supported but did not differ qualitatively from the results of the ≥90 day exposure studies; thus, these studies were not included in the evidence tables. Some of these shorter duration studies are presented in the text of the Toxicological Review and are described in sections such as the "Mechanistic Evidence" to augment the discussion. A more detailed discussion of methodological concerns that were identified will precede each endpoint evaluated in the hazard identification section.

Table LS-3. Questions and relevant experimental information for evaluation of experimental animal studies

Methodological feature	Question(s) considered	Examples of relevant information extracted
Test animal	Based on the endpoint(s) in question, are concerns raised regarding the suitability of the species, strain, or sex of the test animals on study?	Test animal species, strain, sex
Experimental setup	Are the timing, frequency and duration of exposure, as well as animal age and experimental group allocation procedures/ group size for each endpoint evaluation, appropriate for the assessed endpoint(s)?	Age/lifestage of test animals at exposure and all endpoint testing time points Timing and periodicity of exposure and endpoint evaluations; duration of exposure Sample size for each experimental group (e.g., animals; litters; dams) at each endpoint evaluation
Exposure	Are the exposure conditions and controls informative and reliable for the endpoint(s) in question, and are they sufficiently specific to the compound of interest?	Exposure administration techniques (e.g., route; chamber type)
Endpoint evaluation procedures	Do the procedures used to evaluate the endpoint(s) in question conform to established protocols, or are they biologically sound? Are they sensitive for examination of the outcome(s) of interest?	Specific methods for assessing the effect(s) of exposure, including related details (e.g., specific region of tissue/organ evaluated) Endpoint evaluation controls, including those put in place to minimize evaluator bias
Outcomes and data reporting	Were data reported for all pre-specified endpoint(s) and study groups, or were any data excluded from presentation/ analyses?	Data presentation for endpoint(s) of interest

Note: "Outcome" refers to findings from an evaluation (e.g., hypertrophy), whereas "endpoint" refers to the evaluation itself (e.g., liver histopathology).

1. HAZARD IDENTIFICATION

1.1. PRESENTATION AND SYNTHESIS OF EVIDENCE BY ORGAN/SYSTEM

1.1.1. Kidney Effects

Synthesis of Effects in Kidney

This section reviews the studies that investigated whether exposure to ETBE can cause kidney toxicity or cancer in humans or animals. The database examining kidney effects following ETBE exposure contains no human data, and 10 studies are performed in animals, predominantly rats. Studies employing short-term and acute exposures that examined kidney effects are not included in the evidence tables; however, they are discussed in the text if they provided data to support mode of action or hazard identification. EPA externally peer-reviewed six unpublished technical reports prior to their subsequent publication: JPEC (2010a), JPEC, 2010b, JPEC, 2008a, JPEC, 2008c, and the pharmacokinetic studies JPEC (2008g) and JPEC (2008f). No methodological concerns were identified that would lead one or more studies to be considered less informative for assessing human health hazard, although the report by Cohen et al. (2011) was not peer reviewed externally. This report (Cohen et al., 2011) consists of a pathology working group review commissioned by the Lyondell Chemical Company to reexamine kidney histopathology from the JPEC (2010a) [subsequently published as Suzuki et al. (2012)]and JPEC (2007) studies. All reanalysis was conducted in a blinded manner with the exception of the analysis of 2-year tumor data, data from low and intermediate doses in females, and data in all males from the control and high doses. Cohen et al. (2011) did not report different incidences of carcinomas than the original (Suzuki et al., 2012; JPEC, 2010a) study; thus, these data will not be presented twice. Histopathological results from both Cohen et al. (2011) and JPEC will be considered for hazard identification.

The kidney effects observed were increased organ weight, increased severity of histopathological lesions such as chronic progressive nephropathy (CPN), and urine and serum biomarkers (see Table 1-1, Table 1-2, Table 1-3; Figure 1-1, Figure 1-2). No statistically significant increases in renal tumors were observed in chronic bioassays (see Table 1-4). Kidney effects were not observed in the lone mouse study; however, lack of additional mouse studies precludes a conclusion on the species specificity of ETBE-induced kidney effects (Medinsky et al., 1999).

In most of the studies with data available for relative and absolute organ weight comparisons, relative kidney weights are increased to a greater extent than absolute kidney weights (Miyata et al., 2013; Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010b, 2008b, c; Gaoua, 2004b). Regression analysis indicates there is no discernible advantage to presenting absolute or

relative kidney weights (Bailey et al., 2004); thus, both absolute and relative weight were evaluated to make a determination of hazard. Absolute and relative kidney weights were dose-responsively increased in male and female rats following oral exposures of 16 weeks or longer (Fujii et al., 2010)(Miyata et al., 2013; JPEC, 2008c)(Suzuki et al., 2012; JPEC, 2010a). Absolute or relative kidney weight increases in rats were also dose-responsive following inhalation exposures of 13 weeks or longer (JPEC, 2008b)(Medinsky et al., 1999)(Saito et al., 2013; JPEC, 2010b). Short-term studies in rats also observed increased kidney weight (JPEC, 2008a).

The number and size of hyaline droplets were increased in the proximal tubules of male rats, but not females, and the hyaline droplets tested positive for the presence of α_{2u}-globulin (Miyata et al., 2013; JPEC, 2008c, e, f; Medinsky et al., 1999). The significance of this effect, along with other potentially related histopathological effects, such as necrosis, mineralization, and tubular hyperplasia, will be discussed in the succeeding section on Mode of Action.

The incidence of CPN, which was characterized by sclerosis of glomeruli, thickening of the renal tubular basement membranes, inflammatory cell infiltration and interstitial fibrosis, was not increased in any study as a result of ETBE exposure; however, the severity of CPN was exacerbated by ETBE in male and female rats in a 2-year inhalation study and in male rats in a 13-week drinking water study (see Table 1-2)(Cohen et al., 2011; (Saito et al., 2013; JPEC, 2010b); (JPEC), 2007). Increased incidence of urothelial hyperplasia was observed in male rats in two-year studies by both inhalation and oral exposure (Suzuki et al., 2012; JPEC, 2010a; (Saito et al., 2013; JPEC, 2010b). Cohen et al. (2011) attributed this effect to CPN rather than the "direct" result of ETBE treatment. The biological significance of this effect will be discussed in the succeeding Mode of Action Analysis.

The increased kidney weight and CPN in male rats is associated with several changes in urinary and serum biomarkers of renal function (see Table 1-3). CPN elicits a number of changes in urinary and blood serum measures such as proteinuria, blood urea nitrogen, creatinine, and hypercholesterolemia (Hard et al., 2009). Male rat blood concentrations of total cholesterol, blood urea nitrogen (BUN), and creatinine were elevated in 3, 2, and 1 out of 4 chronic and subchronic studies, respectively (Miyata et al., 2013; Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b, 2008c). With respect to female rats, cholesterol and BUN were elevated at the highest dose in one chronic inhalation study, which corresponded with increased CPN (Saito et al., 2013; JPEC, 2010b). The single instance of elevated proteinuria in male and female rats occurred in a chronic inhalation study (Saito et al., 2013; JPEC, 2010b).

The 2-year kidney weight data are not appropriate for hazard identification due the prevalence of age-associated confounders such as CPN and mortality that affect organ weight analysis. CPN is an age-associated disease characterized by cell proliferation and chronic inflammation that results in increased kidney weight (Melnick et al., 2012; Travlos et al., 2011). The majority (64–100%) of the male and female rats in the 2-year oral and inhalation studies were observed to have CPN regardless of ETBE administration (Saito et al., 2013; Suzuki et al., 2012;

JPEC, 2010a, b). In addition, mortality in the 2-year studies was significantly increased in ETBE-treated male and female rats compared with controls following oral and inhalation exposure (see Table 1-21). Causes of death were the result of age-associated diseases, such as CPN and tumors. Using kidney weight data from these 2-year studies would impart bias by selecting animals that survive to the end of the study for organ weight analysis. Thus, the 2-year organ weight data are not appropriate for hazard identification.

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Absolute Weight			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		100	5%
		300	8%
		1000	18%*
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		100	-2%
		300	0%
		1000	7%*

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Absolute Weight (continued)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, males and females (25/group/sex): via P0 dams in utero daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups	P0, Male	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 11%* 15%* 21%*
	F1, Male	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 10% 22%* 58%*
	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -1% 2% 5%
	F1, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 4% 3% 11%*
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks	Male	Dose(mg/kg-d) 0 1000	Percent change compared to control - 19%*

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Absolute Weight (continued)			
Miyata et al. (2013);JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	1%
		25	6%
		100	5%
		400	25%*
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	1%
		25	0%
		100	7%
		400	10%*
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	-4%
		121	5%
		542	18%*
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	3%
		171	10%*
		560	14%*

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Absolute Weight (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	10%
		2090	11%
		6270	18%*
		20,900	16%*
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	1%
		2090	-1%
		6270	4%
		20,900	7%
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		20,900	19%
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		20,900	8%

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Absolute Weight (continued)			
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	7%
		7320	10%*
		20,900	19%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	4%
		7320	12%*
		20,900	21%*
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation - vapor female (40/group): 0, 500, 1750, 5000 ppm(0, 2090, 7320, 20,900 mg/m^3)[b]; male (40/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	9%
		7320	10%
		20,900	5%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	0%
		7320	6%
		20,900	4%

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Absolute Weight (continued)			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	8%*
		6270	17%*
		20,900	22%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	5%
		6270	6%*
		20,900	18%*
Kidney: Relative Weight			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		100	8%*
		300	12%*
		1000	26%*
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		100	-3%
		300	-1%
		1000	2%

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Relative Weight (continued)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, males and females (25/group/sex): via P0 dams in utero daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	11%*
		500	18%*
		1000	28%*
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	10%*
		500	19%*
		1000	58%*
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		250	9%
		500	5%
		1000	3%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Female	0	-
		250	6%
		500	6%
		1000	10%*
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		1000	25%*

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Relative Weight (continued)			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	8%
		25	6%
		100	12%*
		400	21%*
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	7%
		25	4%
		100	11%*
		400	15%*
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		28	0%
		121	12%*
		542	31%*
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		46	13%*
		171	22%*
		560	37%*

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Relative Weight (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	10%
		2090	9%
		6270	20%*
		20,900	24%*
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	8%
		2090	7%
		6270	12%*
		20,900	20%*
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		20,900	15%*
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		20,900	5%

Table 1-1. Evidence pertaining to kidney weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Kidney: Relative Weight (continued)			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	19%*
		6270	26%*
		20,900	66%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	11%*
		6270	16%*
		20,900	51%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).

Table 1-2. Evidence pertaining to kidney nephropathy and histopathological effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Incidence of Chronic Nephropathy			
Cohen et al. (2011) rat, F344/DuCrlCrlj oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] reanalysis of the histopathology from JPEC (2010a) study where animals were dosed daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	49/50
		28	-
		121	-
		542	50/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	45/50
		46	41/50
		171	46/50
		560	46/50
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	49/50
		28	43/50
		121	45/50
		542	48/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	41/50
		46	37/50
		171	37/50
		560	39/50
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	49/50
		2090	50/50
		6270	49/49
		20,900	50/50
		Dose(mg/m^3)	Response (incidence)
	Female	0	32/50
		2090	38/50
		6270	41/50
		20,900	40/50

Table 1-2. Evidence pertaining to kidney nephropathy and histopathological effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Average Severity of Chronic Nephropathy			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (severity)
	Male	0	2.1
		28	2
		121	2
		542	2.4
		Dose(mg/kg-d)	Response (severity)
	Female	0	1.2
		46	1.2
		171	1.5
		560	1.5
Cohen et al. (2011) rat, F344/DuCrlCrlj oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] reanalysis of the histopathology from JPEC 2010 (HERO ID 1561279) study where animals were dosed daily for 104 wks		Dose(mg/kg-d)	Response (severity)
	Male	0	2.08
		28	-
		121	-
		542	2.72
		Dose(mg/kg-d)	Response (severity)
	Female	0	1.14
		46	0.98
		171	1.2
		560	1.36
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (severity)
	Male	0	2.4
		2090	2.6
		6270	2.7
		20,900	3.1*
		Dose(mg/m^3)	Response (severity)
	Female	0	0.9
		2090	1.3
		6270	1.3
		20,900	1.6*

Table 1-2. Evidence pertaining to kidney nephropathy and histopathological effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Average Severity of Chronic Nephropathy as Calculated by EPA			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (severity)
	Male	0	2.1
		28	1.7
		121	1.8
		542	2.3
	Average severity calculated as (grade x # of affected animals)/total # of animals exposed		
		Dose(mg/kg-d)	Response (severity)
	Female	0	1
		46	0.9
		171	1.1
		560	1.2
	Average severity calculated as (grade x # of affected animals)/total # of animals exposed		
Number of CPN Foci			
Cohen et al. (2011) rat, F344/DuCrlCrlj oral - water male (10/group): 0, 250, 1600, 4000, 10000 ppm reanalysis of the histopathology from JPEC 2006 (study No. 0665) study where animals were dosed daily for 13 weeks		Dose(ppm)	Response (foci/rat)
	Male	0	1.2
		250	-
		1600	-
		4000	-
		10000	27.2

Table 1-2. Evidence pertaining to kidney nephropathy and histopathological effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Slight Urothelial Hyperplasia of the Renal Pelvis			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/50
		28	0/50
		121	10/50*
		542	25/50*
	Female urothelial hyperplasia of the renal pelvis not observed		
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	2/50
		2090	5/50
		6270	16/49*
		20,900	41/50*
	Female urothelial hyperplasia of the renal pelvis not observed		
Incidence of Atypical Tubule Hyperplasia			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/50
		28	0/50
		121	0/50
		542	1/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/50
		46	0/50
		171	0/50
		560	2/50

Table 1-2. Evidence pertaining to kidney nephropathy and histopathological effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Incidence of Atypical Tubule Hyperplasia (continued)			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male atypical tubule hyperplasia not observed		
	Female atypical tubule hyperplasia not observed		
Incidence of Papillary Mineralization			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/15
		5	0/15
		25	0/15
		100	1/15
		400	0/15
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/15
		5	-
		25	-
		100	-
		400	0/15
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/50
		28	0/50
		121	16/50*
		542	42/50*
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/50
		46	0/50
		171	1/50
		560	3/50

Table 1-2. Evidence pertaining to kidney nephropathy and histopathological effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Incidence of Papillary Mineralization (continued)			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	0/50
		2090	0/50
		6270	1/49
		20,900	6/50*
Incidence of Papillary Necrosis			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/50
		28	1/50
		121	0/50
		542	2/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/50
		46	1/50
		171	1/50
		560	2/50
Proximal Tubule Proliferation			
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	137%*
		7320	274%*
		20,900	171%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	73%
		7320	64%
		20,900	47%

[a]Conversion performed by study authors.

[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Blood Urea Nitrogen (BUN)			
Miyata et al. (2013);JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	12%
		25	1%
		100	4%
		400	8%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	-5%
		25	-7%
		100	-1%
		400	4%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	3%
		121	20%*
		542	43%*
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	-8%
		171	-5%
		560	-5%

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Blood Urea Nitrogen (BUN) (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	-9%
		2090	-5%
		6270	4%
		20,900	4%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	-5%
		2090	3%
		6270	-8%
		20,900	-4%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	41%*
		6270	45%*
		20,900	179%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	10%
		6270	4%
		20,900	30%*

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Cholesterol			
Miyata et al. (2013);JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	-5%
		25	21%
		100	12%
		400	53%*
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	-7%
		25	-7%
		100	-2%
		400	3%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	-11%
		121	10%
		542	31%*
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	-2%
		171	12%
		560	8%

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Cholesterol (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	8%
		2090	9%
		6270	26%
		20,900	15%
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	7%
		2090	9%
		6270	11%
		20,900	21%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	10%
		6270	29%*
		20,900	52%*
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	-3%
		6270	-4%
		20,900	53%*

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Creatinine			
Miyata et al. (2013);JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	0%
		25	-10%
		100	-3%
		400	0%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	-19%
		25	-12%
		100	-16%
		400	-16%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	0%
		121	17%
		542	17%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	0%
		171	-17%
		560	0%

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Creatinine (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	-13%
		2090	-6%
		6270	-6%
		20,900	-3%
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	0%
		2090	3%
		6270	-9%
		20,900	-9%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	14%*
		6270	29%*
		20,900	71%*
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	0%
		6270	0%
		20,900	0%

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Incidence of Proteinuria			
Miyata et al. (2013);JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days	Male	Dose(mg/kg-d)	Response
		0	10/10
		5	10/10
		25	10/10
		100	10/10
		400	10/10
	Female	Dose(mg/kg-d)	Response
		0	8/10
		5	9/10
		25	7/10
		100	9/10
		400	7/10
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male	Dose(mg/kg-d)	Response
		0	39/39
		28	37/37
		121	34/34
		542	35/35
	Female	Dose(mg/kg-d)	Response
		0	37/37
		46	37/37
		171	38/38
		560	38/38
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Response
		0	3/6
		627	5/6
		2090	5/6
		6270	6/6
		20,900	4/6
	Female	Dose(mg/m^3)	Response
		0	1/6
		627	1/6
		2090	1/6
		6270	2/6
		20,900	2/6

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Incidence of Proteinuria (continued)			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	44/44
		2090	38/38
		6270	40/40
		20,900	31/31
		Dose(mg/m^3)	Response (incidence)
	Female	0	33/38
		2090	39/39
		6270	30/30
		20,900	30/30
Severity of Proteinuria[c]			
Miyata et al. (2013);JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	7%
		25	7%
		100	-13%
		400	0%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	8%
		25	-17%
		100	8%
		400	-17%

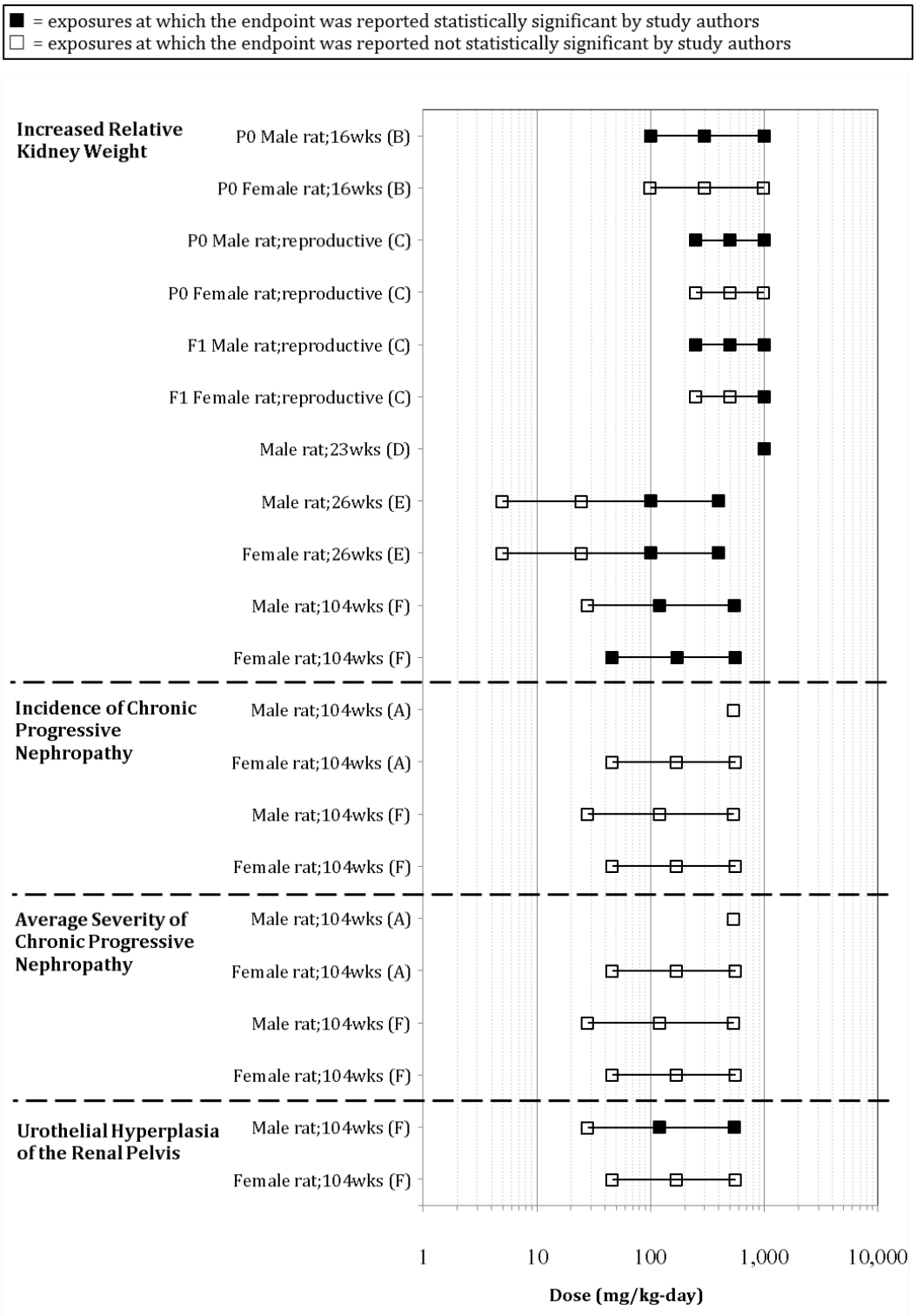
Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Severity of Proteinuria (continued)[c]			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	3%
		121	3%
		542	3%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	7%
		171	7%
		560	11%
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	140%
		2090	140%
		6270	160%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	50%
		2090	0%
		6270	150%
		20,900	50%

Table 1-3. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE (*continued*)

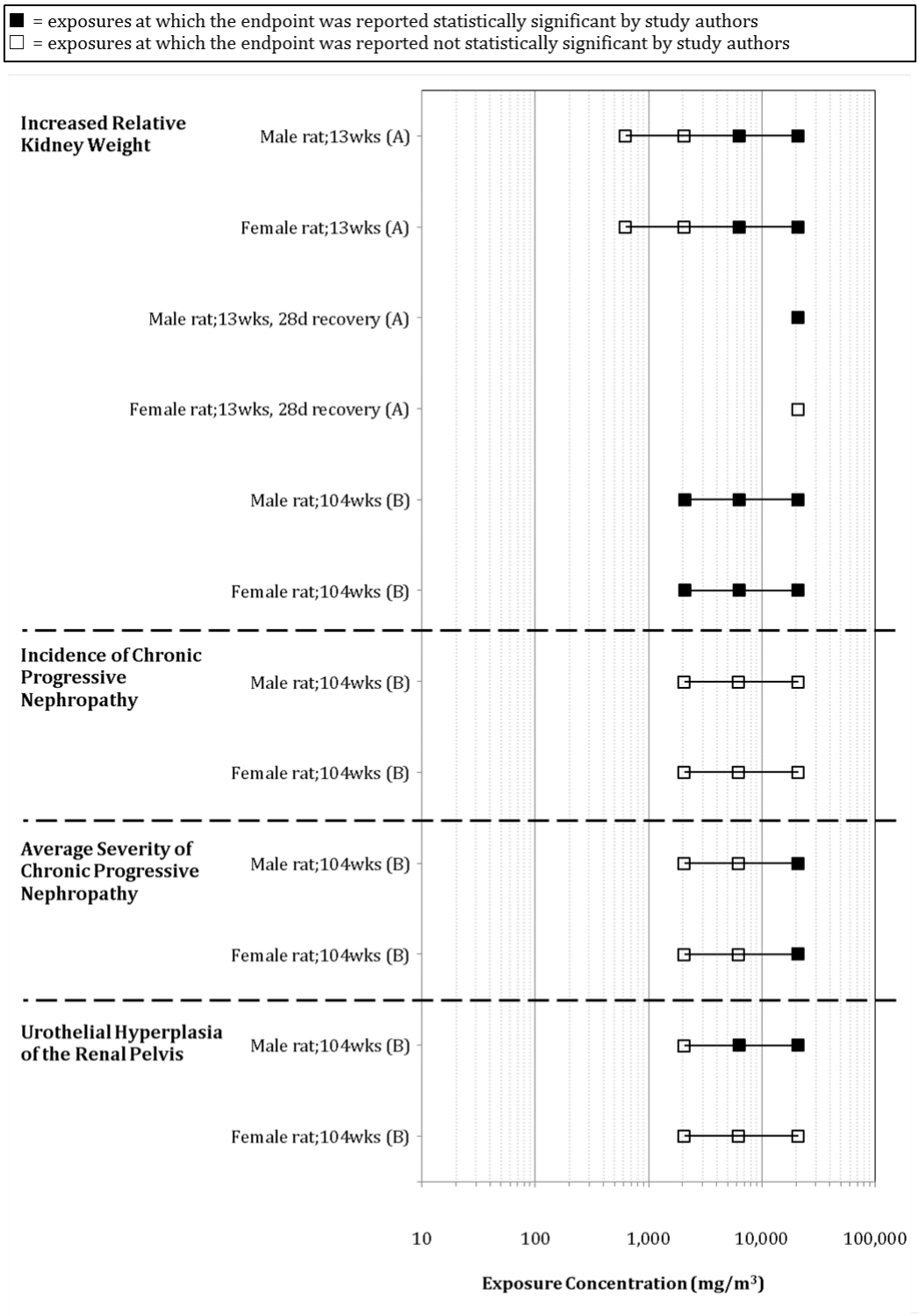
Reference and Dosing Protocol	Results by Endpoint		
Severity of Proteinuria (continued)[c]			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	-5%
		6270	-3%
		20,900	-3%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	11%
		6270	18%
		20,900	21%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
[c]Severity of proteinuria= (1* number of animals with "1+") + (2*number of animals with "2+") + (3 * number of animals with "3+") + (4 * number of animals with "4+")/ total number of animals in group
NR: not reported; *: result is statistically significant (p<0.05) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).



Sources: (A) Cohen et al., 2011 reanalysis of JPEC, 2010a; (B) Fujii et al., 2010; JPEC, 2008e; (C) Gaoua, 2004b; (D) Hagiwara et al., 2011; (E) Miyata et al., 2013; JPEC, 2008c; (F) Suzuki et al., 2012; JPEC, 2010a

Figure 1-1. Exposure-response array of kidney effects following oral exposure to ETBE.



Sources: (A) JPEC, 2008b; (B) Saito et al., 2013; JPEC, 2010b

Figure 1-2. Exposure-response array of kidney effects following inhalation exposure to ETBE.

Table 1-4. Evidence pertaining to kidney tumor effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Renal Cell Carcinoma			
Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d 4 d/wk for 104 wks; observed until natural death		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/60
		250	0/60
		1000	0/60
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	0/60
		1000	0/60
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/50
		28	0/50
		121	0/50
		542	1/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/50
		46	0/50
		171	0/50
		560	1/50
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	0/50
		2090	1/50
		6270	0/49
		20,900	0/50
	Female none were observed		

Table 1-5. Evidence pertaining to kidney tumor promotion by ETBE in animals

Reference and Dosing Protocol		Results by Endpoint	
Renal Transitional Cell Carcinoma			
Hagiwara et al. (2011); JPEC (2008d)		Dose(mg/kg-d)	Response (incidence)
rat, Fischer 344			
oral - gavage	Male	0	1/30
male (12/group): 0, 1000 mg/kg-d		300	0/30
daily for 23 weeks		1000	2/30
[+] no DMBB initiation		0[+]	0/12
		1000[+]	0/12
Renal Tubular Adenoma or Carcinoma			
Hagiwara et al. (2011); JPEC (2008d)		Dose(mg/kg-d)	Response (incidence)
rat, Fischer 344			
oral - gavage	Male	0	11/30
male (30/group): 0, 300, 1000 mg/kg-d		300	6/30
daily for 23 weeks following a 4 week tumor		1000	13/30
initiation by DMBDD		0[+]	0/12
[+] no DMBB initiation		1000[+]	0/12

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant (p<0.05) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
(n): number evaluated from group

Mode of Action Analysis-Kidney Effects

Toxicokinetic considerations relevant to kidney toxicity

ETBE is metabolized by cytochrome P450 (CYP) enzymes to an unstable hemiacetal that decomposes spontaneously into *tert*-butanol and acetaldehyde (Bernauer et al., 1998). Acetaldehyde is further metabolized in the liver and is not thought to play a role in extrahepatic toxicity. The main circulating metabolite is *tert*-butanol, which is filtered from the blood by the kidneys and excreted in urine. Thus, following ETBE exposure, the kidney is exposed to significant concentrations of *tert*-butanol, and kidney effects caused by *tert*-butanol (described in the more detail in the draft IRIS assessment of *tert*-butanol) are also relevant to evaluating the kidney effects observed after ETBE exposure. In particular, similar to ETBE, *tert*-butanol has been reported to causes nephrotoxicity in rats, including effects associated with α_{2u}-globulin nephropathy. However, unlike ETBE, increased renal tumors were reported following chronic drinking water exposure to *tert*-butanol.

α_{2u}-Globulin-related nephropathy

Description of the hypothesized MOA

In the case of male rats treated with ETBE, α_{2u}-globulin was confirmed in the hyaline droplets from multiple studies (Miyata et al., 2013; JPEC, 2008b, c; Medinsky et al., 1999). α_{2u}-Globulin is derived from hepatic synthesis and can be chemically induced to accumulate in the proximal tubule as the result of impaired renal catabolism (U.S. EPA, 1991a). In the context of noncancer kidney toxicity observed after ETBE exposure, this accumulation could lead to various types of nephropathy, including chronic proliferation of the renal tubule epithelium and possibly exacerbation of CPN (U.S. EPA, 1991a).

U.S. EPA (1991a) has described the hypothesized sequence of events in α_{2u}-globulin-associated nephropathy. Chemicals that induce α_{2u}-globulin accumulation do so rapidly. The accumulation of α_{2u}-globulin in the hyaline droplets results in hyaline droplet deposition in the P2 segment of the proximal tubule within 24 hours of exposure. As hyaline droplet deposition continues, single-cell necrosis occurs in the P2 segment which leads to exfoliation of these cells into the tubule lumen within 5 days of chemical exposure. In response to the cell loss, cell proliferation is observed in the P2 segment after 3 weeks and continues for the duration of the exposure. After 2 or 3 weeks of exposure, the cell debris accumulates in the P3 segment of the proximal tubule to form granular casts. Continued chemical exposure for 3 to 12 months leads to the formation of calcium hydroxyapatite in the papilla which results in linear mineralization. After 1 or more years of chemical exposure, these lesions may result in the induction of renal adenomas and carcinomas.

U.S. EPA (1991a) states that two questions must be addressed to determine the extent to which α_{2u}-globulin-mediated processes induce renal effects. First, it must be determined whether or not the α_{2u}-globulin process is occurring in male rats, and therefore could be a factor in renal effects. Because ETBE has not been found to cause kidney tumors in male rats, the second question as to whether the renal effects are solely due to the α_{2u}-globulin process, are a combination of the α_{2u}-globulin process and other carcinogenic processes, or are due primarily to other processes, is not pertinent to this MOA analysis. However, U.S. EPA (1991a) states that if the α_{2u}-globulin process is occurring in male rats, then the associated nephropathy in male rats (described above) would not be an appropriate endpoint to determine noncancer effects occurring in humans due to the specificity of the protein to male rats. In such a case, the characterization of human health hazard for renal toxicity would need to rely on data on other types of nephrotoxic effects in male rats and/or on nephrotoxic effects in female rats or other species.

Based on the information above, the MOA analysis for ETBE-induced renal effects are focused only on the first question of whether or not the α_{2u}-globulin process is occurring in male rats. U.S. EPA (1991a) describes the criteria for determining this as follows:

(1) hyaline droplets are increased in size and number in male rats,

(2) the protein in the hyaline droplets in male rats is α_{2u}-globulin, and

(3) several (but not necessarily all) additional steps in the pathological sequence are present in male rats, such as:

(a) single-cell necrosis,

(b) exfoliation of epithelial cells into the tubular lumen,

(c) granular casts,

(d) linear mineralization, and

(e) tubule hyperplasia.

The available data in male rats will be evaluated in accordance with the MOA framework from the EPA cancer guidelines (U.S. EPA, 2005a). These data are summarized in

Table 1-7 and Figure 1-3 and Figure 1-4.

Table 1-6. Additional kidney effects potentially relevant to mode of action in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Incidence of Hyaline Droplets			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/15
		5	0/15
		25	0/15
		100	4/15*
		400	10/15*
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/15
		5	-
		25	-
		100	-
		400	0/15
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male no hyaline droplets observed		
	Female no hyaline droplets observed		
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male no hyaline droplets observed		
	Female no hyaline droplets observed		

Table 1-6. Additional kidney effects potentially relevant to mode of action in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Incidence of Hyaline Droplets in the Proximal Tube Epithelium			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	0/10
		627	3/10
		2090	8/10*
		6270	8/10*
		20,900	8/10*
	Female no hyaline droplets observed in proximal tubule		
Average Hyaline Droplet Severity			
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (severity)
	Male	0	1.8
		2090	3
		7320	3.2
		20,900	3.8
	Female no hyaline droplets observed		
Incidence of Hyaline Droplets Positive for α_{2u}-globulin			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/1
		5	-
		25	-
		100	2/2
		400	1/1
	Female Incidence of hyaline droplets positive for α_{2u}-globulin not examined in females		
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150,	Male unspecified representative samples reported as "weakly positive" for α_{2u}-globulin		
	Female		

Table 1-6. Additional kidney effects potentially relevant to mode of action in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint
500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	hyaline droplets positive for α_{2u} -globulin not examined in females

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).

Table 1-7. Summary of data informing whether the α_{2u}-globulin process is occurring in male rats exposed to ETBE

Criterion	Duration	Results	Reference
(1) hyaline droplets are increased in size and number	1 wk	(+)	Medinsky et al. (1999)
	4 wk	(+)	Medinsky et al. (1999)
	13 wk	(+)	Medinsky et al. (1999)
	13 wk	+	JPEC (2008b)
	26 wk	+	Miyata et al. (2013); JPEC (2008c)
	104 wk	–	Suzuki et al. (2012)
	104 wk	–	Saito et al. (2013); JPEC (2010b)
(2) the protein in the hyaline droplets is α_{2u}-globulin	1 wk	(+)[a]	JPEC (2008b)
	4 wk	(+)[a]	Medinsky et al. (1999)
	13 wk	(+)[a]	Medinsky et al. (1999)
	13 wk	(+)[a]	JPEC (2008b)
	26 wk	(+)[b]	Miyata et al. (2013); JPEC (2008c)
(3) Several (but not necessarily all) additional steps in the pathological sequence are present in male rats, such as:			
(a) single-cell necrosis	13 wk	–	JPEC (2008b)
	13 wk	–	Medinsky et al. (1999)
	26 wk	–	Miyata et al. (2013); JPEC (2008c)
	104 wk	–	(Suzuki et al., 2012; JPEC, 2010a)
	104 wk	–	Saito et al. (2013); JPEC (2010b)
(b) exfoliation of epithelial cells into the tubular lumen	13 wk	–	JPEC (2008b)
	13 wk	–	Medinsky et al. (1999)
	26 wk	–	Miyata et al. (2013); JPEC (2008c)
	104 wk	–	(Suzuki et al., 2012; JPEC, 2010a)
	104 wk	–	Saito et al. (2013); JPEC (2010b)
(c) granular casts	13 wk	–	JPEC (2008b)
	13 wk	(+)	Cohen et al. (2011)
	13 wk	–	Medinsky et al. (1999)
	26 wk	–	Miyata et al. (2013); JPEC (2008c)
	104 wk	–	(Suzuki et al., 2012; JPEC, 2010a)
	104 wk	–	Saito et al. (2013); JPEC (2010b)
(d) linear mineralization	13 wk	–	JPEC (2008b)
	13 wk	–	Medinsky et al. (1999)
	26 wk	–	Miyata et al. (2013); JPEC (2008c)
	104 wk	+	(Suzuki et al., 2012; JPEC, 2010a) Cohen et al. (2011)
	104 wk	+	Saito et al. (2013); JPEC (2010b)
(e) tubule hyperplasia	13 wk	–	JPEC (2008b)
	13 wk	+/–[c]	Medinsky et al. (1999)
	26 wk	–	Miyata et al. (2013); JPEC (2008c)
	104 wk	–	(Suzuki et al., 2012; JPEC, 2010a)
	104 wk	–	Saito et al. (2013); JPEC (2010b)

+ = Statistically significant change reported in one or more treated groups.
(+) = Effect was reported in one or more treated groups, but statistics not reported.
– = No statistically significant change reported in any of the treated groups.
[a]Unspecified "representative samples" examined.

[b]Three samples from highest two dose groups examined.
[c]Labeling index statistically significantly increased, but no hyperplasia reported.

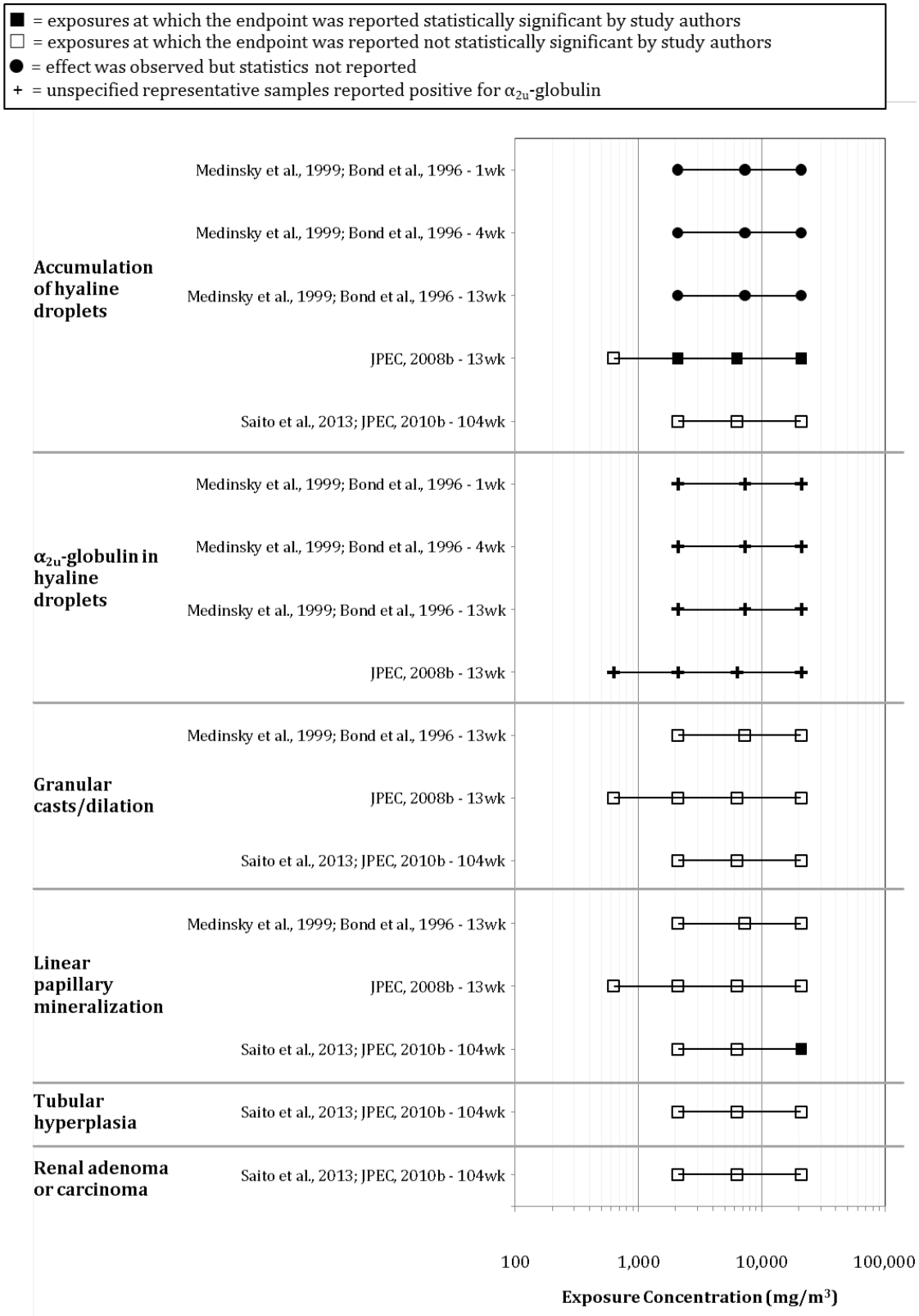


Figure 1-3. ETBE inhalation exposure array of α_{2u}-globulin data in male rats

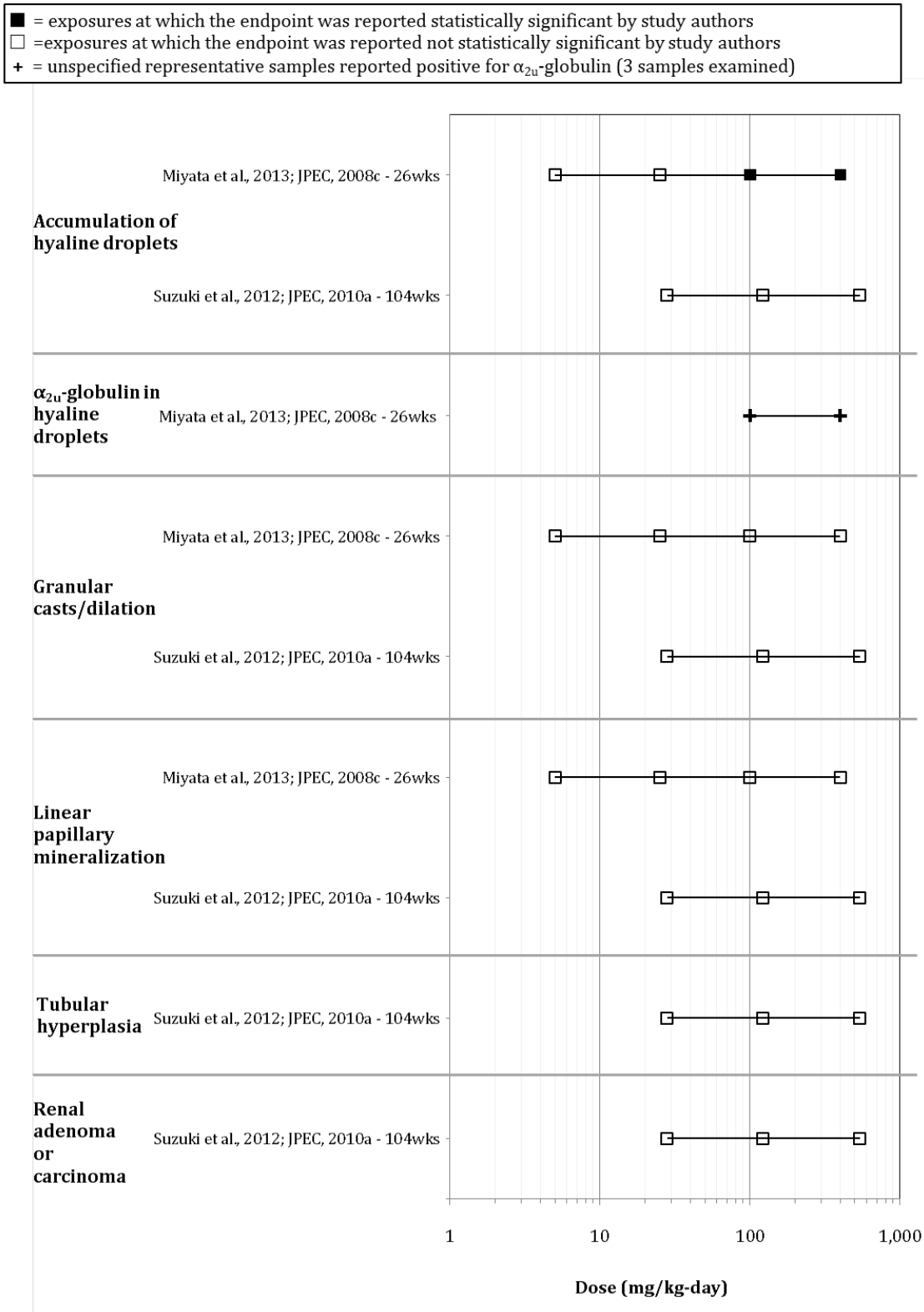


Figure 1-4. ETBE oral exposure array of α_{2u}-globulin data in male rats

Strength, consistency, and specificity of association

The first criterion to consider in determining if the α_{2u}-globulin process is occurring is whether or not hyaline droplets are increased in size and number in male rats. The accumulation of hyaline droplets was observed in all three subchronic ETBE exposure studies, but was not observed in two chronic ETBE studies (see Table 1-6). Accumulation of hyaline droplets in the proximal tubular epithelium of the kidney was observed in 8 of 10 male rats at the 3 highest exposure concentrations of ETBE compared with 0 of 10 in control rats following 90-day inhalation exposure. The increases at these 3 doses were statistically significant; however, none of the animals had hyaline droplet grades over 1 (JPEC, 2008b). Hyaline droplets were statistically significantly increased in 4 of 15 (all grade 1 severity) and 10 of 15 (5 of each grade 1 and 2 severity) male rats at the two highest doses of ETBE, respectively, compared with 0 of 15 controls following oral exposure for 180 days (Miyata et al., 2013; JPEC, 2008c). Finally, a 90-day inhalation ETBE exposure study reported an increase in the grade of hyaline droplets as indicated by severity grades of 1.8, 3.0, 3.2, and 3.8 in the control and 3 ETBE dose groups, respectively (Medinsky et al., 1999).

The second criterion in determining occurrence of the α_{2u}-globulin process is whether the protein in the hyaline droplets in male rats is α_{2u}-globulin. Immunohistological staining to ascertain the protein composition in the hyaline droplets was only performed in ETBE exposure studies that observed accumulation of hyaline droplets. At the two highest doses, (Miyata et al. (2013); JPEC, 2008c) identified hyaline droplets as positive for α_{2u}-globulin in 2/2 and 1/1 animals that were tested for the presence of α_{2u}-globulin. The other two studies also reported that unspecified samples were positive for α_{2u}-globulin (JPEC, 2008b; Medinsky et al., 1999). JPEC (2008b) reported that the samples stained weakly positive for α_{2u}-globulin and that positive α_{2u}-globulin staining was only observed in male rats. No statistical tests were performed on any of these results.

The third criterion in determining occurrence of the α2u-globulin process considers the presence of additional steps in the pathological sequence in male rats (refer to

Table 1-7). The incidence of papillary mineralization was statistically significantly increased in both of the 2-year studies. In the drinking water study, incidence of mineralization was increased from 0/50 in the control animals to 16/50 and 42/50 in the 121- and 542-mg/kg-day dose groups, respectively (Suzuki et al., 2012; JPEC, 2010a). Cohen et al. (2011) further reported that the observed mineralization in (Suzuki et al., 2012; JPEC, 2010a) was linear mineralization. In the inhalation study, incidence of mineralization was 6/50 in the 20,900-mg/m^3 group compared with 0/50 in the control group (Saito et al., 2013; JPEC, 2010b). However, single-cell necrosis, exfoliation of epithelial cells into the tubular lumen, granular casts, and tubule hyperplasia were either absent or not consistently observed across studies. Cohen et al. (2011) reported that at 13 weeks, granular casts were observed in high dose males, while none were observed in controls (no statistical tests performed). Other studies did not report the presence of granular casts. Medinsky et al. (1999) reported increased labeling indices indicative of tubular proliferation, but no hyperplasia, after 1 to 13 weeks of exposure. However, both males and females showed statistically significant increases at shorter durations, and both sexes had elevated labeling indices at 13 weeks, though only the males were statistically significantly increased. Moreover, increased hyperplasia was not observed in any other studies.

In summary, the evidence supports ETBE causing hyaline droplets to be increased in size and number and the accumulating protein being α_{2u}-globulin, but only one of the additional steps in the pathological sequence was consistently observed (linear papillary mineralization), and only after exposure for 2 years. Overall, the strength, consistency, and specificity of the association between ETBE and the hypothesized key events is weak.

Dose-response concordance

The accumulation of hyaline droplets was dose responsive in the 90-day inhalation ETBE exposure study. Hyaline droplets were observed in 0/10, 3/10, 8/10, 8/10, and 8/10 at 0, 627, 2,090, 6,270, and 20,900 mg ETBE/m^3, respectively (JPEC, 2008b). In addition, the incidence of hyaline droplets was dose responsive after a 26-week gavage as indicated by droplets in 0/15, 0/15, 0/15, 4/15, and 10/15 at 0, 5, 25, 100, and 400 mg ETBE/kg-day, respectively (Miyata et al., 2013; JPEC, 2008c). Finally, severity grade of the hyaline droplets exhibited a dose response after a 1-week exposure as indicated by scores of 1.2, 3.4, 4.0, and 4.6 at 0, 2090, 7320, and 20,900 mg ETBE/m^3, respectively (Medinsky et al., 1999).

The available studies that tested for α_{2u}-globulin in the hyaline droplets did not test a sufficient number of samples within a dose group nor were enough dose groups tested for α_{2u}-globulin to perform dose-response analysis. All three studies that tested for α_{2u}-globulin failed to report the actual number of positive samples. For these reasons, no dose response concordance can be established between accumulation of hyaline droplets and α_{2u}-globulin accumulation.

Papillary mineralization was dose-responsively increased following oral ETBE exposure in 0/50, 0/50, 16/50, and 42/50 male rats at doses of 0, 28, 121, and 542 mg/kg-day, respectively (Suzuki et al., 2012; JPEC, 2010a), and in 0/50, 0/50, 1/49, and 6/50 males at ETBE inhalation concentrations of 0, 2090, 6270, and 20,900 mg/m^3 (Saito et al., 2013; JPEC, 2010b). Based on the above data, hyaline droplet deposition was observed at a similar frequency as mineralization following oral ETBE exposure ((Suzuki et al., 2012; JPEC, 2010a); Miyata et al., 2013; JPEC, 2008c); however, hyaline droplet deposition was observed in 80% of animals at the 3 highest inhalation exposure concentrations (JPEC, 2008b) compared with mineralization rates of 0, 2, and 12% at the corresponding doses (Saito et al., 2013; JPEC, 2010b).

Although these results suggest that mineralization is dose responsive following either oral or inhalation ETBE exposure, a stronger dose-response concordance between mineralization and hyaline droplet deposition was observed for oral exposures. Furthermore, as discussed above, the additional steps in the pathological sequence were not observed, so overall there is only weak evidence of dose-response concordance among the hypothesized key events.

Temporal relationship

The accumulation of hyaline droplets is the first endpoint that is observed in α_{2u}-globulin-mediated nephropathy that may occur within 24 hours post-exposure. Droplets were increased after 1, 4, 13, and 26 weeks of exposure (Miyata et al., 2013; JPEC, 2008b, c; Medinsky et al., 1999). Confirmation of α_{2u}-globulin in the droplets was reported after 13 weeks (JPEC, 2008b). Failure to observe α_{2u}-globulin and increased droplet accumulation in the 2-year studies is not unusual because α_{2u}-globulin naturally declines in males around 5 months of age.

Of the other endpoints in the pathological sequence, only papillary mineralization was observed. Mineralization was reported after 2-year oral and inhalation exposures but not in any study employing a shorter exposure. Endpoints such as necrosis, exfoliation of epithelial cells into the tubular lumen, granular casts, and hyperplasia were not observed at the expected subchronic and chronic time points. Due to the absence of the other key effects at the critical time points in the α_{2u}-globulin-mediated pathological sequence, the evidence for temporal relationship among the hypothesized key events is weak.

Biological plausibility and coherence

Both EPA and IARC have accepted the biological plausibility of the α_{2u}-globulin-mediated hypothesis for inducing nephropathy and cancer in male rats (Swenberg and Lehman-McKeeman, 1999; U.S. EPA, 1991a), and those rationales will not be repeated here. More recent retrospective analysis indicates that several steps in the sequence of pathological events are not required for tumor development.

A retrospective analysis has demonstrated that a number of α_{2u}-globulin-inducing chemicals fail to induce many of the pathological sequences in the α_{2u}-globulin pathway (Doi et al., 2007). For

instance, dose-response concordance was not observed for several endpoints such as linear mineralization, tubular hyperplasia, granular casts, and hyaline droplets following exposure to α_{2u}-globulin-inducing chemicals such as d-limonene, decalin, propylene glycol mono-t-butyl ether, and Stoddard solvent IICA (SS IICA). Although some of these chemicals induced dose-responsive effects for a few endpoints, all of them failed to induce a dose response for at least one of the endpoints in the sequence. Furthermore, no endpoint in the pathological sequence was predictive for tumor incidence when considering either the dose responsiveness or the severity. Tumor incidence was not dose responsive following either d-limonene or decalin exposure. Tumor incidence was not correlated with the severity of any one effect in the α_{2u}-globulin sequence as demonstrated by SS IIC which induced some of the most severe nephropathy relative to the other chemicals, but did not significantly increase kidney tumors (Doi et al., 2007). Thus, this analysis suggests that another MOA may be operative for inducing tumors in male rats.

As described above, ETBE is metabolized to *tert*-butanol, so kidney data following *tert*-butanol exposure is also potentially relevant to evaluating the MOA of ETBE. In particular, the effects of *tert*-butanol on α_{2u}-globulin are relevant for evaluating the coherence of the available data on ETBE-induced nephropathy.

Hyaline droplet deposition and linear mineralization were both observed following similar exposure durations to *tert*-butanol and ETBE. After 13 weeks of exposure to *tert*-butanol or ETBE, hyaline droplets were dose-responsively increased. ETBE exposure increased hyaline droplets at lower internal concentrations of *tert*-butanol than by direct *tert*-butanol administration. Similar to hyaline droplets, linear mineralization was increased at an internal *tert*-butanol concentration approximately tenfold lower following ETBE exposure than *tert*-butanol exposure.

Tubule hyperplasia and renal tumors were both observed following 2-year exposure to *tert*-butanol but not ETBE. Tubule hyperplasia occurred at an internal concentration of *tert*-butanol that was similar to the blood concentrations of *tert*-butanol following ETBE exposure (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010b). Similarly, the incidence of renal tumors was increased at three internal concentrations of *tert*-butanol that were achieved in two separate ETBE studies. The failure of internal *tert*-butanol concentrations to induce histopathological lesions early in the α_{2u}-globulin pathological sequence at blood levels that later induced hyperplasia and tumors suggests a lack of coherence across the two data sets.

With regard to the discrepancy in renal tumors between ETBE and *tert*-butanol, it should be noted that the background renal tumor rate in the *tert*-butanol exposure study was high compared with historical values. Renal tumors in the NTP (1995) chronic bioassay of *tert*-butanol, as re-analyzed by Hard et al. (2011) were reported in 4/50 of control male rats, which is much greater than would be expected from historical NTP F344 rat data (0/450) (Dinse and Peddada, 2011). Thus, it is possible that *tert*-butanol treatment served as a promoter of background tumorigenic processes occurring in that experiment and that, had background renal tumor rates in the ETBE

bioassays been higher, renal tumors would have been observed. However, key events in such a "promotion" MOA have not been identified (proliferation does not appear to be a likely key event because ETBE only induces transient increases in cell proliferation).

Conclusions about the hypothesized MOA for α_{2u}-globulin -associated nephropathy

Is the hypothesized MOA sufficiently supported in test animals?

Although ETBE induced an increase in α_{2u}-globulin deposition and increased hyaline droplet accumulation, most of the subsequent steps in the pathological sequence were not observed despite identical study conditions and doses in a number of experiments over a 2-year exposure period. These data failed to provide sufficient evidence that the α_{2u}-globulin process is operative. Since these data do not suggest that α_{2u}-globulin process is operative for ETBE exposures, the extent to which that α_{2u}-globulin is operative will not be examined further. Considering that a retrospective analysis found poor concordance of tumor incidence with the severity of any of the key pathological steps (Doi et al., 2007), the observation that ETBE does not induce renal tumors is not unexpected.

Is the hypothesized MOA relevant to humans?

Because EPA finds that the data are insufficient to demonstrate α_{2u}-globulin nephropathy, the male rat kidney data are relevant for humans.

Which populations or lifestages can be particularly susceptible to the hypothesized MOA?

This question is not applicable.

Alternative MOA hypotheses

Other nephrotoxic responses, such as exacerbation of CPN, urothelial hyperplasia, elevated biochemical markers, and increased kidney weight, are observed in male and/or female rats, suggesting other possible processes are operative for kidney toxicity. Exacerbation of CPN has been proposed to be a rat-specific mechanism of nephrotoxicity that is not relevant to humans (Hard et al., 2009).

CPN is an age-related renal disease of laboratory rodents of unknown etiology that occurs spontaneously in rats, especially the F344, Sprague-Dawley, and Osborne-Mendel strains (Hard et al., 2009). Additional markers associated with CPN include elevated proteinuria and albumin in the urine and increased BUN, creatinine, and cholesterol in the serum (Hard et al., 2009). CPN is frequently more severe in males compared with females. Several of the CPN pathological effects are similar to and can obscure the lesions characteristic of α_{2u}-globulin-related hyaline droplet nephropathy (Webb et al., 1990). Additionally, renal effects of α_{2u}-globulin accumulation can exacerbate the effects associated with CPN (U.S. EPA, 1991a). However, (Webb et al., 1990) suggested that exacerbated CPN was one component of the nephropathy resulting from exposure to

chemicals that induce α_{2u}-globulin nephropathy. Male rat sensitivity has been noted with both CPN and α_{2u}-globulin nephropathy.

Increased severity of CPN occurred in both male and female rats as a result of ETBE exposure, but was statistically significant only in the highest exposure group in the chronic inhalation study. Some of the observed renal lesions in male rats following exposure to ETBE are effects commonly associated with CPN. Cohen et al. (2011) concluded that the observation of slight (or mild) urothelial hyperplasia in the 2-year drinking study conducted by (Suzuki et al., 2012; JPEC, 2010a) was associated with CPN, and not a direct effect of ETBE exposure. However, there was a strong, statistically-significant, treatment-related, dose-response relationship between chronic ETBE exposure and increased incidence of urothelial hyperplasia in male rats in both the inhalation and oral studies (Suzuki et al., 2012; JPEC, 2010a), (Saito et al., 2013; JPEC, 2010b)). The severity of CPN also increased with ETBE exposure, although the dose-response relationship is very weak (only statistically significant at the highest dose in the inhalation study; trend test was not significant). The very different dose-response relationships argue against their being a close association. Moreover, even if urothelial hyperplasia were associated with CPN, there is no evidence to support that it is independent of ETBE treatment, given the robust dose-response relationships. Therefore, the data are insufficient to dismiss urothelial hyperplasia as causally related to ETBE exposure.

The underlying mechanisms regulating CPN and its exacerbation are not well understood, and to date, there is no scientific consensus on the relevance of CPN in rats to human health hazard (Melnick et al., 2012; Hard et al., 2009). Moreover, no key events for the exacerbation of CPN have been identified, so no MOA analysis can be performed. Therefore, kidney effects from ETBE exposure associated with CPN are considered relevant to humans.

Summary of Kidney Toxicity

The data that report kidney effects following oral and inhalation ETBE exposure are entirely from experimental rodent studies. Several noncancer effects in the kidney have been observed across multiple studies; chronic bioassays did not find treatment-related increases in renal tumors.

Kidney weights were consistently increased in male and female rats at several doses following subchronic and chronic gavage and inhalation exposures (Miyata et al., 2013; JPEC, 2008b, c; Medinsky et al., 1999). Regarding oral exposure, male kidney weights were more consistently increased across all exposure durations than females; however, both sexes responded similarly following inhalation exposures. The magnitude of the increases in kidney weight was moderate, with maximal changes in relative or absolute weights that were less than twofold. Several studies observing statistically significant increases at multiple exposure levels are consistent with a monotonic dose-response relationship. In mice, only one subchronic study was available, and it reported no changes in kidney weights (Medinsky et al., 1999), but the lack of additional mouse studies precludes a conclusion on the species specificity of ETBE-induced kidney

weight changes. In rats, chronic kidney weights were increased similarly to subchronic studies but were not considered for hazard assessment due to age-associated confounding factors (e.g., CPN); therefore a temporal relationship cannot be determined for this endpoint.

Histopathological analysis observed increased CPN lesions in male rats after a 13-week oral exposure and increased CPN severity in male and female rats after a 2-year inhalation exposure (Cohen et al., 2011; JPEC, 2010b); however, this was only observed at the highest tested doses. Urothelial hyperplasia was observed in male rats after 2-year inhalation or oral exposures (Suzuki et al., 2012; JPEC, 2010a), (Saito et al., 2013; JPEC, 2010b)). Although Cohen et al. (2011) attributed this finding to CPN, independent of ETBE exposure, the robust dose-response relationship (especially as compared to that for CPN) suggests it is a treatment-related effect.

Additional evidence of altered kidney function included elevated blood concentrations of total cholesterol, BUN, and creatinine in rats (Miyata et al., 2013; JPEC, 2010a, b, 2008c). These biochemistry markers were increased more consistently in males than females. Males had dose-related increases at several biochemistry endpoints, and these increases in biochemistry markers occurred at lower doses than lesions of nephropathy, consistent with the expected relationship between early markers of altered function and observable histopathology. Elevations in biochemical markers of kidney disease were greater in males than females, consistent with males' greater sensitivity to changes in kidney weights and histopathological changes, further adding to the biological coherence of the available data on kidney toxicity.

MOA analysis determined that the data are insufficient to conclude that the nephropathy observed in male rats is mediated by α_{2u}-globulin. The available data also precluded establishing any other MOA for ETBE-induced kidney toxicity. Therefore, in the absence of information indicating otherwise, EPA considered the male and female kidney effects observed in experimental animals to be relevant to assessing human health hazard. EPA identified kidney effects as a human hazard of ETBE exposure.

1.1.2. Liver Effects

Synthesis of Effects in Liver

This section reviews the studies that investigated whether exposure to ETBE can cause liver toxicity or cancer in humans or animals. The database for ETBE-induced liver effects includes 10 studies conducted in animals, all but one performed in rats. Studies employing short-term and acute exposures that examined liver effects are not included in the evidence tables; however, they are discussed in the text if they provided data to support mode of action or hazard identification. No methodological concerns were identified that would lead one or more studies to be considered less informative for assessing human health hazard.

Chronic and subchronic studies by both the oral and inhalation routes reported consistent statistically-significant, dose-related increases in liver weights (see

Table 1-8; Figure 1-5, Figure 1-6). Liver weight and body weight have been demonstrated to be proportional and liver weight normalized to body weight is optimal for data analysis (Bailey et al., 2004); thus, only relative liver weight is presented and considered in the determination of hazard. Relative liver weights were consistently increased in males in 8 of 9 studies and 6 of 8 studies for females; however, statistically significant increases frequently occurred only at the highest tested concentration with modest increases in relative liver weight ranging from 17–27% in males and 8–18% in females. Relative liver weights in rats were increased at the only highest dose following oral exposures of 16 weeks or longer (Miyata et al., 2013; Fujii et al., 2010; JPEC, 2008c). Inhalation exposure increased liver weight at the highest dose in female rats following 13 week exposure (JPEC, 2008b) and was dose responsively increased following 2 year exposure (Saito et al., 2013; JPEC, 2010b). Short-term studies observed similar effects on liver weight (JPEC, 2008a; White et al., 1995).

Centrilobular hypertrophy was inconsistently increased throughout the database (see Table 1-9; Figure 1-5, Figure 1-6). A 26-week oral gavage study (Miyata et al., 2013; JPEC, 2008c) in rats and three 13-week inhalation studies in mice and rats (Weng et al., 2012; JPEC, 2008b; Medinsky et al., 1999) demonstrated a statistically significant increase in centrilobular hypertrophy at the highest dose, but 2-year oral or inhalation studies in rats failed to induce a similar effect. Following a 2-year inhalation exposure to ETBE, acidophilic and basophilic preneoplastic lesions were increased in males, but not females, at the highest tested dose (Saito et al., 2013; JPEC, 2010b). After 2-year drinking water exposure to ETBE, an increasing, but not significant, trend in basophilic preneoplastic lesions was observed in the liver of male rats, but not in female rats (Suzuki et al., 2012; JPEC, 2010a).

Analysis of serum liver enzymes demonstrated inconsistent results across exposure routes (see Table 1-10; Figure 1-5, Figure 1-6). Gamma-glutamyl transpeptidase (GGT) was significantly increased in male rats at one dose following oral exposure and the two highest doses following inhalation exposure in 2-year studies (JPEC, 2010a, b). GGT was not significantly affected in female rats in any study. No consistent dose-related changes were observed in aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphatase (ALP) liver enzymes following either oral or inhalation exposure of any duration.

Data on liver tumor induction by ETBE are presented in Table 1-11. Liver adenomas and carcinomas (combined) were increased in male rats, but not females, following 2-year inhalation exposure (Saito et al., 2013; JPEC, 2010b). No significant increase in tumors was observed following 2 year oral exposure (Suzuki et al., 2012; JPEC, 2010a; Maltoni et al., 1999) . An initiation-promotion study by gavage in male F344 rats suggest tumor promotion activity by ETBE (Hagiwara et al., 2011).

Several factors associated with the 2-year organ weight data confound consideration for hazard identification. As mentioned previously in the discussion of kidney effects, mortality was a

confounding factor in 2-year studies. In addition, neoplastic and non-neoplastic lesions were observed in the livers of all treatment groups in both oral and inhalation studies which further confound organ weight data. For instance, the non-neoplastic lesion bile duct hyperplasia was observed at varying levels of severity in 100% of males surviving to 104 weeks (Suzuki et al., 2012; JPEC, 2010a). Inhalation exposure significantly increased adenomas and carcinomas at the highest dose which corresponded to increased liver weights (Saito et al., 2013; JPEC, 2010b). Altogether, these observations preclude including 2-year liver weight data for hazard identification. However, organ weight data obtained from studies of shorter duration that are not confounded by these age-associated factors may be appropriate for hazard identification.

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Liver: Absolute Weight			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		100	-3%
		300	-1%
		1000	13%*
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		100	-1%
		300	3%
		1000	14%*

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Liver: Absolute Weight (continued)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, male (25/group): 0, 250, 500, 1000 mg/kg-d P0 dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d P0 dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	2%
		500	2%
		1000	17%*
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	0%
		500	14%*
		1000	27%*
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		250	-1%
		500	4%
		1000	6%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Female	0	-
		250	1%
		500	3%
		1000	10%*
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		1000	21%*

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Liver: Absolute Weight (continued)			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	-2%
		25	7%
		100	4%
		400	19%
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	-4%
		25	-1%
		100	2%
		400	9%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		28	-11%*
		121	-4%
		542	2%
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		46	-5%
		171	-2%
		560	-10%

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Liver: Absolute Weight (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	5%
		2090	6%
		6270	4%
		20,900	2%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	-3%
		2090	-8%
		6270	-2%
		20,900	5%
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		20,900	13%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		20,900	11%
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	6%
		7320	14%*
		20,900	32%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	2%
		7320	9%

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
		20,900	26%*
Liver: Absolute Weight (continued)			
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation - vapor female (40/group): 0, 500, 1750, 5000 ppm(0, 2090, 7320, 20,900 mg/m^3)[b]; male (40/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	4%
		7320	13%*
		20,900	18%*
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	2%
		7320	19%*
		20,900	33%*
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	1%
		6270	11%*
		20,900	10%
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	-3%
		6270	-8%
		20,900	1%

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Liver: Relative Weight			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		100	1%
		300	3%
		1000	21%*
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		100	-2%
		300	2%
		1000	8%*
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	3%
		500	6%
		1000	24%*
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	0%
		500	11%*
		1000	25%*
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		250	10%
		500	8%
		1000	4%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Female	0	-
		250	3%

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
		500	6%
		1000	9%*
Liver: Relative Weight (continued)			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		1000	27%*
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	5%
		25	7%
		100	9%
		400	17%*
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	1%
		25	1%
		100	4%
		400	12%*

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Liver: Relative Weight (continued)			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	-8%
		121	3%*
		542	12%*
	Study authors stated that increased relative liver weights were due to significantly lowered final body weights of treated groups; individual animal data were not available to confirm statistical analysis conducted by study authors (e.g., 3% statistically significant increase in males at the mid-dose).		
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	4%
		171	9%
		560	8%
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	4%
		2090	-1%
		6270	6%
		20,900	18%*
		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	5%
		2090	5%
		6270	5%
		20,900	10%

Table 1-8. Evidence pertaining to liver weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Liver: Relative Weight (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		20,900	7%
		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		20,900	9%*
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	9%*
		6270	19%*
		20,900	49%*
	Study authors stated that increased relative liver weights were due to significantly lowered final body weights of treated groups; individual animal data were not available to confirm statistical analysis conducted by study authors (e.g., 1% statistically significant increase in females at the mid-dose).		
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	3%
		6270	1%*
		20,900	30%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).

Table 1-9. Evidence pertaining to liver histopathology effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Acidophilic Foci in Liver			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	14/50
		28	12/50
		121	17/50
		542	13/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	2/50
		46	2/50
		171	1/50
		560	0/50
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	31/50
		2090	28/50
		6270	36/49
		20,900	39/50*
		Dose(mg/m^3)	Response (incidence)
	Female	0	2/50
		2090	1/50
		6270	4/50
		20,900	2/50

Table 1-9. Evidence pertaining to liver histopathology effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Basophilic Foci in Liver			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	14/50
		28	18/50
		121	20/50
		542	22/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	36/50
		46	25/50*
		171	31/50
		560	30/50*
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	18/50
		2090	10/50
		6270	13/49
		20,900	33/50*
		Dose(mg/m^3)	Response (incidence)
	Female	0	36/50
		2090	31/50
		6270	32/50
		20,900	28/50
Bile Duct Hyperplasia			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	49/50
		28	47/50
		121	48/50
		542	47/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	1/50
		46	4/50
		171	4/50
		560	3/50

Table 1-9. Evidence pertaining to liver histopathology effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Bile Duct Hyperplasia (continued)			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	48/50
		2090	44/50
		6270	46/49
		20,900	41/50
		Dose(mg/m^3)	Response (incidence)
	Female	0	5/50
		2090	8/50
		6270	7/50
		20,900	6/50
Centrilobular Hypertrophy			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21		Dose(mg/kg-d)	Response (incidence)
	P0, Male	0	0/25
		250	0/25
		500	0/25
		1000	3/25
		Dose(mg/kg-d)	Response
	P0, Female	0	0/25
		250	0/25
		500	0/25
		1000	0/25
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/15
		5	0/15
		25	0/15
		100	0/15
		400	6/15*
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/15
		5	0/15
		25	0/15
		100	0/15
		400	6/15*

Table 1-9. Evidence pertaining to liver histopathology effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Centrilobular Hypertrophy (continued)			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] daily for 104 wks	Male	Dose(mg/kg-d)	Response (incidence)
		0	0/50
		28	0/50
		121	0/50
		542	0/50
	Female	Dose(mg/kg-d)	Response (incidence)
		0	0/50
		46	0/50
		171	0/50
		560	0/50
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Response (incidence)
		0	0/10
		627	0/10
		2090	0/10
		6270	0/10
		20,900	4/10*
	Female	Dose(mg/m^3)	Response (incidence)
		0	0/10
		627	0/10
		2090	0/10
		6270	0/10
		20,900	6/10*
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Response
		0	0/6
		20,900	0/6
	Female	Dose(mg/m^3)	Response
		0	0/6
		20,900	0/6

Table 1-9. Evidence pertaining to liver histopathology effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Centrilobular Hypertrophy (continued)			
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	0/11
		2090	0/11
		7320	0/11
		20,900	0/11
		Dose(mg/m^3)	Response (incidence)
	Female	0	0/10
		2090	0/11
		7320	0/11
		20,900	0/11
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation - vapor female (40/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (40/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	0/15
		2090	0/15
		7320	2/15
		20,900	8/10*
		Dose(mg/m^3)	Response (incidence)
	Female	0	0/13
		2090	2/15
		7320	1/15
		20,900	9/14*
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	0/50
		2090	0/50
		6270	0/49
		20,900	0/50
		Dose(mg/m^3)	Response (incidence)
	Female	0	0/50
		2090	0/50
		6270	0/50
		20,900	0/50

Table 1-9. Evidence pertaining to liver histopathology effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Centrilobular Hypertrophy (continued)			
Weng et al. (2012) mice, C57BL/6 inhalation - vapor female (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber, 6 hr/d, 5 d/wk for 13 wks; generation methods were not reported, but analytical methods (gas chromatograph) and concentration were reported	Male	Dose(mg/m^3)	Response (incidence)
		0	1/5
		2090	0/5
		7320	0/5
		20,900	5/5*
	Female	Dose(mg/m^3)	Response (incidence)
		0	0/5
		2090	0/5
		7320	1/5
		20,900	5/5*
Weng et al. (2012) mice, ALDH2-/- inhalation - vapor female (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber, 6 hr/d, 5 d/wk for 13 wks; generation methods were not reported, but analytical methods (gas chromatograph) and concentration were reported	Male	Dose(mg/m^3)	Response (incidence)
		0	0/5
		2090	3/5
		7320	2/5
		20,900	5/5*
	Female	Dose(mg/m^3)	Response (incidence)
		0	0/5
		2090	0/5
		7320	0/5
		20,900	4/5*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant (p<0.05) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
(n): number evaluated from group

Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Alanine Aminotransferase (ALT)			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	10%
		25	48%
		100	13%
		400	35%
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	11%
		25	21%
		100	46%
		400	21%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		28	-17%
		121	2%
		542	-4%
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		46	-10
		171	-15
		560	-26

Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Alanine Aminotransferase (ALT) (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	9%
		2090	0%
		6270	5%
		20,900	12%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	-1%
		2090	11%
		6270	-5%
		20,900	26%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	53%
		6270	-3%
		20,900	24%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	2%
		6270	-5%
		20,900	4%*

Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Alkaline Phosphatase (ALP)			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	2%
		25	12%
		100	-7%
		400	27%
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	6%
		25	-21%
		100	-18%
		400	-19%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		28	-5%
		121	3%
		542	0%
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		46	-16%
		171	2%
		560	-15%

Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Alkaline Phosphatase (ALP) (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	13%
		2090	12%
		6270	-12%
		20,900	-9%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	-3%
		2090	-12%
		6270	-7%
		20,900	5%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	0%
		6270	-21%*
		20,900	-5%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	12%
		6270	-4%
		20,900	4%

Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Aspartate Aminotransferase (AST)			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	16%
		25	19%
		100	20%
		400	23%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	10%
		25	13%
		100	19%
		400	4%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	-21%
		121	-3%
		542	-1%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	-19%
		171	-17%
		560	-46%*

Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Aspartate Aminotransferase (AST) (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	3%
		2090	1%
		6270	-7%
		20,900	4%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	2%
		2090	-95%
		6270	12%
		20,900	0%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	29%
		6270	-16%
		20,900	-2%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	22%
		6270	10%
		20900	18%*

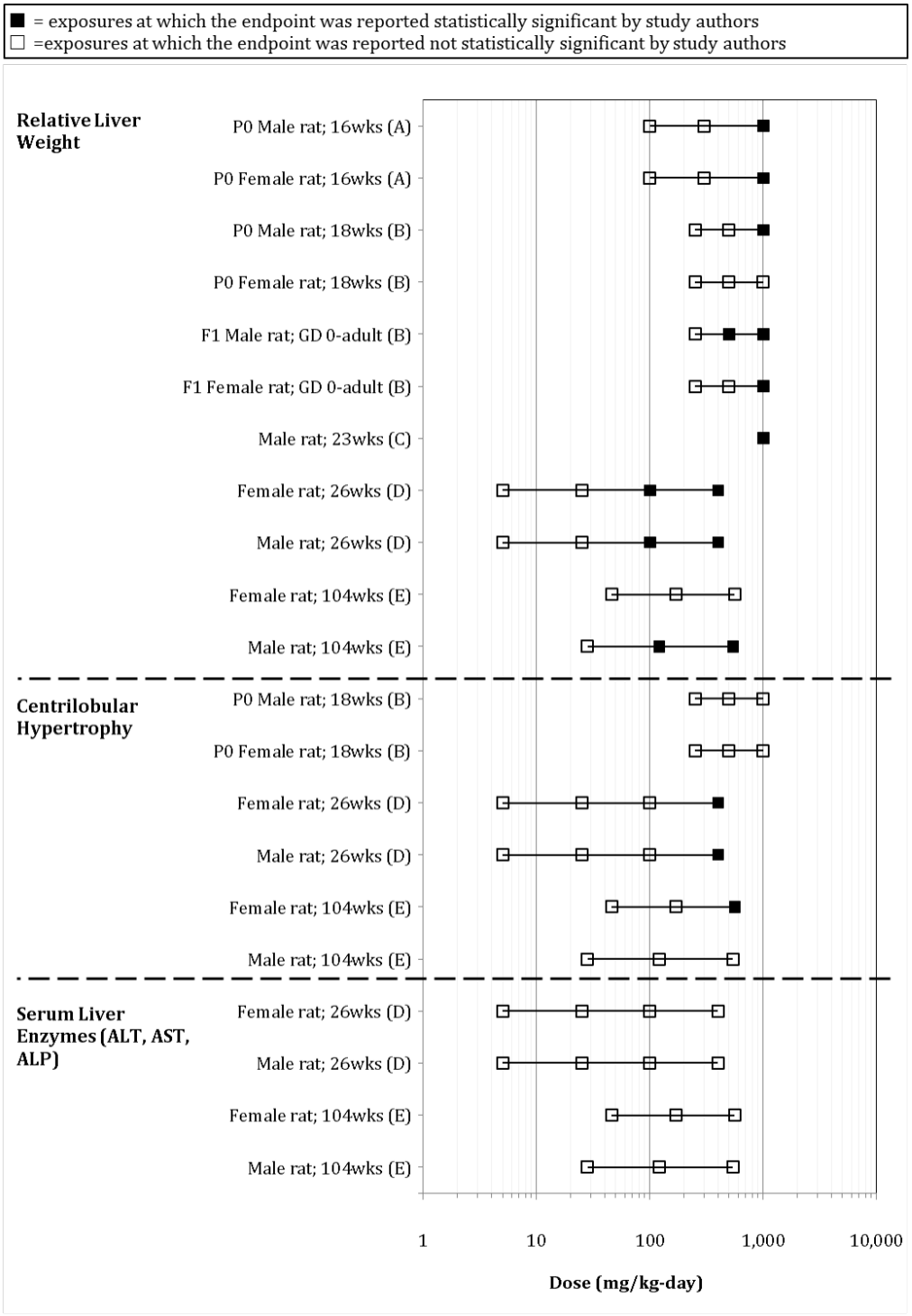
Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Gamma-Glutamyl Transpeptidase (GGT)			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		5	25%
		25	50%
		100	25%
		400	100%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		5	40%
		25	20%
		100	0%
		400	-20%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		28	0%
		121	43%*
		542	29%
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	0%
		171	0%
		560	33%

Table 1-10. Evidence pertaining to liver biochemistry effects in animals exposed to ETBE (*continued*)

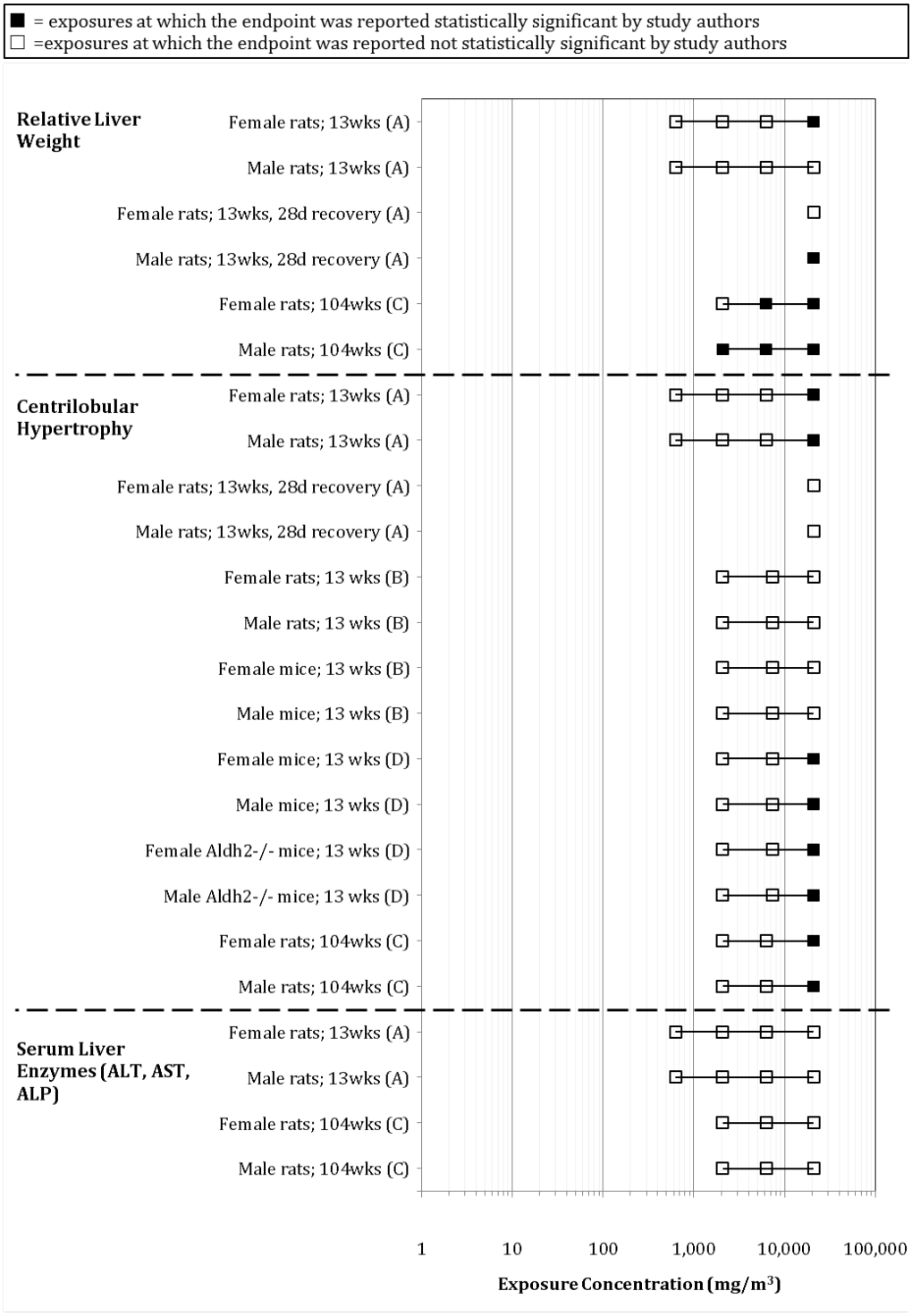
Reference and Dosing Protocol	Results by Endpoint		
Gamma-Glutamyl Transpeptidase (GGT) (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		627	11%
		2090	0%
		6270	11%
		20,900	-100%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		627	25%
		2090	12%
		6270	25%
		20,900	25%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	33%
		6270	50%*
		20,900	200%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	50%
		6270	0%
		20,900	150%

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
(n): number evaluated from group
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).



Sources: (A) Fujii et al., 2010; JPEC, 2008e (B) Gaoua, 2004b (C) Hagiwara et al., 2011 (D) Miyata et al., 2013; JPEC, 2008c (E) Suzuki et al., 2012; JPEC, 2010a

Figure 1-5. Exposure-response array of liver effects following oral exposure to ETBE.



Sources: (A) JPEC, 2008b (B) Medinsky et al., 1999; Bond et al., 1996 (C) Saito et al., 2013; JPEC, 2010b (D) Weng et al., 2012

Figure 1-6. Exposure-response array of liver effects following inhalation exposure to ETBE.

Table 1-11. Evidence pertaining to liver tumor effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint				
Hepatocellular Adenoma and Carcinoma					
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Incidence				
		Dose (mg/kg-d)	Adenoma	Carcinoma	Adenoma or Carcinoma
	Male	0	2/50	2/50	4/50
		28	0/50	0/50	0/50
		121	0/50	0/50	0/50
		542	0/50	0/50	0/50
		Dose (mg/kg-d)	Adenoma	Carcinoma	Adenoma or Carcinoma
	Female	0	0/50	0/50	0/50
		46	0/50	0/50	0/50
		171	0/50	0/50	0/50
		560	1/50	0/50	1/50
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Incidence				
		Dose (mg/m^3)	Adenoma	Carcinoma	Adenoma or Carcinoma
	Male	0	0/50	0/50	0/50
		2090	2/50	0/50	2/50
		6270	1/50	0/50	1/50
		20,900	9/50*	1/50	10/50*
		Dose (mg/m^3)	Adenoma	Carcinoma	Adenoma or Carcinoma
	Female	0	1/50	0/50	1/50
		2090	0/50	0/50	0/50
		6270	1/50	0/50	1/50
		20,900	1/50	0/50	1/50

Table 1-11. Evidence pertaining to liver tumor effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Liver Neoplasm			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (30/group): 0, 300, 1000 mg/kg-d daily for 23 weeks following a 4 week tumor initiation by DMBDD [+] no DMBB initiation		Dose(mg/kg-d)	Response (incidence)
	Male	0	1/30
		300	1/30
		1000	6/30*
		0[+]	0/12
		1000[+]	0/12
Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d 4 d/wk for 104 wks; observed until natural death NOTE: These tumor data were not re-analyzed by Malarkey and Bucher (2011)		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/60
		250	0/60
		1000	0/60
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	0/60
		1000	0/60

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant (p<0.05) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
(n): number evaluated from group

Mode of Action Analysis- Liver Effects

Toxicokinetic considerations relevant to liver toxicity and tumors

ETBE is metabolized by cytochrome P450 (CYP) enzymes to an unstable hemiacetal that decomposes spontaneously into *tert*-butanol and acetaldehyde (Bernauer et al., 1998). Acetaldehyde is further metabolized in the liver by ALDH2, whereas *tert*-butanol undergoes systemic circulation and is ultimately excreted in urine. Thus, following ETBE exposure, the liver is exposed to both acetaldehyde and *tert*-butanol, so the liver effects caused by *tert*-butanol (described in the more detail in the draft IRIS assessment of *tert*-butanol) and acetaldehyde are also relevant to evaluating the liver effects observed after ETBE exposure.

tert-Butanol induces thyroid and kidney tumors in rodents, but has not been observed to affect the incidence of liver tumors following a 2-year oral exposure. Whereas there are some data suggesting *tert*-butanol may be genotoxic, the overall evidence is inadequate to establish a conclusion. No study has reported that *tert*-butanol causes centrilobular hypertrophy or that it

activates nuclear receptors. Therefore, a role for *tert*-butanol in liver carcinogenesis of ETBE does not appear likely. No mode of action information is available for *tert*-butanol-induced noncancer liver effects.

On the other hand, acetaldehyde is genotoxic and mutagenic (IARC, 1999a), and acetaldehyde produced in the liver as a result of ethanol metabolism has been suggested as a contributor to ethanol-related liver toxicity and cancer (Setshedi et al., 2010). Additional discussion on the potential role of acetaldehyde in the liver carcinogenesis of ETBE is provided below.

Receptor-mediated effects

ETBE exposure consistently increased both relative and absolute liver weights in male and female rats. In addition, ETBE increased hepatocellular adenomas and carcinomas in males exposed via inhalation for 2 years (Saito et al., 2013; JPEC, 2010b). These studies did not report consistent effects on liver function as demonstrated by a lack of concordant changes in serum liver enzyme levels. However, several studies have demonstrated that ETBE increases centrilobular hypertrophy and preneoplastic lesions, which may lead to tumorigenesis. This process was investigated in several studies to determine whether nuclear receptor activation is involved.

Centrilobular hypertrophy is induced through a number of possible mechanisms, of which many are via nuclear hormone receptors such as peroxisome proliferator-activated receptor α (PPARα), pregnane X receptor (PXR), and the constitutive androstane receptor (CAR). The sequence of key events hypothesized for PPARα induction of liver tumors is as follows: activation of PPARα, upregulation of peroxisomal genes, expression of PPARα-mediated growth and apoptosis, disrupted cell proliferation and apoptosis, peroxisome proliferation, preneoplastic foci, and tumors (Klaunig et al., 2003). The sequence of key events hypothesized for CAR-mediated liver tumors is as follows: CAR activation, altered gene expression as a result of CAR activation, increased cell proliferation, clonal expansion leading to altered foci, and liver adenomas and carcinomas (Elcombe et al., 2014). PXR does not have an established MOA but is hypothesized to progress from PXR activation to liver tumors in a similar manner as CAR, which would include PXR activation, cell proliferation, hypertrophy, CYP3A induction, and clonal expansion resulting in foci development. One study that exposed male rats to a high and low concentration of ETBE via gavage twice per day for 2 weeks reported that several key sequences in these aforementioned pathways were affected (Kakehashi et al., 2013).

PPAR

The data suggest that PPAR may be involved in ETBE-induced liver tumors (Kakehashi et al., 2013). For instance, mRNA expression was statistically significantly elevated for PPARα and PPARγ after 1 week of exposure but not after 2 weeks. In addition, a number of PPARα-mediated proteins involved in lipid and xenobiotic metabolism were upregulated in the liver after 2 weeks of exposure such as ACOX1, CYP4A2, and ECH1. DNA damage (8-OHdG) and apoptosis (ssDNA) were also

statistically significantly increased after 2 weeks at the highest concentration of ETBE. Cell proliferation was unchanged after 1 week and significantly decreased after 2 weeks. The number of peroxisomes per hepatocyte was increased greater than fivefold after 2 weeks of treatments. Finally, the incidences of basophilic and acidophilic foci were significantly increased in males after 2 years of inhalation exposure to ETBE (Saito et al., 2013; JPEC, 2010b).

Altogether, a number of key sequences in the PPAR pathway were observed in the Kakehashi et al. (2013) and (Saito et al., 2013; JPEC, 2010b) studies; however, several steps in the pathway were either not observed or not examined. For instance, selective clonal expansion was not examined in any study. Furthermore, the cell proliferation and apoptosis data were contrary to what would be expected if a PPAR MOA were operative. Cell proliferation was decreased after 2 weeks of exposure; no other time points in the data set were available (Kakehashi et al., 2013). In addition, PPAR agonists typically decrease rates of apoptosis early in the process, which is in contrast to the increased rate of apoptosis observed after 2 weeks of ETBE exposure (Kakehashi et al., 2013). Perturbation of cell proliferation and apoptosis are both required steps for MOA and future studies with longer exposures could address this data gap. Overall, these data are suggestive but not adequate for establishing a PPAR MOA for liver tumorigenesis.

CAR/PXR

Kakehashi et al. (2013) reported a number of CAR and PXR-mediated events following ETBE exposure. After 2 weeks of exposure at the high dose of ETBE, PXR- and CAR-regulated xenobiotic metabolic enzymes were upregulated, including Cyp2b1, Cyp2b2, Cyp3a1, and Cyp3a2 as determined by mRNA and/or protein expression. Other PXR/CAR-regulated genes such as Sult1d1, Ugt2b5, and Ugt1a1 also had elevated mRNA expression after 1 and 2 weeks of exposure which all suggest activation of PXR and CAR. As described above for Kakehashi et al. (2013), cell proliferation was reduced, and apoptosis was increased following ETBE exposure, in contrast to what is expected during the CAR/PXR sequence of events. There were several data gaps that were not evaluated such as a lack of clonal expansion and gap junction communication. These data provide evidence that PXR and CAR are activated in the liver following ETBE exposure; however, due to crosstalk of PXR and CAR on downstream effects such as cell proliferation, preneoplastic foci, and apoptosis, it is not possible to determine the relative contribution of each pathway in tumorigenesis. The data do not provide enough information to determine dose-response concordance or temporal associations, which are critical for establishing a MOA. Furthermore, the available data from this study do not allow for parsing which effects are induced by PPAR or CAR/PXR activation. Altogether, these data are inadequate to establish a CAR/PXR MOA for inducing liver tumors.

Acetaldehyde-mediated liver toxicity and genotoxicity

Another possible MOA for increased tumors could be due to the production of acetaldehyde in the liver, the primary site for ETBE metabolism. Acetaldehyde produced as a result of

metabolism of alcohol consumption is considered carcinogenic to humans by IARC (1999a), though there is not sufficient evidence that acetaldehyde formed in this manner causes liver carcinogenesis (IARC, 2012). Acetaldehyde administered directly has been demonstrated to increase the incidence of carcinomas following inhalation exposure in the nasal mucosa and larynx of rats and hamsters. Furthermore, acetaldehyde has induced sister chromatid exchanges in Chinese hamster ovary cells, gene mutations in mouse lymphomas, and DNA strand breaks in human lymphocytes IARC (1999a). Acetaldehyde has been shown to have an inhibitory effect on PPARα transcriptional activity (Venkata et al., 2008). The effect of acetaldehyde on CAR or PXR activation has not been established. Additionally, the acetaldehyde metabolic enzyme aldehyde dehydrogenase 2 (ALDH2) is polymorphic in the human population, which contributes to enhanced sensitivity to the effects of acetaldehyde, particularly esophageal cancer, among some subpopulations such as East Asians (IARC, 2012; Brennan et al., 2004). However, the importance of this polymorphism for hepatocarcinogenesis is unclear.

Several studies have examined the role of acetaldehyde and the metabolizing enzyme ALDH2 in genotoxicity and centrilobular hypertrophy following ETBE exposure. Ninety-day inhalation exposure to ETBE significantly increased the incidence of centrilobular hypertrophy in Aldh2 KO mice compared with wild type (WT) (Weng et al., 2012). Hepatocyte DNA damage as determined by DNA strand breaks and oxidative base modification was increased at the highest concentration of ETBE exposure in the WT males, but not in WT females. Measures of DNA damage were all statistically significantly exacerbated in both male and female Aldh2 KO mice (Weng et al., 2012). Further demonstrating enhanced genotoxic sensitivity in males compared with females, erythrocyte micronucleus assays and oxidative DNA damage in leukocytes were only observed to be statistically significantly increased and dose responsive in male Aldh2 KO mice (Weng et al., 2013). Altogether, while these data are suggestive of a potential role for acetaldehyde in the increased liver tumor response observed in male rats exposed to ETBE, the available data are inadequate to establish acetaldehyde-mediated mutagenicity as a MOA for ETBE-induced liver tumors.

Summary of mode of action analysis

The available mechanistic data provide some evidence that two nuclear receptor-mediated pathways (PPAR and CAR/PXR) may contribute to both the hypertrophy and tumorigenesis observed in ETBE-treated males. These studies do not provide any evidence on the relative contributions of either of these pathways in the development of liver tumors. Several reviews suggest that the PPAR, PXR, and/or CAR pathways induce liver tumors in a manner that is not relevant to humans (Elcombe et al., 2014; Klaunig et al., 2003) although this conclusion has been questioned (Guyton et al., 2009). The available data are inadequate to conclude that the liver tumors observed in rats are caused by one of these nuclear receptor-mediated pathways.

Therefore, given the available data, ETBE-induced liver tumors in male rats are considered relevant to humans.

Evidence also suggests that metabolism of ETBE to acetaldehyde may contribute to ETBE-induced liver carcinogenesis. For instance, enhancement of ETBE-induced liver toxicity and genotoxicity has been reported in Aldh2-deficient mice, which have an impaired ability to metabolize acetaldehyde (Weng et al., 2013; Weng et al., 2012). Additionally, lack of ALDH2 is directly relevant to the substantial human subpopulation that is deficient in the ALDH2 isozyme. Given the known genotoxicity and carcinogenicity of acetaldehyde (IARC, 2012), these data are suggestive of a role for acetaldehyde in ETBE-induced liver tumorigenesis. However, the available data are inadequate to establish acetaldehyde-mediated mutagenicity as a MOA for ETBE-induced liver tumors.

Summary of Liver Toxicity

Evidence for ETBE-induced noncancer liver effects is available from rat and mouse studies. Several endpoints such as increased liver weight and liver enzymes were more severely affected in males compared with females (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Noncancer effects were observed in subchronic oral and inhalation studies. One chronic inhalation study observed increased hepatocellular tumors in male rats (Suzuki et al., 2012; JPEC, 2010a).

Relative liver weights were consistently increased in males in 8 of 9 studies and 6 of 8 studies for females; however, statistically significant increases frequently occurred only at the highest tested concentration with modest increases in relative liver weight ranging from 17-27% in males and 8-18% in females. Centrilobular hypertrophy also was observed at the same high doses in males and females after 13-week and 26-week inhalation and oral exposure, respectively. No other accompanying pathologies were observed. A significant dose-related increase in GGT was only observed in one 2-year inhalation study in male rats; no other consistent changes in liver enzymes were observed in males or females.

Given the modest organ weight changes, lack of dose response with other liver endpoints, and poor temporal correlation indicative of accumulating damage, EPA concluded that the evidence does not support liver effects as a potential human hazard of ETBE exposure.

With respect to liver carcinogenicity, one 2-year inhalation rat study observed increased hepatocellular adenomas and carcinomas in males at the highest tested dose (Saito et al., 2013; JPEC, 2010b). Although only one carcinoma was observed, the adenomas have the potential to transform into malignant carcinomas (McConnell et al., 1986). However, increases in liver tumors were not observed either in a 2-year oral drinking water bioassay in rats in the same laboratory or in an additional cancer bioassay in rats performed by oral gavage. A mechanistic study conducted by gavage in rats observed ETBE-related increases in liver tumors following initiation by DMBDD, suggesting that ETBE exposure can promote liver tumors (Hagiwara et al., 2011). Additional mechanistic data on the role of PPAR, PXR, and CAR activation in liver tumorigenesis were

inadequate to conclude that these pathways mediate tumor formation. Additional mechanistic studies reported that lack of ALDH2 enhanced ETBE-induced liver toxicity and genotoxicity (Weng et al., 2013; Weng et al., 2012). These findings are consistent with genotoxicity being mediated by the ETBE metabolite acetaldehyde, which is genotoxic and considered carcinogenic when produced as a result of metabolism from ingested ethanol (IARC, 2012). Overall, available mechanistic data provide some biological plausibility to the liver carcinogenicity of ETBE. Section 1.2.2 discusses the overall weight of evidence for ETBE carcinogenicity.

1.1.3. Reproductive and Developmental Effects

Synthesis of reproductive and developmental toxicity

This section reviews the studies that investigated whether exposure to ETBE can cause reproductive or developmental toxicity in humans or animals. The database examining reproductive or developmental effects following ETBE exposure contains no human data, but is comprised of animal data primarily from rats. Three studies evaluated reproductive effects: a one-generation study, two-generation study, and subchronic study. In addition, there were two short-term studies evaluating effects on reproductive hormones and effects on oocytes. Reproductive organs were also evaluated in a subchronic study and four chronic studies that evaluated reproductive organs with no significant effects observed. Five studies evaluated developmental effects (three developmental studies, a one-generation reproductive study, and a two-generation reproductive study). One preliminary reproductive and developmental study is not discussed because it was superseded by two later studies within the same laboratory. Methodological concerns were identified with the Weng et al., 2014 study and included the lack of reported experimental blinding for histophathological examinations and the lack of standard terminology for reporting sperm effects which reduced confidence in these endpoints. No other methodological concerns were identified that would lead one or more studies to be considered less informative for assessing human health hazard.

Reproductive effects

Reproductive endpoints that were reported include oocyte viability, sex hormones, seminiferous tubules, and sperm effects. Sperm parameters in rats were not affected by ETBE in either generation of the two-generation study (Gaoua, 2004b) or in wild-type mice (Weng et al., 2014) (see Table 1-13; Figure 1-7, Figure 1-8). Sperm effects as measured by percent change in sperm heads and sperm motility (number of sperm that were mobile, number of sperm that were static, sperm with rapid movement) were observed in Aldh2 knockout or heterozygous mice but not in wild type (Weng et al., 2014). Lack of data on the biological relevance of reduced sperm motility reduced the possibility that this finding is a potential hazard. Short-term studies did not observe any effects on the number of oocytes recovered from ovulating female rats or in the ability

of the oocytes to be fertilized (Berger and Horner, 2003) nor was there an effect on testosterone levels (de Peyster et al., 2009); however, male rats had a statistically significant increase in estradiol levels (de Peyster et al., 2009). No effects from ETBE were observed on the seminiferous tubules (Weng et al., 2014). No additional reproductive effects have been reported.

Table 1-12. Evidence pertaining to female reproductive effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Delivery Index (pups delivered/implantations)			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -7% -4% -3%
Fertility Index			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	P0, Male	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 14% 9% 5%
	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 14% 9% 5%

Table 1-12. Evidence pertaining to female reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Fertility Index (continued)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	-9%
		500	-4%
		1000	9%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	0%
		500	-4%
		1000	4%
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		250	-9%
		500	-4%
		1000	9%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Female	0	-
		250	5%
		500	0%
		1000	9%
Postimplantation Loss			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		250	33%
		500	14%
		1000	51%

Table 1-12. Evidence pertaining to female reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Litter Size			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		250	-1%
		500	4%
		1000	-1%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Female	0	-
		250	0%
		500	0%
		1000	2%
Oocytes Fertilized			
Berger and Horner (2003) rat, Simonson albino oral - water P0, female (NR): 0, 0.3 % (estimated to be 0, 1887 mg/kg-d) daily for 2 weeks; then oocytes fertilized in vitro		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		1887	-2%
	Treatment with ETBE did not affect the percentage of oocytes fertilized.		
Oocytes Recovered Per Ovulating Female			
Berger and Horner (2003) rat, Simonson albino oral - water P0, female (NR): 0, 0.3 % (estimated to be 0, 1887 mg/kg-d) daily for 2 weeks; then oocytes fertilized in vitro		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		1887	-3%
	ETBE had no effect on the percentage of females ovulating or number of oocytes per ovulating female.		
Estradiol			
de Peyster et al. (2009) rat, Fischer 344 oral - gavage P0, male (12/group): 0, 600, 1200, 1800 mg/kg-d daily for 14 days		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		600	29%
		1200	106%*
		1800	105%*

*: result is statistically significant ($p<0.05$) based on analysis of data by study authors.

-: for controls, no response relevant; for other doses, no quantitative response reported.

(n): number evaluated from group.

Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Sperm Heads (Testicular)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	-5%
		500	-6%
		1000	-4%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	-3%
		500	5%
		1000	-1%
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (NR): 0, 50, 200, 500 ppm (209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		209	-13%
		836	-15%
		2090	-13%
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (NR): 0, 50, 200, 500 ppm (209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		209	-8%
		836	-16%*
		2090	-23%*
Weng et al. (2014) mice, Aldh2 heterogeneous inhalation - vapor male (NR): 0, 50, 200, 500 ppm (209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		209	0%
		836	-46%*
		2090	-53%*

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Sperm Heads (Testicular) (continued)			
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - 1% 1% -9%
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - -25%* -26%* -26%*
Sperm Motility (Epididymal)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups	P0, Male	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 0% -1% -2%
	F1, Male	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 3% 10% 4%
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male no significant change (results in figure only)		

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint
Sperm Motility (Epididymal) (continued)	
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male significantly decreased at 500 ppm (2090 mg/m^3) (results in figure only)
Weng et al. (2014) mice, Aldh2 heterogeneous inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male significantly decreased at >=200 ppm (836 mg/m^3) (results in figures only)
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male no significant change (results in figure only)
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male significantly decreased at all doses (results in figure only)

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Sperm Normal Morphology (Epididymal)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	0%
		500	4%
		1000	3%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	2%
		500	2%
		1000	5%
Sperm Production (Daily, Testicular)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	-5%
		500	-6%
		1000	-4%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	-3%
		500	5%
		1000	-1%
Sperm with Rapid Movement			
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male no significant change (results in figure only)		

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint
Sperm with Rapid Movement (continued)	
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male significantly decreased at 500 ppm (2090 mg/m^3) (results in figure only)
Weng et al. (2014) mice, Aldh2 heterogeneous inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male significantly decreased at >=200 ppm (836 mg/m^3) (results in figure only)
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male significant decrease in the 5000 ppm (20,900 mg/m^3) group (results in figure only)
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male significantly decreased at all doses (results in figure only)
Sperm, Static	
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (NR): 0, 50, 200, 500 ppm dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male no significant change (results in figure only)

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint
Sperm, Static (continued)	
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male significantly increased at 500 ppm (2090 mg/m^3) (results in figure only)
Weng et al. (2014) mice, Aldh2 heterogeneous inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male significantly increased at >=200 ppm (836 mg/m^3) (results in figure only)
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male no significant change (results in figure only)
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male significantly increased at all doses (results in figure only)

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Spermatozoa Count (Epididymal)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	2%
		500	1%
		1000	-1%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	-7%
		500	-3%
		1000	-5%
Testosterone			
de Peyster et al. (2009) rat, Fischer 344 oral - gavage P0, male (12/group): 0, 600, 1200, 1800 mg/kg-d daily for 14 days		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		600	50%
		1200	26%
		1800	-34%
Atrophy of the Seminiferous Tubules in the Right Testis			
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male no effects were observed (data not provided)		
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male no effects observed (data not provided)		

Table 1-13. Evidence pertaining to male reproductive effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Atrophy of the Seminiferous Tubules in the Right Testis (continued)			
Weng et al. (2014) mice, Aldh2 heterogeneous inhalation - vapor male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2090 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 9 wk; methods were stated to be described in Weng et al., 2012	Male no effects observed (data not provided)		
Weng et al. (2014) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male	Dose(mg/m^3) 0 2090 7320 20,900	Response (incidence) 1/5 0/5 2/5 3/5
Weng et al. (2014) mice, Aldh2-/- inhalation - vapor male (5/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods were stated to be described in Weng et al., 2012	Male	Dose(mg/m^3) 0 2090 7320 20,900	Response (incidence) 2/5 5/5 5/5 5/5

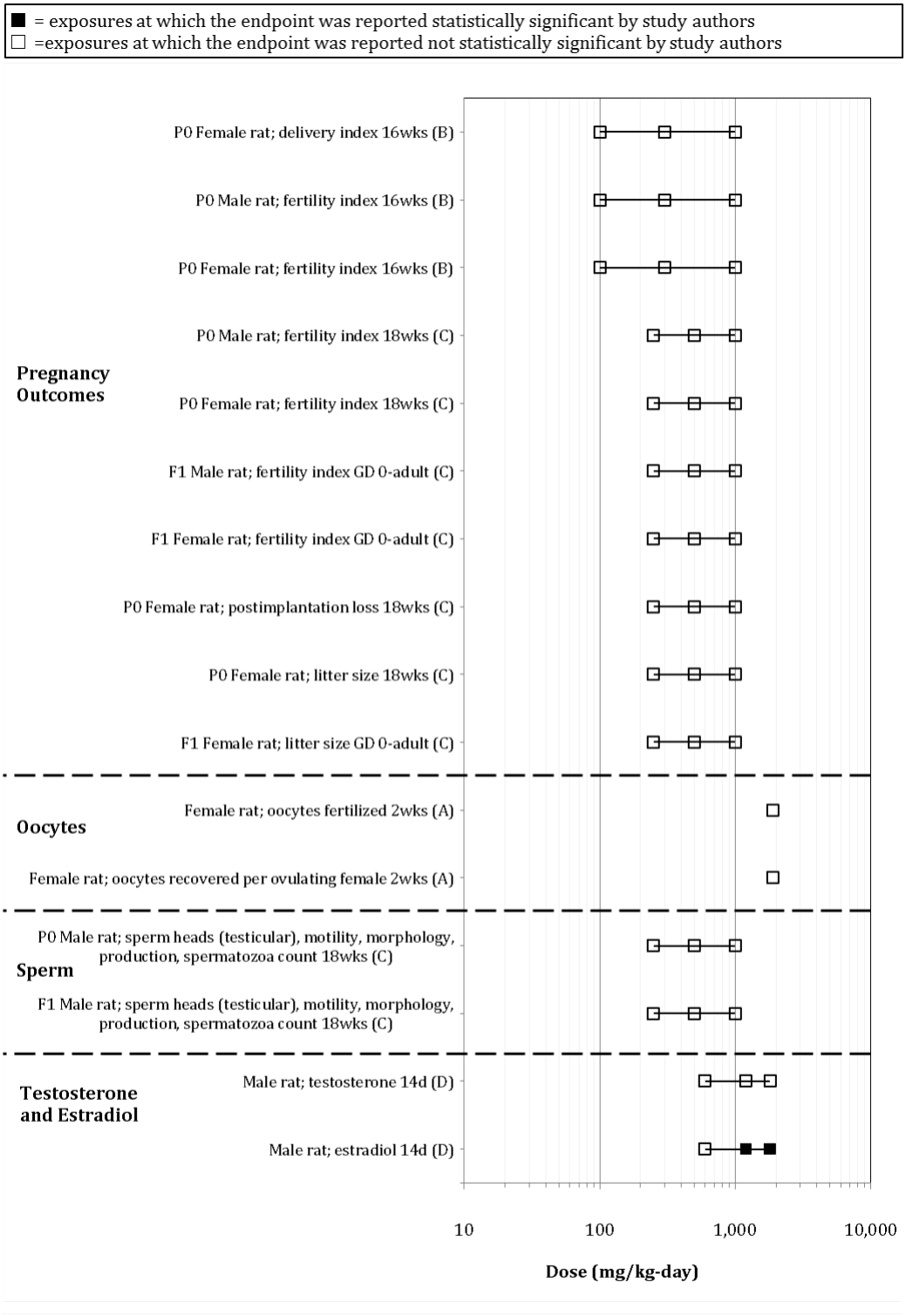
[a]4.18 mg/m^3 = 1 ppm.

*: result is statistically significant ($p<0.05$) based on analysis of data by study authors.

-: for controls, no response relevant; for other doses, no quantitative response reported.

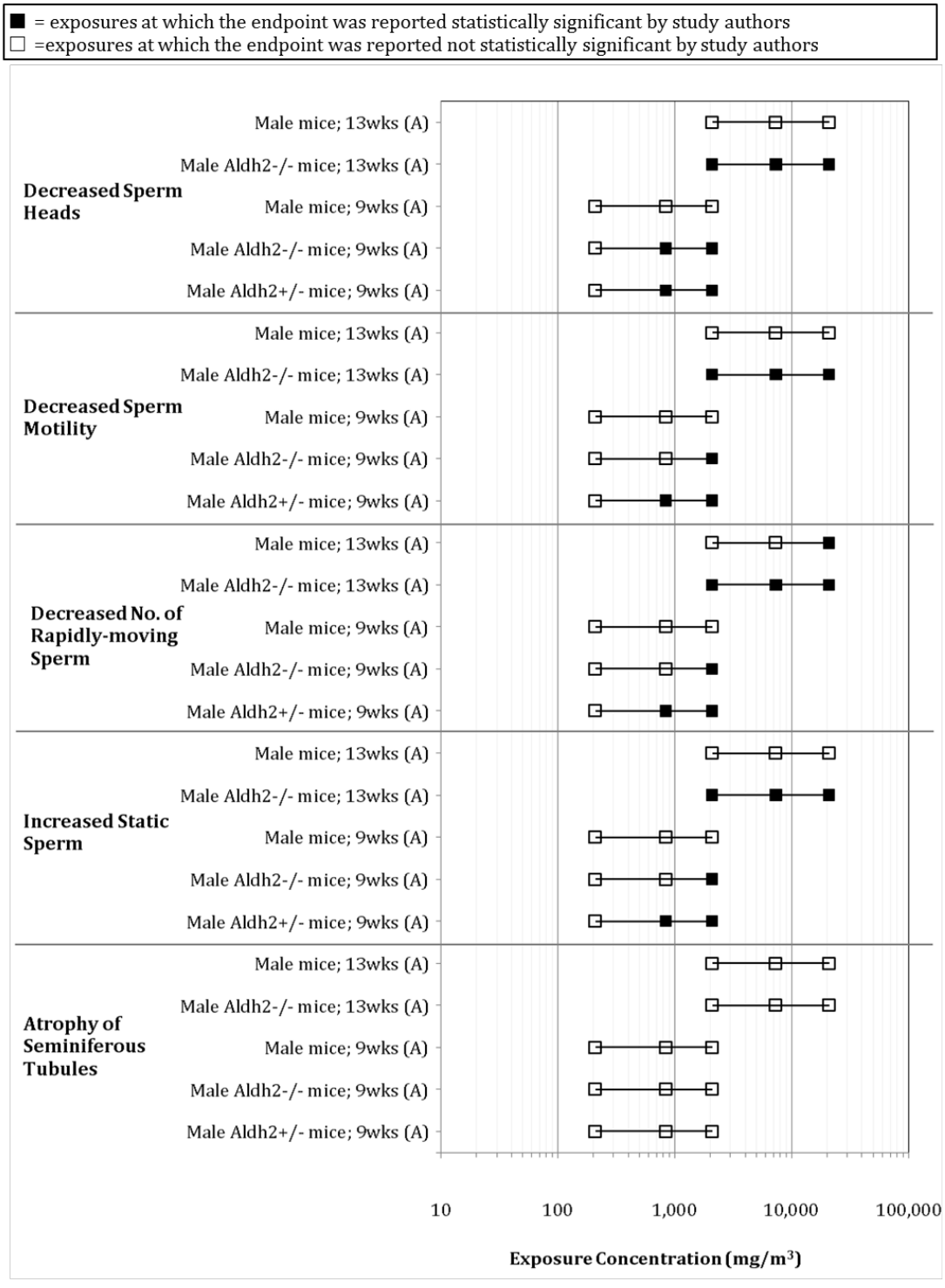
(n): number evaluated from group.

Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).



Sources: (A) Berger et al., 2003 (B) Fujii et al., 2010; JPEC, 2008e (C) Gaoua, 2004b (D) de Peyster et al., 2009

Figure 1-7. Exposure-response array of reproductive effects following oral exposure to ETBE



Source: (A) Weng et al., 2014

Figure 1-8. Exposure-response array of reproductive effects following inhalation exposure to ETBE

Developmental effects

Developmental endpoints that were evaluated include pup survival and growth of fetus and pups. Two studies indicated maternal toxicity associated with exposure to ETBE based on decreases in maternal body weight (Asano et al., 2011; Gaoua, 2004a). However, one of the studies was in rabbits, and EPA's (1991b) developmental guidelines indicate that body weight change is not a useful indicator of maternal toxicity in rabbits. In addition, this same dose did not cause maternal toxicity in rat studies (Aso et al., 2014; Asano et al., 2011; Fujii et al., 2010; Gaoua, 2004b).

There was no significant effects of ETBE on pup survival as measured by pre- or post-implantation loss (Aso et al., 2014; Asano et al., 2011; Gaoua, 2004a), number of live births (Asano et al., 2011; JPEC, 2008h), pup viability at PND 4 including total litter loss (Fujii et al., 2010; Gaoua, 2004b), or lactational index (also called viability index on PND 21) (Fujii et al., 2010; Gaoua, 2004b).

Fetal and pup growth were also not affected by ETBE treatment (Aso et al., 2014; Asano et al., 2011; Fujii et al., 2010). Fujii et al. (2010) did not observe any effects in physical development or reflex ontogeny in the F1 offspring in a one-generation reproductive study nor was there an effect on sexual maturity observed in a two-generation study (Gaoua, 2004b). In section 1.1.1, increased kidney weights in F1 offspring are discussed. No differences were observed in external, skeletal, or visceral variations or malformations (Aso et al., 2014; Asano et al., 2011). Aso et al. (2014) reported a significant increase in rudimentary lumbar ribs, but the result was within the historical control range and vanished after birth.

Table 1-14. Evidence pertaining to prenatal developmental effects in animals following exposure to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Maternal Body Weight Gain (GD0-20)			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage F1, combined (NR): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -4% 8% 12%*
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 2% 3% 3%
	F1, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -1% -3% -6%
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -7% -4% -7%

Table 1-14. Evidence pertaining to prenatal developmental effects in animals following exposure to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Maternal Body Weight Gain (GD0-28)			
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand oral - gavage F1, combined (24/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d dams exposed from GD6 to GD27	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -13% 0% -38%*
Maternal Body Weight Gain (GD5-20)			
Gaoua (2004a) rat, Sprague-Dawley oral - gavage P0, female (24/group): 0, 250, 500, 1000 mg/kg-d dams exposed from GD5 to GD19	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -4% -3% -17%*
Postimplantation Loss[a]			
Gaoua (2004a) rat, Sprague-Dawley oral - gavage P0, female (24/group): 0, 250, 500, 1000 mg/kg-d dams exposed daily from GD5 to GD19	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 27% 38% 44%
Postimplantation Loss (Resorptions/Implantations)			
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 24% -28% -14%

Table 1-14. Evidence pertaining to prenatal developmental effects in animals following exposure to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Postimplantation Loss Per Litter			
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand oral - gavage F1, combined (24/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d dams exposed from GD6 to GD27	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 3% -36% -21%
Preimplantation Loss[b]			
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 38% 21% 82%
Gaoua (2004a) rat, Sprague-Dawley oral - gavage P0, female (24/group): 0, 250, 500, 1000 mg/kg-d dams exposed daily from GD5 to GD19	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -15% -17% -5%

[a]Post-implantation loss = (resorptions + dead fetus/ total implantations) × 100, calculated per litter.
[b]Pre-implantation loss = (corpora lutea-implantations/corpora lutea) × 100, calculated per litter.
*: result is statistically significant ($p<0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
(n): number evaluated from group.
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).

Table 1-15. Evidence pertaining to postnatal developmental effects in animals following exposure to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Live Births			
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -8% -12% -5%
Live Fetuses Per Litter			
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand oral - gavage F1, combined (24/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d dams exposed from GD6 to GD27	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 1% 8% -12%
Viability Index PND 4			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage F1, combined (NR): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	F1, Combined	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -1% 2% -10%

Table 1-15. Evidence pertaining to postnatal developmental effects in animals following exposure to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Viability Index PND 4 (continued)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -5% -16% 0%
	F1, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -3% -1% -5%
Total Litter Loss PND 4			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage F1, combined (NR): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Response (litters) 0/21 0/22 0/23 3/22
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Response 0/23 1/21 3/22 0/25
	F1, Female	Dose(mg/kg-d) 0 250 500 1000	Response (incidence) 0/21 1/21 0/22 1/20

Table 1-15. Evidence pertaining to postnatal developmental effects in animals following exposure to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Lactation Index[a]			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage F1, combined (NR): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -1% -1% -5%
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups	P0, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -3% 2% 5%
	F1, Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - 1% 2% 2%
Gravid Uterus Weight			
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand oral - gavage F1, combined (24/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d dams exposed from GD6 to GD27	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 4% 5% -16%

Table 1-15. Evidence pertaining to postnatal developmental effects in animals following exposure to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Fetal Body Weight			
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19	F1, Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		100	1%
		300	3%
		1000	1%
	F1, Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		100	0%
		300	2%
		1000	5%
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand oral - gavage F1, combined (24/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d dams exposed from GD6 to GD27	F1, Males	Dose(mg/kg-d)	Percent change compared to control
		0	-
		100	0%
		300	1%
		1000	-4%
	F1, Females	Dose(mg/kg-d)	Percent change compared to control
		0	-
		100	1%
		300	3%
		1000	-4%

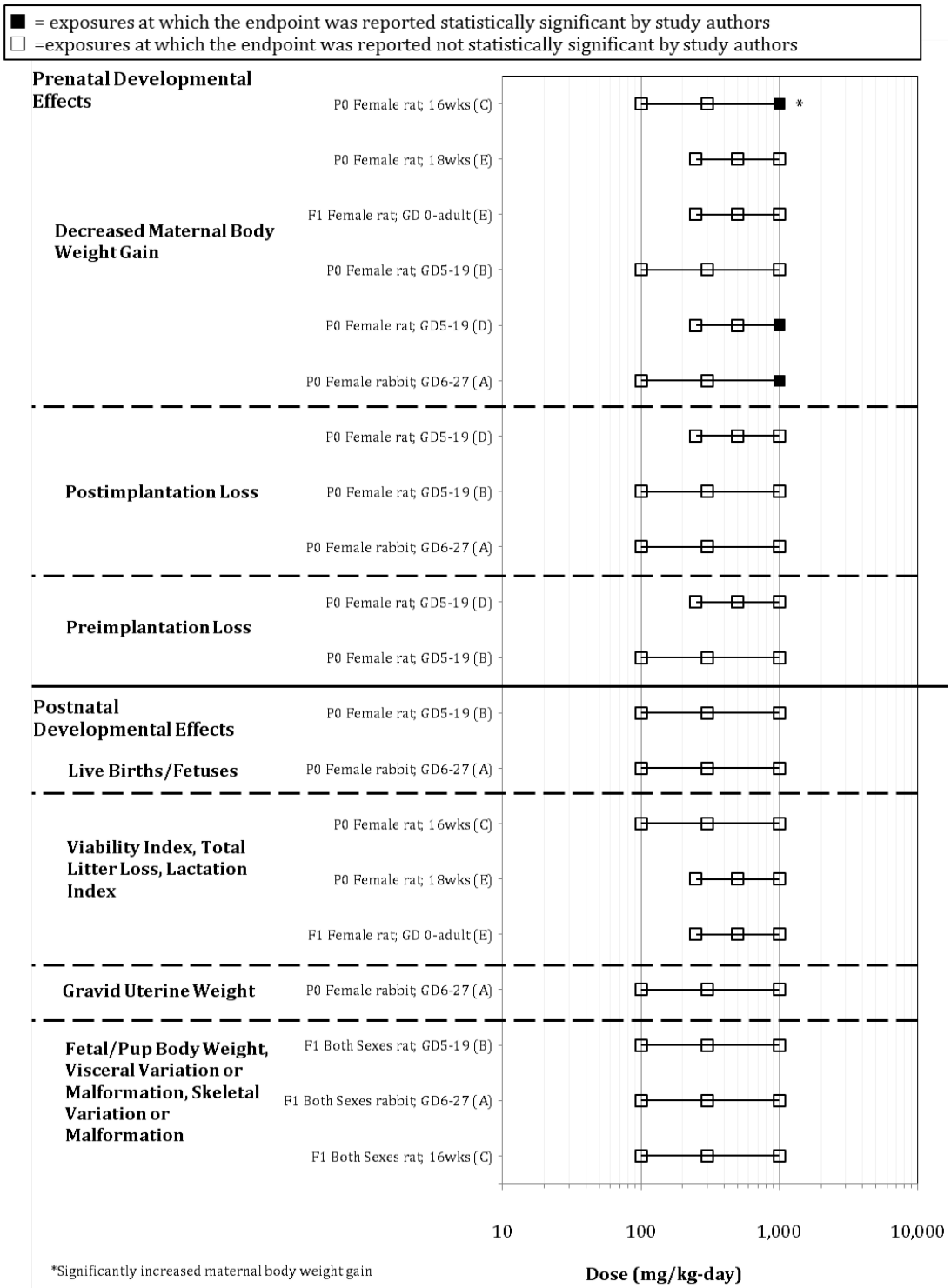
Table 1-15. Evidence pertaining to postnatal developmental effects in animals following exposure to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Body Weight (PND 21)			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage F1, male (84-92/group): 0, 100, 300, 1000 mg/kg-d dams exposed daily from GD0 to lactational day 21; F1 weanlings selected for observation of sexual development continued treatment for approximately 4 weeks		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		100	0%
		300	0%
		1000	0%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Female	0	-
		100	-1%
		300	-1%
		1000	1%
External Malformation			
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19		Dose(mg/kg-d)	Response (incidence)
	F1, Combined	0	0/285
		100	0/263
		300	0/251
		1000	0/270
Skeletal Variation or Malformation			
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19		Dose(mg/kg-d)	Response (incidence)
	F1, Combined	0	9/139
		100	3/126
		300	3/119
		1000	29/131
	mostly rudimentary lumbar rib, incidence was within historical range		

Table 1-15. Evidence pertaining to postnatal developmental effects in animals following exposure to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint
Skeletal Variation or Malformation (continued)	
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand oral - gavage F1, combined (24/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d dams exposed from GD6 to GD27	F1, Combined There were no significant differences in the incidence of skeletal malformations or variations.
Visceral Variation or Malformation	
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand oral - gavage F1, combined (24/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d dams exposed from GD6 to GD27	F1, Combined There was no significant difference in the incidence of fetuses with visceral malformations or variations, but there was a slight (dose-related) increase in the incidence of an absent right atrioventricular valve.
Aso et al. (2014); JPEC (2008h) rat, CRL:CD(SD) oral - gavage F1, combined (251-285/group): 0, 100, 300, 1000 mg/kg-d; F1, female (119-159/group): 0, 100, 300, 1000 mg/kg-d; P0, female (24/group): 0, 100, 300, 1000 mg/kg-d; F1, male (126-136/group): 0, 100, 300, 1000 mg/kg-d dams treated daily from GD5 to GD19	F1, Combined Dose(mg/kg-d): 0 — Response (incidence): 6/146 100 — 8/137 300 — 4/132 1000 — 8/139

[a]Lactation index = (pups alive at day 21/pups at day 4) × 100; LI is the same as viability index on day 21.
NR: not reported; *: result is statistically significant (p<0.05) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
(n): number evaluated from group.
Percentage change compared to control = (treated value − control value) ÷ control value × 100.



Sources: (A) Asano 2011; JPEC, 2008h (B) Aso 2014; JPEC, 2008g (C) Fujii, 2010; JPEC, 2008e (D) Gaoua, 2004a (E) Gaoua, 2004b

Figure 1-9. Exposure-response array of developmental effects following oral exposure to ETBE

Mechanistic Evidence

No mechanistic evidence is available for reproductive or developmental effects.

Summary of reproductive and developmental toxicity

The evidence for reproductive and developmental effects is entirely from animal studies. Reproductive endpoints were not consistently affected across studies. Subchronic but not chronic exposures to ETBE decreased rapid sperm movement at the highest tested dose. However, Aldh2 knockout or heterozygous mice had reduced number of sperm heads and sperm motility effects (i.e., number of sperm that were mobile, number of sperm that were static, sperm with rapid movement) associated with ETBE (Weng et al., 2014). These effects suggest that populations with Aldh2 polymorphism may be sensitive to reproductive effects (discussed in section 1.2.3). A single short-term exposure study reported an increase in estradiol levels in male rats that did not exhibit a dose response(de Peyster et al., 2009).

Of the endpoints assessed in two studies evaluating developmental effects, reduced maternal body weight was the only statistically significant effect reported (Asano et al., 2011; Gaoua, 2004a). This effect was not dose-responsive, was inconsistently observed, and did not correspond to any other maternal effects or effects in offspring.

EPA concluded that the evidence does not support reproductive or developmental effects as a potential human hazard of ETBE exposure.

1.1.4. Carcinogenicity (other than in the kidney or liver)

Synthesis of carcinogenicity data (other than in the kidney or liver)

This section reviews the studies that investigated whether exposure to ETBE can cause cancers (other than in the kidney or liver) in humans or animals. Tumorigenicity in the liver and kidney were previously discussed in the relevant organ-specific section and will not be discussed in this section. The database for ETBE carcinogenicity consists of only animal data: three 2-year studies, one 23-week initiation study, and one 31-week initiation study performed in rats (Hagiwara et al., 2013; Saito et al., 2013; Suzuki et al., 2012; Hagiwara et al., 2011; Malarkey and Bucher, 2011; JPEC, 2010a, b; Maltoni et al., 1999) (see Table 1-16, Table 1-17; Figure 1-9, Figure 1-10). One study conducted by Maltoni et al. (1999) had several methodological limitations such as only two treatment groups, nonstandard histopathological diagnoses, a nonstandard 4-day dosing schedule, and greater than expected mortality in treated groups and controls compared with other laboratories. In response to these concerns, a pathology working group (PWG) sponsored by U.S. EPA and the National Toxicology Program (NTP) reviewed the histopathological data (Malarkey and Bucher, 2011). In addition to recalculating tumor incidences, the PWG found that the respiratory infections in the study animals confound interpretation of leukemia and lymphoma. Thus, U.S. EPA will use the Malarkey and Bucher (2011) data when considering carcinogenicity in

place of the published Maltoni et al. (1999) study and will not consider leukemia and lymphoma from this study.

Following 2-year exposure to ETBE, the incidence of leiomyomas was increased in the uterus of rats in the high-dose group Maltoni et al. (1999). Malignant schwannomas in the uterus were increased only at the lowest dose and no significant trend was observed. Leiomyomas and a carcinoma were observed in uterine/vaginal tissue, but no significant trend was observed (Malarkey and Bucher, 2011). A statistically significant increase in incidence of neoplastic lesions was observed in the thyroid of male rats following subchronic exposure to ETBE after a 4-week tumor initiation exposure to DMBDD (Hagiwara et al., 2011). An increase in carcinomas of the urinary bladder also occurred (Hagiwara et al., 2013); however, subchronic exposure to ETBE via gavage without initiation using DMBDD treatment did not result in tumor development in any of the organs that previously demonstrated tumorigenicity (Hagiwara et al., 2011). The incidence of neoplastic lesions in the thyroid was dose-dependently increased, which demonstrate that ETBE possesses tumor promotion potential (Hagiwara et al., 2011). While increased incidences of tumorigenicity were observed in Hagiwara et al. (2011), a chronic drinking water study and chronic inhalation study failed to demonstrate significant increases in the incidence of tumors in any of these tissues (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010b).

Mechanistic Evidence

Available mechanistic evidence was previously discussed in the context of kidney and liver tumors (Sections 1.1.1 and 1.1.2).

Summary of Carcinogenicity Evidence

The evidence for carcinogenic effects not of the liver or kidney is all from rat studies. Tumor initiation increased the incidence of thyroid adenomas and carcinomas and urinary bladder carcinomas in male rats (Hagiwara et al., 2011); however, these results were not observed in the three 2-year bioassays. A statistically significant increase in the trend of uterine leiomyomas and leimyosarcomas was not observed (Malarkey and Bucher, 2011). Malignant schwannomas were increased at the lowest dose in the uterus/vagina in one study but these neoplasms arise from nervous tissue and are not specific to uterine tissue (Malarkey and Bucher, 2011). Low survival rates at 104 weeks (approximately 25%) in control groups confounds these data because it cannot be determined if tumors in the control group were not observed due to premature death. In addition, these results differed from two other 2-year bioassays, one oral and one inhalation (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). No methodological problems that could lead to false negative outcomes were identified in these two bioassays.

Confidence in the data demonstrating an increase in the incidence of schwannomas is low due to the lack of a similar effect in two other well-conducted studies. No mechanistic evidence is available to suggest that nervous tissue or uterine tissue are targets for ETBE carcinogenicity.

Table 1-16. Evidence pertaining to tumor promotion by ETBE in animals

Reference and Dosing Protocol	Results by Endpoint		
Colon Adenoma or Carcinoma			
Hagiwara et al. (2011); JPEC (2008d)		Dose(mg/kg-d)	Response (incidence)
rat, Fischer 344			
oral - gavage	Male	0	25/30
male (30/group): 0, 300, 1000 mg/kg-d		300	21/30
daily for 23 weeks following a 4 week tumor		1000	28/30*
initiation by DMBDD		0[+]	0/12
[+]no DMBDD initiation		1000[+]	0/12
Forestomach Papillomas			
Hagiwara et al. (2011); JPEC (2008d)		Dose(mg/kg-d)	Response (incidence)
rat, Fischer 344			
oral - gavage	Male	0	0/30
male (30/group): 0, 300, 1000 mg/kg-d		300	4/30
daily for 23 weeks following a 4 week tumor		1000	3/30
initiation by DMBDD		0[+]	0/12
[+]no DMBDD initiation		1000[+]	0/12
Thyroid Gland Adenoma or Carcinoma			
Hagiwara et al. (2011); JPEC (2008d)		Dose(mg/kg-d)	Response (incidence)
rat, Fischer 344			
oral - gavage	Male	0	8/30
male (30/group): 0, 300, 1000 mg/kg-d		300	17/30*
daily for 23 weeks following a 4 week tumor		1000	20/30*
initiation by DMBDD		0[+]	0/12
[+]no DMBDD initiation		1000[+]	0/12
Urinary Bladder Carcinoma			
Hagiwara et al. (2013)		Dose(mg/kg-d)	Response (incidence)
rat, F344/DuCrlCrlj			
oral - water	Male	0	5/30
male (30/group): 0, 100, 300, 500, 1000 mg/kg-d		100	7/30
daily for 31 weeks beginning one week after a 4		300	6/30
wk exposure to BBN		500	14/30*
		1000	9/26
Urinary Bladder Papilloma			
Hagiwara et al. (2013)		Dose(mg/kg-d)	Response (incidence)
rat, F344/DuCrlCrlj			
oral - water	Male	0	21/30
male (30/group): 0, 100, 300, 500, 1000 mg/kg-d		100	13/30
daily for 31 weeks beginning one week after a 4		300	17/30
wk exposure to BBN		500	17/30
		1000	21/26

Table 1-16. Evidence pertaining to tumor promotion by ETBE in animals (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Urinary Bladder Papilloma or Carcinoma			
Hagiwara et al. (2013) rat, F344/DuCrlCrlj oral - water male (30/group): 0, 100, 300, 500, 1000 mg/kg-d daily for 31 weeks beginning one week after a 4 wk exposure to BBN		Dose(mg/kg-d)	Response (incidence)
	Male	0	24/30
		100	18/30
		300	20/30
		500	25/30
		1000	21/26
Urinary Bladder Papillamotosis			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks following a 4 week tumor initiation by DMBDD [+]no DMBDD initiation		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/30
		300	0/30
		1000	10/30*
		0[+]	0/12
		1000[+]	2/12

Table 1-17. Evidence pertaining to carcinogenic effects (in tissues other than liver or kidney) in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Papillomas of the Oral Mucosa/Tongue			
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d reanalysis of data from Maltoni et al. (1999) where animals were dosed 4 d/wk for 104 weeks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/60
		250	0/60
		1000	0/60
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	0/60
		1000	0/60
Squamous Cell Carcinoma of Oral Mucosa/Tongue			
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d reanalysis of data from Maltoni et al. (1999) where animals were dosed 4 d/wk for 104 weeks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/60
		250	0/60
		1000	0/60
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	0/60
		1000	0/60

Table 1-17. Evidence pertaining to carcinogenic effects (in tissues other than liver or kidney) in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Thyroid Follicular Adenocarcinoma			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/50
		28	1/50
		121	0/50
		542	0/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/50
		46	1/50
		171	0/50
		560	0/50
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	0/50
		2090	0/50
		6270	0/50
		20,900	0/50
		Dose(mg/m^3)	Response (incidence)
	Female	0	1/50
		2090	1/50
		6270	1/50
		20,900	0/50
Thyroid Adenocarcinoma			
Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d 4 d/wk for 104 wks; observed until natural death; NOTE: These tumor data were not re-analyzed by Malarkey and Bucher (2011)		Dose(mg/kg-d)	Response (incidence)
	Male	0	0/60
		250	0/60
		1000	0/60
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	0/60
		1000	1/60

Table 1-17. Evidence pertaining to carcinogenic effects (in tissues other than liver or kidney) in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Thyroid Follicular Adenoma			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Male	0	1/50
		28	0/50
		121	0/50
		542	0/50
		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/50
		46	0/50
		171	0/50
		560	0/50
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Male	0	1/50
		2090	0/50
		6270	1/50
		20,900	2/50
		Dose(mg/m^3)	Response (incidence)
	Female	0	0/50
		2090	0/50
		6270	0/50
		20,900	0/50
Endometrial Stromal Sarcoma			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Female	0	6/50
		46	9/50
		171	3/50
		560	7/50
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Female	0	2/50
		2090	2/50
		6270	3/50
		20,900	2/50

Table 1-17. Evidence pertaining to carcinogenic effects (in tissues other than liver or kidney) in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Carcinoma of the Uterus/Vagina			
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d reanalysis of data from Maltoni et al. (1999) where animals were dosed 4 d/wk for 104 weeks		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	1/60
		1000	0/60
Uterine Leiomyosarcoma			
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d reanalysis of data from Maltoni et al. (1999) where animals were dosed 4 d/wk for 104 weeks		Dose(mg/kg-d)	Response (incidence)
	Female	0	1/60
		250	0/60
		1000	0/60
Uterine Leiomyoma			
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d reanalysis of data from Maltoni et al. (1999) where animals were dosed 4 d/wk for 104 weeks		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	0/60
		1000	3/60
Schwannoma of the Uterus/Vagina			
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d reanalysis of data from Maltoni et al. (1999) where animals were dosed 4 d/wk for 104 weeks		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	7/60
		1000	2/60
Uterine Adenocarcinoma			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Female	0	1/50
		46	0/50
		171	2/50
		560	2/50

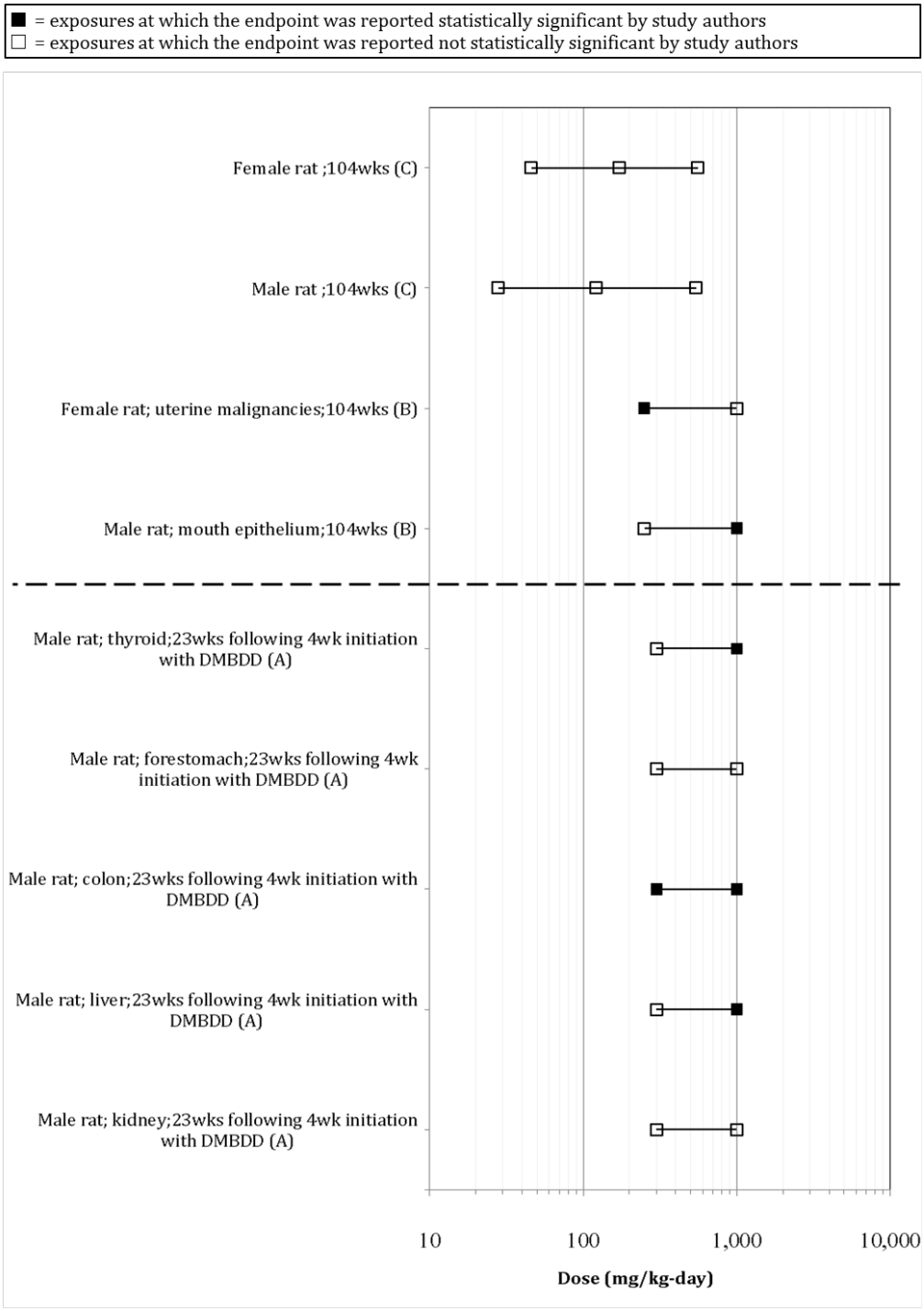
Table 1-17. Evidence pertaining to carcinogenic effects (in tissues other than liver or kidney) in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Uterine Adenocarcinoma (continued)			
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Response (incidence)
	Female	0	2/50
		2090	3/50
		6270	1/50
		20,900	4/50
Uterine Fibroma			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Response (incidence)
	Female	0	1/50
		46	0/50
		171	0/50
		560	0/50
Uterine Carcinoma			
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d 4 d/wk for 104 wks; observed until natural death		Dose(mg/kg-d)	Response (incidence)
	Female	0	0/60
		250	1/60
		1000	0/60

[a]Conversion performed by study authors.

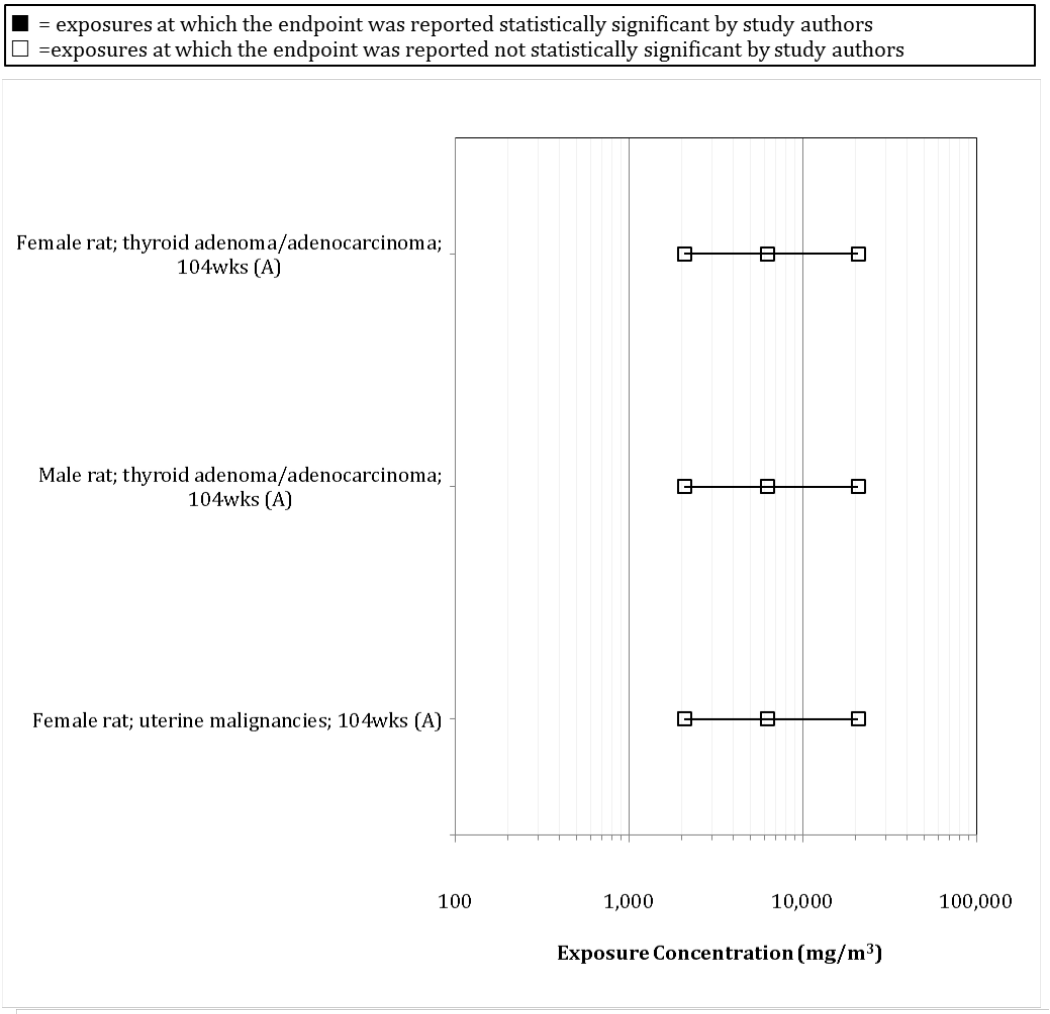
[b]4.18 mg/m^3 = 1 ppm.

[*]Statistically significant ($p \leq 0.05$) based on analysis of data conducted by study authors.



Sources: (A) Hagiwara et al., 2011; JPEC 2008d (B) Maltoni et al., 1999; Malarkey et al., 2011 (reanalysis of Maltoni et al., 1999) (C) Suzuki et al., 2012; JPEC, 2010a

Figure 1-9. Exposure-response array of carcinogenic effects following oral exposure to ETBE



Source: (A) Saito et al., 2013; JPEC, 2010b

Figure 1-10. Exposure-response array of carcinogenic effects following inhalation exposure to ETBE

1.1.5. Other Toxicological Effects

Synthesis of other toxicity data

The database for effects other than kidney, liver, reproductive, and cancer contain only 11 rodent studies. All selected studies employed inhalation, oral gavage, or drinking water exposures for ≥90 days. Shorter duration multiple exposure studies that examined immunological endpoints were also included. No studies were removed for methodological concerns.

Body weight

As presented in Table 1-18, body weights were significantly reduced compared with vehicle controls following 2-year oral and inhalation exposures to ETBE (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Reductions were also reported in studies of exposure durations shorter than 2 years (Hagiwara et al., 2011; Banton et al., 2011; Fujii et al., 2010; Gaoua, 2004b; JPEC, 2008b, c; Medinsky et al., 1999); however, these effects were frequently not statistically significant. Food consumption did not correlate well with body weight (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Water consumption was reduced in the 2-year oral exposure study (JPEC, 2010a). Palatability and reduced water consumption due to ETBE exposure may contribute to the reduced body weight, particularly for oral exposures. Ptyalism, which is frequently observed with unpalatable chemicals following gavage, was observed in rats gavaged for 18 weeks (Gaoua, 2004b). Body weight changes are poor indicators of systemic toxicity but are important when evaluating relative organ weight changes. Because body weight was most severely affected in 2-year studies, and 2-year organ weights are not appropriate for analysis as stated in Sections 1.1.1 and 1.1.2, this endpoint will not be considered further.

Adrenal weight

Adrenal weights were increased in 13-week and 26-week studies (see Table 1-19). For instance, a 13-week drinking water study found that relative adrenal weights were increased in male and female rats (Medinsky et al., 1999). In another study, absolute adrenal weights were increased in male rats (Hagiwara et al., 2011). None of the observed organ weight changes corresponded with functional or histopathological changes.

Immune system

Immunological endpoints yielded inconsistent results in a number of studies (see Table 1-20). Relative spleen weights were increased in male rats following 2-year oral and inhalation exposures to ETBE (Suzuki et al., 2012; JPEC, 2010b). CD3+, CD4+, and CD8+ T cells were reduced in male mice after 6 or 13 weeks of ETBE exposure via inhalation (Li et al., 2011). An analysis of antibody response reported that the number of IgM^+ splenic antibody forming cells was not significantly affected after a 28-day oral exposure to ETBE followed by sheep red blood cell

immunization (Banton et al., 2011). No other indicators of histopathological or functional changes were reported with a single chemical exposure.

Mortality

Mortality was significantly increased in male and female rats following a 2-year ETBE inhalation exposure (Saito et al., 2013; JPEC, 2010b) but not significantly affected following a 2-year drinking water exposure (Suzuki et al., 2012; JPEC, 2010a). Increased mortality in male rats correlated with increased CPN severity in the kidney. Increased mortality in females was attributed to pituitary tumors by the study authors; however, pituitary tumors were not dose responsively increased by ETBE exposure. Survival was also reduced in a chronic gavage study at the highest exposure in males and females at 72 weeks (data not shown); however, by 104 weeks survival in controls was approximately 25% in males and 28% in females which is much lower than anticipated for a 2-year study (Maltoni et al., 1999). Thus, additional confounding factors such as chronic respiratory infections may have contributed to the reduced survival. These data do not suggest that mortality was increased in these studies due to excessively high exposure concentrations of ETBE.

Mechanistic Evidence

No relevant mechanistic data are available for these endpoints.

Summary of other toxicity data

EPA concluded that the evidence does not support body weight changes, adrenal and immunological effects, and mortality as potential human hazards of ETBE exposure.

Table 1-18. Evidence pertaining to body weight effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Body Weight			
Banton et al. (2011) rat, Sprague-Dawley oral - gavage female (10/group): 0, 250, 500, 1000 mg/kg-d daily for 28 consecutive days		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		250	3%
		500	5%
		1000	-1%
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21 P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		100	-4%
		300	-4%
		1000	-7%
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		100	1%
		300	1%
		1000	5%

Table 1-18. Evidence pertaining to body weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Body Weight (continued)			
Gaoua (2004b) rat, Sprague-Dawley oral - gavage P0, male (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1000 mg/kg-d daily for a total of 18 weeks beginning 10 weeks before mating until PND 21 F1, male (25/group): 0, 250, 500, 1000 mg/kg-d dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups F1, female (24-25/group): 0, 250, 500, 1000 mg/kg-d P0 dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		250	-1%
		500	-3%
		1000	-5%*
		Dose(mg/kg-d)	Percent change compared to control
	F1, Male	0	-
		250	0%
		500	3%
		1000	1%
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		250	-7%
		500	-2%
		1000	0%
		Dose(mg/kg-d)	Percent change compared to control
	F1, Female	0	-
		250	-2%
		500	-3%
		1000	2%
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		1000	-5%*

Table 1-18. Evidence pertaining to body weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Body Weight (continued)			
Miyata et al. (2013);JPEC (2008c) rat, CRL:CD(SD) oral - gavage female (15/group): 0, 5, 25, 100, 400 mg/kg-d; male (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	-6%
		25	0%
		100	-5%
		400	2%
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		5	-5%
		25	-2%
		100	-2%
		400	-3%
Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d 4 d/wk for 104 wks; observed until natural death	Male no significant difference at any dose		
	Female no significant difference at any dose		
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a] daily for 104 wks	Male	Dose(mg/kg-d)	Percent change compared to control
		0	-
		28	-4%
		121	-7%*
		542	-9%*
	Female	Dose(mg/kg-d)	Percent change compared to control
		0	-
		46	-10%*
		171	-11%*
		560	-17%*

Table 1-18. Evidence pertaining to body weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Body Weight (continued)			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 627 2090 6270 20,900	Percent change compared to control - 0% 1% -1% -7%
	Female	Dose(mg/m^3) 0 627 2090 6270 20,900	Percent change compared to control - -6% -7% -7% -11%
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 20,900	Percent change compared to control - 3%
	Female	Dose(mg/m^3) 0 20,900	Percent change compared to control - 4%
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - 2% 4% 2%
	Female	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - -3% 3% 6%*

Table 1-18. Evidence pertaining to body weight effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Body Weight (continued)			
Medinsky et al. (1999); Bond et al. (1996b) mice, CD-1 inhalation - vapor female (40/group): 0, 500, 1750, 5000 ppm(0, 2090, 7320, 20,900 mg/m^3)[b]; male (40/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	0%
		7320	-1%
		20,900	-3%
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	-2%
		7320	-1%
		20,900	2%
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported		Dose(mg/m^3)	Percent change compared to control
	Male	0	-
		2090	-7%*
		6270	-7%*
		20,900	-26%*
		Dose(mg/m^3)	Percent change compared to control
	Female	0	-
		2090	-6%*
		6270	-10%*
		20,900	-23%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
Percent change compared to controls calculated as 100 × ((treated value – control value) ÷ control value).

Table 1-19. Evidence pertaining to adrenal effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Adrenal Gland: Absolute Weight			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks	Male	Dose(mg/kg-d) 0 1000	Percent change compared to control - 16%*
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - 11% 9% 34%*
	Female	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - 7% 7% 18%*
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation - vapor female (40/group): 0, 500, 1750, 5000 ppm(0, 2090, 7320, 20,900 mg/m^3)[b]; male (40/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - 0% 50% 0%
	Female	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - -8% 8% -8%
Adrenal Gland: Relative Weight			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks	Male	Dose(mg/kg-d) 0 1000	Percent change compared to control - 19%*

Table 1-20. Evidence pertaining to immune effects in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Sheep red blood cell- specific IgM Antibody Forming Cells/10^6 Spleen Cells			
Banton et al. (2011) rat, Sprague-Dawley oral - gavage female (10/group): 0, 250, 500, 1000 mg/kg-d daily for 28 consecutive days immunized i.v. 4 days prior to sacrifice with sheep red blood cells	Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -21% 42% 8%
Sheep red blood cell-specific IgM Antibody Forming Cells/Spleen			
Banton et al. (2011) rat, Sprague-Dawley oral - gavage female (10/group): 0, 250, 500, 1000 mg/kg-d daily for 28 consecutive days immunized i.v. 4 days prior to sacrifice with sheep red blood cells	Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -20% 36% 8%
Number of CD3+ T cells			
Li et al. (2011) mice, C57BL/6 inhalation – vapor male (6/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d /wk over 6 wks; generation method not reported; analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -14% -13% -24%*
Li et al. (2011) mice, 129/SV inhalation - vapor male (6/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 6 wks; generation method not reported; analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -18%* -16% -21%*

Table 1-20. Evidence pertaining to immune effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Number of CD4+ T cells			
Li et al. (2011) mice, C57BL/6 inhalation - vapor male (6/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 6 wks; generation method not reported; analytical concentration and method were reported		Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -15% -11% -23%*
Li et al. (2011) mice, 129/SV inhalation - vapor male (6/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 6 wks; generation method not reported; analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -16% -11% -17%*
Li et al. (2011) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 13 wks; generation method not reported; analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -9% -17%* -24%*
Li et al. (2011) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 13 wks; generation method not reported; analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -11% -28%* -37%*
Number of CD8+ T cells			
Li et al. (2011) mice, C57BL/6 inhalation - vapor male (6/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 6 wks	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -12% -13%* -23%*

Table 1-20. Evidence pertaining to immune effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Number of CD8+ T cells (continued)			
Li et al. (2011) mice, 129/SV inhalation - vapor male (6/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 6 wks; generation method not reported; analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -13% -14% -25%
Li et al. (2011) mice, C57BL/6 inhalation - vapor male (5/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hrs/d for 5 d/wk over 13 wks; generation method not reported; analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20900	Percent change compared to control - -8% -12% -20%
Spleen: Absolute Weight			
Banton et al. (2011) rat, Sprague-Dawley oral - gavage female (10/group): 0, 250, 500, 1000 mg/kg-d daily for 28 consecutive days	Female	Dose(mg/kg-d) 0 250 500 1000	Percent change compared to control - -3% -15% -9%
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	P0, Male	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - -4% -2% 0%
	P0, Female	Dose(mg/kg-d) 0 100 300 1000	Percent change compared to control - 0% -2% -1%
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks	Male	Dose(mg/kg-d) 0 1000	Percent change compared to control - -5%

Table 1-20. Evidence pertaining to immune effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Spleen: Absolute Weight (continued)			
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male	Dose(mg/kg-d) 0 628 121 542	Percent change compared to control - -3% 19% 39%
	Female	Dose(mg/kg-d) 0 46 171 560	Percent change compared to control - -35% -1% -50%*
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 627 2090 6270 20,900	Percent change compared to control - 0% 7% -1% -9%
	Female	Dose(mg/m^3) 0 627 2090 6270 20,900	Percent change compared to control - -9% -2% -5% 1%
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 20,900	Percent change compared to control - 10%
	Female	Dose(mg/m^3) 0 20,900	Percent change compared to control - 6%

Table 1-20. Evidence pertaining to immune effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Spleen: Absolute Weight (continued)			
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation - vapor female (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b]; male (48/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - 6% 3% 5%
	Female	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - -3% 3% 0%
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation - vapor female (40/group): 0, 500, 1750, 5000 ppm(0, 2090, 7320, 20,900 mg/m^3)[b]; male (40/group): 0, 500, 1750, 5000 ppm (0, 2090, 7320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - -5% 0% -15%
	Female	Dose(mg/m^3) 0 2090 7320 20,900	Percent change compared to control - -11% -2% -11%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 6270 20,900	Percent change compared to control - 4% 32% 17%
	Female	Dose(mg/m^3) 0 2090 6270 20,900	Percent change compared to control - 5% -39% -43%*

Table 1-20. Evidence pertaining to immune effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Spleen: Relative Weight			
Banton et al. (2011) rat, Sprague-Dawley oral - gavage female (10/group): 0, 250, 500, 1000 mg/kg-d daily for 28 consecutive days		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		250	0%
		500	-18%
		1000	0%
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral - gavage P0, male (24/group): 0, 100, 300, 1000 mg/kg-d daily for 16 weeks beginning 10 weeks prior to mating P0, female (24/group): 0, 100, 300, 1000 mg/kg-d daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21		Dose(mg/kg-d)	Percent change compared to control
	P0, Male	0	-
		100	-1%
		300	2%
		1000	8%
		Dose(mg/kg-d)	Percent change compared to control
	P0, Female	0	-
		100	-2%
		300	-3%
		1000	-5%
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral - gavage male (12/group): 0, 1000 mg/kg-d daily for 23 weeks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		1000	0%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks		Dose(mg/kg-d)	Percent change compared to control
	Male	0	-
		628	2%
		121	28%
		542	55%*
		Dose(mg/kg-d)	Percent change compared to control
	Female	0	-
		46	-35%
		171	3%*
		560	-45%

Table 1-20. Evidence pertaining to immune effects in animals exposed to ETBE (*continued*)

Reference and Dosing Protocol	Results by Endpoint		
Spleen: Relative Weight			
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3); male (NR): 0, 150, 500, 1500, 5000 ppm (0, 627, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	0%
		2090	5%
		6270	1%
		20,900	-2%
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		627	-3%
		2090	5%
		6270	1%
		20,900	12%
JPEC (2008b) rat, CRL:CD(SD) inhalation - vapor female (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b]; male (6/group): 0, 5000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks followed by a 28 day recovery period; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		20,900	6%
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		20,900	0%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	15%
		6270	43%*
		20,900	66%*
	Female	Dose(mg/m^3)	Percent change compared to control
		0	-
		2090	30%
		6270	-31%
		20,900	-25%

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors
-: for controls, no response relevant; for other doses, no quantitative response reported
(n): number evaluated from group

Table 1-21. Evidence pertaining to mortality in animals exposed to ETBE

Reference and Dosing Protocol	Results by Endpoint		
Survival at 104 wks			
Maltoni et al. (1999) rat, Sprague-Dawley oral - gavage female (60/group): 0, 250, 1000 mg/kg-d; male (60/group): 0, 250, 1000 mg/kg-d 4 d/wk for 104 wks; observed until natural death	Male	Dose(mg/m^3) 0 250 1000	Percent change compared to control - -8% -54%
	Female	Dose(mg/m^3) 0 250 1000	Percent change compared to control - -8% 18%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral - water female (50/group): 0, 625, 2500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a]; male (50/group): 0, 625, 2500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a] daily for 104 wks	Male	Dose(mg/kg-d) 0 628 121 542	Percent change compared to control - -3% -11% -11%
	Female	Dose(mg/kg-d) 0 46 171 560	Percent change compared to control - 3% 6% 6%
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation - vapor female (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b]; male (50/group): 0, 500, 1500, 5000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	Male	Dose(mg/m^3) 0 2090 6270 20,900	Percent change compared to control - -14% -9% -32%*
	Female	Dose(mg/m^3) 0 2090	Percent change compared to control - 3%

Reference and Dosing Protocol	Results by Endpoint	
	6270	-21%*
	20,900	-21%*

[a]Conversion performed by study authors.

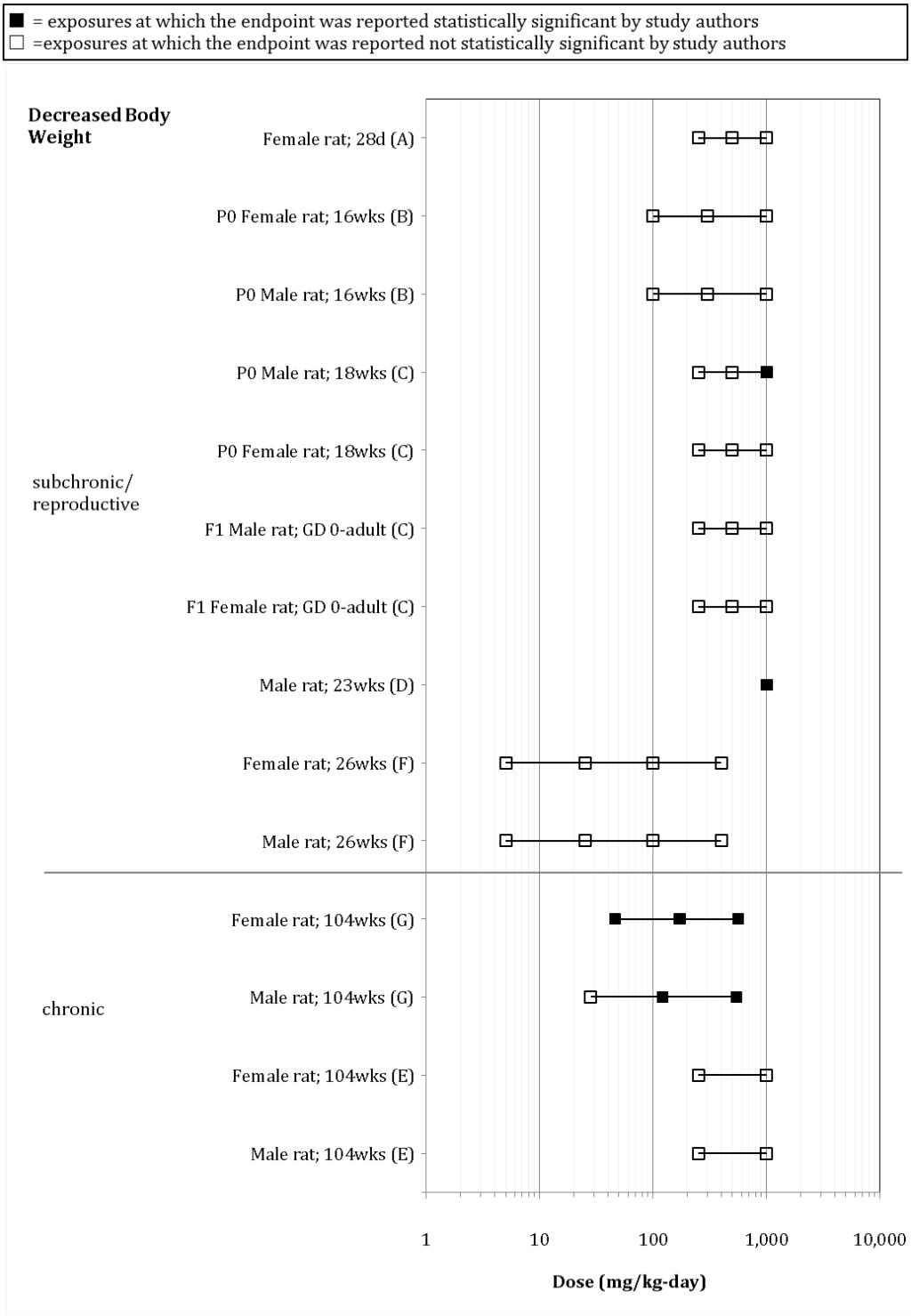
[b]4.18 mg/m^3 = 1 ppm.

NR: not reported; *: result is statistically significant ($p<0.05$) based on analysis of data by study authors

-: for controls, no response relevant; for other doses, no quantitative response reported

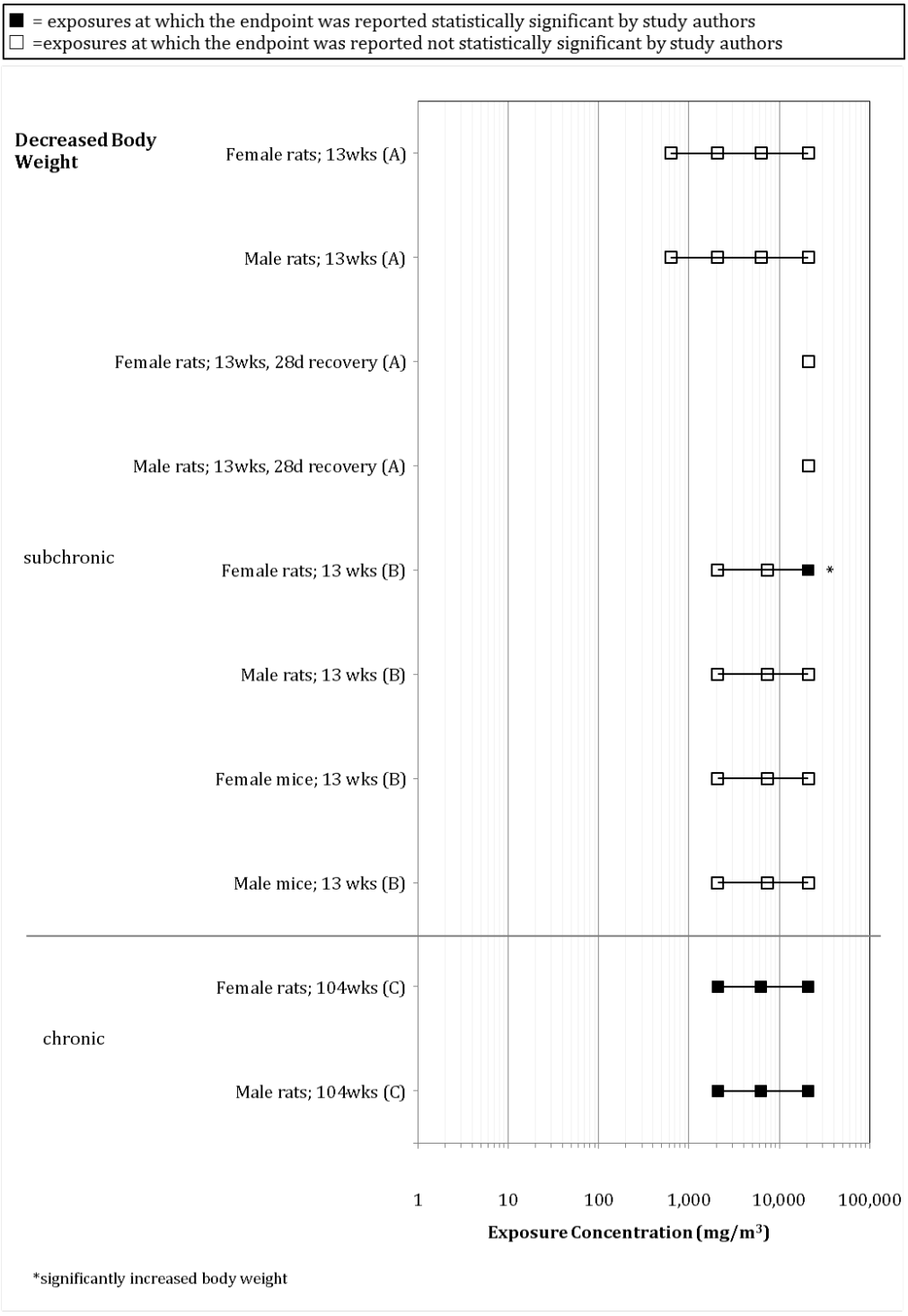
(n): number evaluated from group

1



Sources: (A) Banton et al., 2011 (B) Fujii et al., 2010; JPEC, 2008e (C) Gaoua, 2004b (D) Hagiwara et al., 2011 (E) Maltoni et al., 1999 (F) Miyata et al., 2013; JPEC, 2008c (G) Suzuki et al., 2012; JPEC, 2010a

Figure 1-11. Exposure-response array of body weight effects following oral exposure to ETBE



Sources: (A) JPEC, 2008b (B) Medinsky et al., 1999; Bond et al., 1996 (C) Saito et al., 2013; JPEC, 2010b

Figure 1-12. Exposure-response array of body weight effects following inhalation exposure to ETBE

1.2. INTEGRATION AND EVALUATION

1.2.1. Effects Other Than Cancer

The evidence for noncancer effects associated with ETBE is entirely from rodent studies. Kidney and liver were the most frequently affected endpoints following oral and inhalation exposure to ETBE.

Changes in kidney parameters were consistently observed but the magnitude of change was generally moderate while males had greater severity of effects compared with females. Overall, there was consistency across multiple measures of potential kidney toxicity, including organ weight increases, exacerbated CPN, urothelial hyperplasia, and increases in serum markers of kidney function such as cholesterol, BUN, and creatinine. Additionally, effects were consistently observed across routes of exposure, species, and sex although male rats appear more sensitive than female rats, and rats in general appear more sensitive than mice. Mechanistic data were insufficient to establish a mode of action, and thus these effects are considered relevant to humans. EPA identified kidney effects as a human hazard of ETBE exposure.

Increased liver weight and centrilobular hypertrophy in male and female rats were consistently observed across studies. However, no additional histopathological findings were observed, and only one serum marker of liver toxicity (GGT) was elevated, while other markers (AST, ALT, and ALP) were not. The magnitude of change for these noncancer effects was mild to moderate and, except for organ weight data, did not exhibit consistent dose-response relationships. Mechanistic data suggest ETBE exposure leads to activation of several nuclear receptors, but a relationship between receptor activation and liver toxicity has not been established for ETBE. Additionally, mechanistic data suggest possible susceptibility related to reduced clearance of acetaldehyde, a metabolite of ETBE, as discussed below in Section 1.2.3. EPA concluded that the evidence does not support liver effects as a potential human hazard of ETBE exposure. Thus, these effects were not considered further for dose-response analysis and the derivation of reference values. Potential for liver carcinogenicity is discussed in the following section.

EPA concluded that the evidence does not support body weight changes, adrenal, immunological, reproductive and developmental effects, and mortality as potential human hazards of ETBE exposure. Thus, these effects were not considered further for dose-response analysis and the derivation of reference values.

1.2.2. Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for ETBE provides "suggestive evidence of carcinogenic potential." This is based on induction of hepatocellular adenomas and carcinomas (combined) at the highest dose in male F344 rats by inhalation (Saito et al., 2013; JPEC, 2010b), but not in female rats in the same study or in either sex of two strains of rats exposed orally to ETBE (Suzuki et al., 2012; Malarkey and Bucher, 2011; JPEC,

2010a; Maltoni et al., 1999). Additionally, there is an absence of data in other experimental species or in humans, and limited mechanistic data.

EPA evaluated the available mechanistic data and concluded that the evidence related to putative pathways PPAR, PXR, and CAR was insufficient to determine the role these pathways play, if any, in tumor formation. Genotoxicity data for ETBE and its metabolite *tert*-butanol are inadequate to form a conclusion about ETBE's potential for genotoxicity. Additional mechanistic studies reported that deficient function of Aldh2 enhanced ETBE-induced genotoxicity in hepatocytes and leukocytes (Weng et al., 2013; Weng et al., 2012). These findings are consistent with genotoxicity being mediated by the ETBE metabolite acetaldehyde, which is directly genotoxic (IARC, 1999) and considered carcinogenic when produced as a result of metabolism from ingested ethanol (IARC, 2012). A mechanistic study conducted by gavage in rats reported ETBE-related increases in thyroid, urinary bladder, and liver tumors following initiation by DMBDD, suggesting that ETBE exposure promotes tumors (Hagiwara et al., 2011). Thus, these mechanistic data provide some biological plausibility to the carcinogenicity of ETBE.

The chronic gavage bioassay reported an increased incidence of schwannomas (Malarkey and Bucher, 2011; Maltoni et al., 1991), but confidence in these data are low as the increase was small, only observed at the lowest dose, and not accompanied by any mechanistic data supporting their biological plausibility.

As emphasized in the Cancer Guidelines (U.S. EPA, 2005a), selection of the cancer descriptor followed a full evaluation of the available evidence. The descriptor of "suggestive evidence of carcinogenic potential" is appropriate when a concern for potential carcinogenic effects in humans is raised, but the data are judged to be insufficient for a stronger conclusion. Exposure to ETBE produced a clearly positive tumor response at only one tissue (liver), one dose (highest), and one sex/species combination (male rats). Thus, these data correspond most closely to one of the examples in the Cancer Guidelines (U.S. EPA, 2005a) for the descriptor of "suggestive evidence of carcinogenic potential;" i.e., "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'likely to be carcinogenic to humans'." Overall, the cancer descriptor "suggestive evidence of carcinogenic potential" is plausible given that some concern for carcinogenic effects in humans is raised by the presence of a single positive result at one dose in one study and some biological plausibility provided by the available mechanistic data, including the metabolism of ETBE to acetaldehyde.

The Cancer Guidelines (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there are convincing toxicokinetic data that absorption does not occur by other routes. In the case of ETBE, the positive tumor response was in a tissue (liver) remote from the site of absorption

(respiratory tract). Although both oral and inhalation routes have been tested, all the bioassays were in a single species (rats). Absorption of ETBE via inhalation, oral, or dermal routes either has been demonstrated experimentally or is expected based on chemical properties. Therefore, the conclusion that ETBE presents "suggestive evidence of carcinogenic potential" applies to all routes of exposure.

1.2.3. Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

Genetic polymorphisms of ALDH, the enzyme that oxidizes acetaldehyde to acetic acid, may also affect potential ETBE liver toxicity. The virtually inactive form, ALDH2*2, is responsible for alcohol intolerance and is found in about one-half of all East Asians (Brennan, 2002). This variant is associated with slow metabolism of acetaldehyde and, hence, extended exposure to a genotoxic compound. With respect to ETBE exposure, the ALDH2*2 variant should increase any type of risk associated with acetaldehyde produced by ETBE metabolism because it will prolong internal exposure to this metabolite. As demonstrated in several in vivo and in vitro genotoxic assays in *Aldh2* knockout mice, genotoxicity was significantly increased compared with wild type controls following ETBE exposure to similar doses where both cancer and noncancer effects were observed (Weng et al., 2014; Weng et al., 2013; Weng et al., 2012; Weng et al., 2011). Studies in *Aldh2* knockout mice observed elevated blood concentrations of acetaldehyde following ETBE exposure compared with wild type mice (Weng et al., 2013) as well as increased alterations to sperm and male reproductive tissue (Weng et al., 2014) and increased severity of centrilobular hypertrophy (Weng et al., 2013; Weng et al., 2012). Notably, a consistent finding in these studies was increased severity of genotoxicity in males compared with females which corresponds with increased incidence of hepatic tumors only in male rats (Saito et al., 2013; JPEC, 2010b). No mode-of-action information exists to account for the sex discrepancies in genotoxic effects. Finally, (IARC, 2012; IARC (1999b)) identified acetaldehyde produced as a result of ethanol metabolism as the predominant cause of carcinogenesis in the upper aerodigestive tract and esophagus following ethanol ingestion, with effects amplified by deficient acetaldehyde metabolism in humans. Altogether, these data present plausible evidence that diminished Aldh2 activity yields health effect outcomes that are more severe than those in wild type counterparts. It is reasonable to assume similar outcomes could occur in sensitive human populations.

No other specific potential polymorphic-related susceptibility issues were reported in the literature. CYP2A6 is likely to be the P450 isoenzyme in humans to cleave the ether bond in ETBE. It also exists in an array of variants, and it is clear that at least one variant (2A6*4) has no catalytic activity (Fukami et al., 2004); however, the effect of this variability on ETBE toxicity is unknown. Finally, specific age-related susceptibility to ETBE is not indicated by the data.

2. DOSE-RESPONSE ANALYSIS

2.1. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

The reference dose (RfD) (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

2.1.1. Identification of Studies and Effects for Dose-Response Analysis

EPA identified kidney effects as a human hazard of ETBE exposure. Studies were evaluated using general study quality characteristics (as discussed in Section 6 of the Preamble) to help inform the selection of studies from which to derive toxicity values. Rationale for selection of studies and effects representative of this hazard is summarized below.

Human studies are preferred over animal studies when quantitative measures of exposure are reported and the reported effects are determined to be associated with exposure. However, there are no available human occupational or epidemiological studies of oral exposure to ETBE.

Animal studies were evaluated to determine which studies provided: (a) the most relevant routes and durations of exposure; (b) multiple exposure levels that informed the shape of the dose-response curve; and (c) the power to detect effects at low exposure levels (U.S. EPA, 2002). The database for ETBE includes several studies and data sets that are suitable for use in deriving reference values. Specifically, effects associated with ETBE exposure in animals included observations of organ weight and histological changes in the kidney in several chronic and subchronic studies, mostly in rats. Sufficient data were available to develop a PBPK model in rats for both oral and inhalation exposure in order to perform route-to-route extrapolation, so rat studies from both routes of exposure were considered for dose-response analysis.

Kidney Toxicity

The kidney was identified as the only human hazard of ETBE exposure based on findings of organ weight changes, histopathology (nephropathy, urothelial hyperplasia), and altered serum biomarkers (cholesterol, creatinine, BUN) in rats. The most consistent findings across studies were for kidney weight changes and urothelial hyperplasia. In the case of kidney weight changes, numerous chronic and subchronic studies investigated this endpoint following oral and inhalation exposure (Miyata et al., 2013; Saito et al., 2013; Suzuki et al., 2012; Hagiwara et al., 2011; Fujii et al.,

2010; JPEC, 2010b, 2008b, c; Gaoua, 2004b; Medinsky et al., 1999). For urothelial hyperplasia, chronic studies by both inhalation and oral exposure reported this effect to be increased with treatment in male rats (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Changes in serum biomarkers lacked consistency and strength of association and were not considered for modeling.

Hagiwara et al. (2011), with only one dose group, was not considered further given its concordance with multiple other rat studies that had multiple groups. Additionally, as discussed in Section 1.1.1, 2-year organ weight data were not considered suitable due to the prevalence of age-associated confounders. Therefore, only the urothelial hyperplasia data from the JPEC (2010a) [selected data published as Suzuki et al. (2012)] and JPEC (2010b) [selected data published as Saito et al. (2013)] studies were considered for dose-response analysis. These and the remaining studies, JPEC (2008c) [selected data published as Miyata et al. (2013)], Gaoua (2004b), Fujii et al. (2010), JPEC (2008b), Medinsky et al. (1999), and Suzuki et al. (2012) , are discussed further below.

Oral studies

The (Suzuki et al., 2012; JPEC, 2010a) study treated male and female F344 rats (50/sex/dose group) with ETBE via drinking water at dose levels of 0, 28, 121, or 542 mg/kg-day in males for 104 consecutive weeks. Increased incidence of slight urothelial hyperplasia was only observed in males and significantly increased at 121 and 542 mg/kg-day. Similar effects were not observed in females.

The JPEC (2008c) study treated male and female Crl:CD(SD) rats (15/sex/dose group) with ETBE via gavage at dose levels of 0, 5, 25, 100, or 400 mg/kg-day daily for 180 consecutive days (26 weeks). Relative kidney weight was significantly increased in males and females treated with 100 or 400 mg/kg-day. Abnormal histopathological findings in the kidney (basophilic tubules and hyaline droplets) were observed in male rats, but not in female rats. As discussed in Section 1.1.1., although an increase in α_{2u}-globulin was measured by immunohistochemical staining, there was inadequate evidence to conclude that the observed kidney effects are the result of α_{2u}-globulin accumulation.

A two-generation reproductive toxicity study of ETBE was conducted in rats by Gaoua (2004b). Sprague-Dawley rats (25/sex/dose group) were administered ETBE via gavage for 18 weeks at dose levels of 0, 250, 500, or 1000 mg/kg-day that commenced 10 weeks before mating and continued throughout the 2-week mating period, gestation, and end of lactation (PND 21) for a total of 18 weeks. Absolute and relative kidney weights were increased in all dose groups in males, which was associated with the presence of acidophilic globules in renal tissue from 5/6 males examined. In addition, tubular basophilia (4/6), peritubular fibrosis (3/6), and proteinaceous casts (1/6) were observed in kidneys of male rats at the high dose. Similar microscopic effects in females were not observed.

A one-generation reproductive toxicity study of ETBE was conducted in rats by Fujii et al. (2010). Male and female Crl:CD(SD) rats (24/sex/dose group) were administered ETBE via gavage

at dose levels of 0, 100, 300, or 1000 mg/kg-day beginning 10 weeks prior to F0 mating and continuing throughout the reproduction period (mating, gestation, and lactation). Treatment durations were stated to be approximately 16 weeks for males and 17 weeks for females but ranged up to 20 weeks in animals that took longer to mate. Kidney weights were significantly increased in F0 males and females at 1000 mg/kg-day. F0 males had a dose-dependent increase in relative kidney weight with statistically significant increases in all three dose groups.

Inhalation studies

The (Saito et al., 2013; JPEC, 2010b) study treated male and female F344 rats (50/sex/dose group) with ETBE via inhalation at dose levels of 0, 2090, 6270, or 20,900 mg/m^3 in males and females for 104 consecutive weeks. Increased incidences of slight urothelial hyperplasia were only observed in males and significantly increased at 6270 and 20,900 mg/m^3. Similar effects were not observed in females.

In a subchronic-duration inhalation study, JPEC (2008b) exposed male and female Crl:CD(SD) rats to ETBE via whole-body inhalation exposure at 0, 626.8, 2089, 6268, or 20,894 mg/m^3 for 6 hours/day, 5 days/week, for 13 weeks (65 exposures total). There were no significant differences in body weight throughout the study period for males or females. Significant increases in relative kidney weights occurred in male and female rats exposed to 6268 or 20,894 mg/m^3 ETBE compared with controls. After a recovery period of 28 days, the only remaining effect observed was an increase in kidney weight in high-dose males.

Medinsky et al. (1999) exposed male and female F344 rats in whole-body chambers to 0, 2089, 8358, or 16,717 mg/m^3 ETBE 6 hours/day, 5 days/week, for 13 weeks. At termination, body weights of female rats in the 16,717-mg/m^3 group were significantly higher than controls, but body weights of other groups, both male and female, did not differ significantly from those of controls. Slight, but statistically significant, increases in various clinical chemistry parameters were observed, but these effects were reported to be of uncertain toxicological significance.

Medinsky et al. (1999) also exposed male and female CD-1 mice in whole-body chambers to 0, 2089, 7313, or 20,894 mg/m^3 ETBE for 6 hours/day, 5 days/week, for 13 weeks. No statistically significant effects were noted in the kidney.

2.1.2. Methods of Analysis

No biologically based dose-response models are available for ETBE. In this case, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, all models available in EPA's Benchmark Dose Software (BMDS) were evaluated. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b), the benchmark dose (BMD) and the 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) of 10% change from the control

mean (Relative Deviation; RD) for organ weight data in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparison across endpoints, studies, and assessments. A benchmark response (BMR) of 10% extra risk was considered appropriate for the quantal data on incidences of slight urothelial hyperplasia. The estimated BMDLs were used as points of departure (PODs). Further details including the modeling output and graphical results for the best fit model for each endpoint can be found in Appendix C of the Supplemental Information.

In general, absolute and relative kidney weight data may both be considered appropriate endpoints for analysis. Body weight, which may impact interpretation of relative organ weights, was not significantly affected in the studies chosen. Based on a historical review of 26 studies of 1-month exposed control rats, Bailey et al. (2004) concluded that neither absolute kidney weight nor relative kidney:body (or kidney:brain) weight are optimal for evaluating organ weight changes. As neither approach is preferred, both were considered to be appropriate for BMD analysis.

PODs from Oral Studies

Human equivalent doses (HEDs) for oral exposures were derived from the PODs estimated from the laboratory animal data as described in EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011). In this guidance, EPA advocates a hierarchy of approaches for deriving HEDs from data in laboratory animals, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches can include using chemical-specific information in the absence of a complete physiologically based toxicokinetic model. As discussed in Appendix D of the Supplemental Information, several rat physiologically based pharmacokinetic (PBPK) models for ETBE have been developed and published, but a validated human PBPK model for ETBE for extrapolating doses from animals to humans is not available. In lieu of either chemical-specific models or data to inform the derivation of human equivalent oral exposures, a body weight scaling to the ¾ power (i.e., BW$^{3/4}$) approach is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans for the purpose of deriving an oral RfD. BW$^{3/4}$ scaling was not employed for deriving HEDs from studies in which doses were administered directly to early postnatal animals, because of the absence of information on whether allometric (i.e., body weight) scaling holds when extrapolating doses from neonatal animals to adult humans due to presumed toxicokinetic and/or toxicodynamic differences between lifestages (U.S. EPA, 2011; Hattis et al., 2004).

Consistent with EPA guidance (U.S. EPA, 2011), the PODs estimated based on effects in adult animals are converted to HEDs employing a standard dosimetric adjustment factor (DAF) derived as follows:

$$DAF = (BW_a^{1/4} / BW_h^{1/4})$$

where:

BW_a = animal body weight

BW_h = human body weight

Using a standard BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAFs for rats is 0.24. The DAF would be applied to the POD identified for effects in adult rats as follows to yield a POD_{HED} (see Table 2-1):

POD_{HED} = Laboratory animal dose (mg/kg-day) × DAF

Table 2-1 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD for each data set discussed above.

Table 2-1. Summary of derivation of PODs

Endpoint and Reference	Species/ Sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{ADJ}[b] (mg/kg-d)	POD_{HED}[c] (mg/kg-d)
Kidney							
Increased urothelial hyperplasia (Suzuki et al., 2012; JPEC, 2010a)	Male Fischer rats	Quantal-Linear	10%	79.3	60.5	60.5	14.5
Increased absolute kidney weight JPEC (2008c); Miyata et al. (2013)	Male Sprague-Dawley rats	Linear	10% RD	176	115	115	27.6
Increased relative kidney weight JPEC (2008c); Miyata et al. (2013)	Male Sprague-Dawley rats	NOAEL (25 mg/kg-d) (6% ↑ in kidney weight)				25	6.0
Increased absolute kidney weight JPEC (2008c); Miyata et al. (2013)	Female Sprague-Dawley rats	Exponential (M4)	10% RD	224	57	57	13.7
Increased relative kidney weight JPEC (2008c); Miyata et al. (2013)	Female Sprague-Dawley rats	Hill	10% RD	191	20	20	4.8

Table 2-1. Summary of derivation of PODs (*continued*)

Endpoint and Reference	Species/ Sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{ADJ}[b] (mg/kg-d)	POD_{HED}[c] (mg/kg-d)
Increased absolute kidney weight (P0 generation) Gaoua (2004b)	Male Sprague-Dawley rats	Hill	10% RD	244	94	94	22.6
Increased relative kidney weight (P0 generation) Gaoua (2004b)	Male Sprague-Dawley rats	Hill	10% RD	224	137	137	32.9
Increased absolute kidney weight (P0 generation) Gaoua (2004b)	Female Sprague-Dawley rats	Exponential (M2)	10% RD	1734	1030	1030	247
Increased relative kidney weight (P0 generation) Gaoua (2004b)	Female Sprague-Dawley rats	NOAEL (1000 mg/kg-d) (5% ↑ in kidney weight)				1000	240
Increased absolute kidney weight (F1 generation) Gaoua (2004b)	Male Sprague-Dawley rats	Polynomial 3°	10% RD	318	235	235	56.4
Increased relative kidney weight (F1 generation) Gaoua (2004b)	Male Sprague-Dawley rats	LOAEL (250 mg/kg-d) (10% ↑ in kidney weight)				250	60
Increased absolute kidney weight (F1 generation) Gaoua (2004b)	Female Sprague-Dawley rats	Exponential (M2)	10% RD	978	670	670	161
Increased relative kidney weight (F1 generation) Gaoua (2004b)	Female Sprague-Dawley rats	NOAEL (500 mg/kg-d) (6% ↑ in kidney weight)				500	120
Increased absolute kidney weight (P0 generation) Fujii et al. (2010)	Male Sprague-Dawley rats	Hill	10% RD	435	139	139	33.4
Increased relative kidney weight (P0 generation) Fujii et al. (2010)	Male Sprague-Dawley rats	Hill	10% RD	243	129	129	31.0

Table 2-1. Summary of derivation of PODs (*continued*)

Endpoint and Reference	Species/ Sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{ADJ}[b] (mg/kg-d)	POD_{HED}[c] (mg/kg-d)
Increased absolute kidney weight (P0 generation) Fujii et al. (2010)	Female Sprague-Dawley rats	Polynomial 2°	10% RD	1094	905	905	217
Increased relative kidney weight (P0 generation) Fujii et al. (2010)	Female Sprague-Dawley rats	Polynomial 2°	10% RD	1751	1254	1254	301

[a]For modeling details, see Appendix C of the Supplemental Information.
[b]For studies in which animals were not dosed daily, administered doses were adjusted to calculate the TWA daily doses prior to BMD modeling.
[c]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011).
[d]BMD modeling failed to successfully calculate a BMD value (see Appendix C of the Supplemental Information).
RD = relative deviation; NA = not applicable

PODs from Inhalation Studies – Use of PBPK Model for Route-to-route Extrapolation

A PBPK model for ETBE and its metabolite *tert*-butanol in rats has been developed, as described in Appendix B of the Supplemental Information. Using this model, route-to-route extrapolation of the inhalation BMCLs to derive oral PODs was performed as follows. First, the internal dose in the rat at each inhalation $BMCL_{ADJ}$ (already adjusted to continuous exposure) was estimated using the PBPK model to derive an "internal dose BMDL." Then, the oral dose concentration (assuming continuous exposure) that led to the same internal dose in the rat was estimated using the PBPK model. The resulting BMDL already reflects a continuous exposure so it is equivalent to a POD_{ADJ}, described above. This value was then converted to a human equivalent dose POD using the formula previously described in "PODs from oral studies":

$$POD_{HED} = POD_{ADJ}\ (mg/kg\text{-}day) \times DAF$$

A critical decision in the route-to-route extrapolation is the selection of the internal dose metric to use that established "equivalent" oral and inhalation exposures. For ETBE-induced kidney effects, the four options are the concentration of *tert*-butanol in blood, the rate of *tert*-butanol metabolism, the rate of ETBE metabolism, and the concentration of ETBE in blood. Note that using a kidney concentration for ETBE or *tert*-butanol will lead to the same route-to-route extrapolation relationship as using blood concentration of ETBE or *tert*-butanol, respectively, because the distribution from blood to kidney is independent of route. The major systemically available metabolite of ETBE is *tert*-butanol, which has also been shown to cause kidney toxicity, so *tert*-butanol is a plausible dose metric. There are no data to suggest that metabolites of *tert*-butanol

mediate its renal toxicity, so the rate of *tert*-butanol metabolism is not a supported dose metric. The other metabolite of ETBE is acetaldehyde, but it is largely produced in the liver, and its systemic availability is limited due to its rapid clearance. Therefore, the rate of metabolism of ETBE is not supported as a dose metric. The final dose metric option is ETBE blood concentration. Although it is possible that *tert*-butanol contributes to the kidney effects of ETBE, it is clear that ETBE alone cannot fully account for the kidney effects, given the presence of systemically available *tert*-butanol following ETBE exposure. Therefore, *tert*-butanol in blood was selected as the best available dose metric for route-to-route extrapolation, while recognizing that some uncertainty remains as to whether it can fully account for the kidney effects of ETBE.

Table 2-2 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD for each inhalation data set discussed above.

Table 2-2. Summary of derivation of oral PODs derived from route-to-route extrapolation from inhalation exposures

Endpoint and reference	Species/sex	BMR	$BMCL_{ADJ}$ (mg/m^3)	Internal dose[a] (mg/L)	Equivalent POD_{ADJ} (mg/kg-d)	Equivalent POD_{HED}[b] (mg/kg-d)
Kidney						
Increased urothelial hyperplasia (Saito et al., 2013; JPEC, 2010b)	Male F344 rats	10%	268	3.40	93.7	22.5
Increased absolute kidney weight JPEC (2008b)	Male Sprague-Dawley rats	10%	12	0.12	4.24	1.02
Increased relative kidney weight JPEC (2008b)	Male Sprague-Dawley rats	10%	99	1.19	34.9	8.38
Increased absolute kidney weight JPEC (2008b)	Female Sprague-Dawley rats	10%	2969	103	1110	266
Increased relative kidney weight JPEC (2008b)	Female Sprague-Dawley rats	10%	236	2.96	82.8	19.9
Increased absolute kidney weight Medinsky et al. (1999)	Male F344 rats	10%	450	6.06	158	37.9
Increased absolute kidney weight Medinsky et al. (1999)	Female F344 rats	10%	609	8.60	213	51.1

[a]Average blood concentration of *tert*-butanol under continuous inhalation exposure to ETBE at the BMDL (from Table 2-1).

[b]Continuous ETBE oral human equivalent dose that leads to the same average blood concentration of *tert*-butanol as continuous inhalation exposure to ETBE at the BMCL (see text for details).

2.1.3. Derivation of Candidate Values

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), also described in the Preamble, five possible areas of uncertainty and variability were considered. An explanation follows.

An intraspecies uncertainty factor, UF_H, of 10 was applied to all PODs to account for potential differences in toxicokinetics and toxicodynamics in the absence of information on the variability of response in the human population following oral exposure to ETBE.

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to all PODs because $BW^{3/4}$ scaling is used to extrapolate oral doses from laboratory animals to humans. Although $BW^{3/4}$ scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's $BW^{3/4}$ guidance (U.S. EPA, 2011) recommends use of an uncertainty factor of 3.

A subchronic to chronic uncertainty factor, UF_S, differs depending on the exposure duration. For rodent studies, exposure durations of 90 days (or 13 weeks) are generally considered subchronic, so a UF_S of 10 was applied for studies of 13 weeks. In the case of the studies of 16–26 week duration, the magnitude of change observed in kidney weights was similar to the effect observed at 104 weeks. This suggests a maximum effect may have been reached by 16-26 weeks. However, the 104 week kidney data are confounded due to age-associated factors, so this comparison may not be completely reliable. Additionally, some, but not all, markers of kidney toxicity appear to be more severely affected by ETBE at 2 years (e.g., BUN). Thus, a UF_S of 3 was applied for studies of 16-26 week duration to account for this uncertainty and a UF_S of 1 was applied to 2 year studies.

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because either the POD was a NOAEL or a BMDL. When the POD is a BMDL, the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, BMRs of a 10% change in absolute or relative kidney weight and a 10% extra risk of urothelial hyperplasia were selected under an assumption that they represent minimal biologically significant changes. When the POD was a LOAEL, a UF_L of 10 was applied.

A database uncertainty factor, UF_D, of 1 was applied to all PODs. The ETBE toxicity database includes two chronic toxicity studies in rats (Suzuki et al., 2012; JPEC, 2010a)(Saito et al., 2013; JPEC, 2010b), several 13-26 week toxicity studies in mice and rats (Miyata et al., 2013; Medinsky et al., 1999; JPEC, 2008b), prenatal developmental toxicity studies in rats and rabbits (Aso et al., 2014; Asano et al., 2011), and both single- and multi-generation reproductive studies and developmental studies in rats (Fujii et al., 2010; Gaoua, 2004a; Gaoua, 2004b). Additionally, the available mouse study observed effects that were less severe than those in rats, suggesting that mice are not more sensitive than rats. Although most of the studies are in rats, the ETBE database adequately covers all major systemic effects, including reproductive and developmental effects, and does not suggest

that additional studies would lead to identification of a more sensitive endpoint or a lower POD. Therefore, a database UF_D of 1 was applied.

Table 2-3 is a continuation of Tables 2-1 and 2-2 and summarizes the application of UFs to each POD to derive a candidate value for each data set. The candidate values presented in the table below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative oral reference value for a specific hazard and subsequent overall RfD for ETBE.

Figure 2-1 presents graphically the candidate values, UFs, and PODs, with each bar corresponding to one data set described in Table 2-3.

Table 2-3. Effects and corresponding derivation of candidate values

Endpoint and Reference	POD_{HED}[a] (mg/kg-d)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Kidney									
Increased urothelial hyperplasia; male rat Suzuki et al. (2012); JPEC (2010a)	14.5	$BMDL_{10\%}$	3	10	1	1	1	30	5×10^{-1}
Increased urothelial hyperplasia; male rat Saito et al. (2013); JPEC (2010b)	22.5	$BMDL_{10\%}$	3	10	1	1	1	30	8×10^{-1}
Increased absolute kidney weight; male rat JPEC (2008c); Miyata et al. (2013)	28	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{-1}
Increased relative kidney weight; male rat JPEC (2008c); Miyata et al. (2013)	6.0	NOAEL	3	10	1	3	1	100	6×10^{-2}
Increased absolute kidney weight; female rat JPEC (2008c); Miyata et al. (2013)	14	$BMDL_{10\%}$	3	10	1	3	1	100	1×10^{-1}
Increased relative kidney weight; female rat JPEC (2008c); Miyata et al. (2013)	4.8	$BMDL_{10\%}$	3	10	1	3	1	100	5×10^{-2}
Increased absolute kidney weight; P0 male rat Gaoua (2004b)	23	$BMDL_{10\%}$	3	10	1	3	1	100	2×10^{-1}
Increased relative kidney weight; P0 male rat Gaoua (2004b)	33	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{-1}
Increased absolute kidney weight; P0 female rat Gaoua (2004b)	250	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{0}
Increased relative kidney weight; P0 female rat Gaoua (2004b)	240	NOAEL	3	10	1	3	1	100	2×10^{0}
Increased absolute kidney weight; F1 male rat Gaoua (2004b)	56.4	$BMDL_{10\%}$	3	10	1	3	1	100	6×10^{-1}

Endpoint and Reference	POD_{HED}[a] (mg/kg-d)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Increased relative kidney weight; F1 male rat Gaoua (2004b)	60	LOAEL	3	10	10	3	1	1000	6×10^{-2}
Increased absolute kidney weight; F1 female rat Gaoua (2004b)	161	$BMDL_{10\%}$	3	10	1	3	1	100	2×10^{0}
Increased relative kidney weight; F1 female rat Gaoua (2004b)	120	NOAEL	3	10	1	3	1	100	1×10^{0}
Increased absolute kidney weight; male rat Fujii et al. (2010)	33	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{-1}
Increased relative kidney weight; male rat Fujii et al. (2010)	31	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{-1}
Increased absolute kidney weight; female rat Fujii et al. (2010)	220	$BMDL_{10\%}$	3	10	1	3	1	100	2×10^{0}
Increased relative kidney weight; female rat Fujii et al. (2010)	300	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{0}
Increased absolute kidney weight; male rat JPEC (2008b)	1.02	$BMDL_{10\%}$	3	10	1	10	1	300	3×10^{-3}
Increased relative kidney weight; male rat JPEC (2008b)	8.38	$BMDL_{10\%}$	3	10	1	10	1	300	3×10^{-2}
Increased absolute kidney weight; female rat JPEC (2008b)	266	$BMDL_{10\%}$	3	10	1	10	1	300	9×10^{-1}
Increased relative kidney weight; female rat JPEC (2008b)	19.9	$BMDL_{10\%}$	3	10	1	10	1	300	7×10^{-2}
Increased absolute kidney weight; male rat Medinsky et al. (1999)	37.9	$BMDL_{10\%}$	3	10	1	10	1	300	1×10^{-1}

Endpoint and Reference	POD_{HED}[a] (mg/kg-d)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Increased absolute kidney weight; female rat Medinsky et al. (1999)	51.1	$BMDL_{10\%}$	3	10	1	10	1	300	2×10^{-1}

1

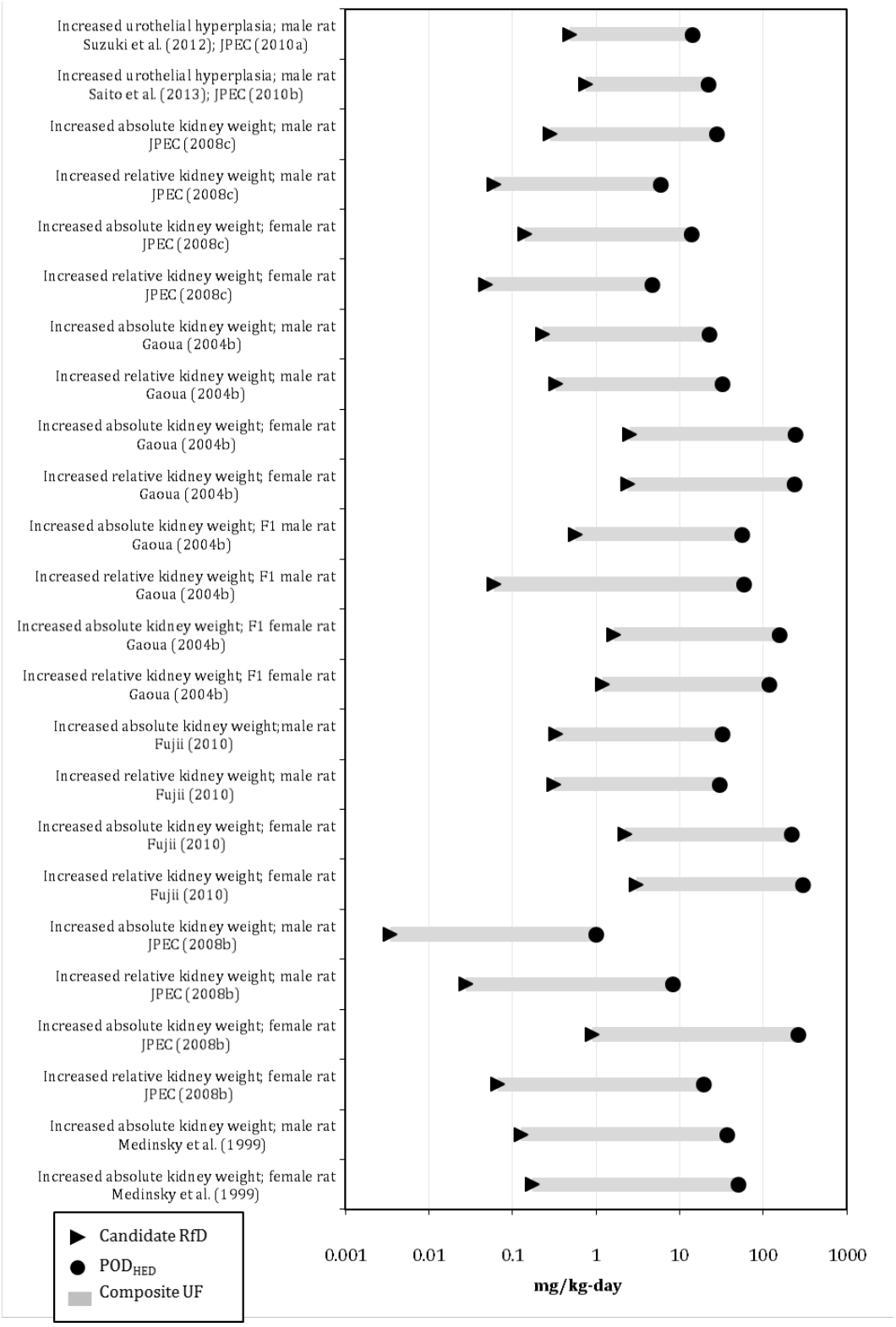


Figure 2-1. Candidate values with corresponding POD and composite UF

2.1.4. Derivation of Organ/System-Specific Reference Doses

Table 2-4 distills the candidate values from Table 2-3 into a single value for the kidney. Organ-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Kidney Toxicity

For ETBE, candidate reference values were for several different effects in both sexes, spanning a range from 3×10^{-3} to 3×10^{0} mg/kg-day, for an overall thousand range. Selection of a point estimate considered multiple aspects, including study design and consistency across estimates. The only data from a chronic study are for urothelial hyperplasia in male rats, exposed via inhalation or oral routes (Suzuki et al., 2012; JPEC, 2010a)(Saito et al., 2013; JPEC, 2010b). This is a specific indicator of kidney toxicity, and is synonymous with the transitional epithelial hyperplasia observed after chronic *tert*-butanol exposure NTP (1995). Additionally, estimated benchmark doses are consistent between the two chronic ETBE studies, with the benchmark dose estimated from the oral study within less than twofold of the benchmark dose derived by PBPK model-based route-to-route extrapolation from the inhalation study. On the other hand, data on kidney weight changes are limited to studies of 13-26 week duration, and the estimated benchmark doses are highly variable across studies.

Taken together, these observations suggest that the most appropriate basis for a kidney-specific RfD would be the results in male rats from the chronic studies (Suzuki et al., 2012; JPEC, 2010a)(Saito et al., 2013; JPEC, 2010b). For the RfD, the results from the oral study (Suzuki et al., 2012; JPEC, 2010a) are preferred, though it is notable that the two candidate values are very similar. Therefore, to estimate an exposure level below which kidney toxicity from ETBE exposure is not expected to occur, the candidate value for increased incidence of urothelial hyperplasia in male rats from (Suzuki et al., 2012; JPEC, 2010a) of **5×10^{-1} mg/kg-day** is proposed as the kidney-specific reference dose for ETBE. Confidence in this kidney-specific RfD is high. The POD is based on modeled benchmark dose estimates, and the candidate value is derived from a well-conducted GLP study, involving a sufficient number of animals per group, assessing a wide range of kidney endpoints. A candidate value for the same endpoint of urothelial hyperplasia based on route-to-route extrapolation from the inhalation study (Saito et al., 2013; JPEC, 2010b) is 8×10^{-1} mg/kg-day, differing from the recommended kidney-specific RfD by less than twofold.

Table 2-4. Organ/system-specific RfDs and proposed overall RfD for ETBE

Effect	Basis	RfD (mg/kg-day)	Exposure description	Confidence
Kidney toxicity	Increased urothelial hyperplasia	5×10^{-1}	Chronic	HIGH
Proposed overall RfD	**Increased urothelial hyperplasia**	**5×10^{-1}**	**Chronic**	**HIGH**

2.1.5. Selection of the Proposed Overall Reference Dose

For ETBE, only kidney effects were identified as a hazard; thus a single organ/system-specific reference dose was derived. Therefore, the kidney-specific RfD of 5×10^{-1} mg/kg-day is also proposed as an estimated exposure level below which deleterious effects from ETBE exposure are not expected to occur. The overall reference dose is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole including potentially susceptible subgroups (U.S. EPA, 2002).

2.1.6. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The overall confidence in this RfD is high. Confidence in the principal study JPEC (2008c) is high. This study was well conducted, complied with OECD guidelines for GLP studies, involved a sufficient number of animals per group (including both sexes), and assessed a wide range of tissues and endpoints. Confidence in the database is high; the available studies evaluated a comprehensive array of endpoints and there is no indication that additional studies would lead to identification of a more sensitive endpoint. Reflecting high confidence in the principal study and high confidence in the database, confidence in the overall RfD for ETBE is high.

2.1.7. Previous IRIS Assessment

An oral assessment for ETBE was not previously available on IRIS.

2.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The inhalation reference concentration (RfC) (expressed in units of mg/m^3) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or

the 95% lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

2.2.1. Identification of Studies and Effects for Dose-Response Analysis

EPA identified kidney effects as a human hazard of ETBE exposure. Studies were evaluated using general study quality characteristics (as discussed in Section 6 of the Preamble) to help inform the selection of studies from which to derive toxicity values. Rationale for selection of studies and effects representative of this hazard is summarized below.

Human studies are preferred over animal studies when quantitative measures of exposure are reported and the reported effects are determined to be associated with exposure. Data on the effects of inhaled ETBE in humans is limited to a few 2-hour inhalation studies at doses up to 208.9 mg/m^3 (Nihlén et al., 1998; Vetrano, 1993). These studies were not considered for dose-response assessment, because they are of acute duration and did not investigate effects in the kidney.

Animal studies were evaluated to determine which provided, (a) the most relevant routes and durations of exposure, (b) multiple exposure levels to inform the shape of the dose-response curve, and (c) the power to detect effects at low exposure levels (U.S. EPA, 2002). Sufficient data were available to develop a PBPK model in rats for both oral and inhalation exposure to perform route-to-route extrapolation, so rat studies from both routes of exposure were considered for dose-response analysis. The database for ETBE includes several studies and data sets that are suitable for use in deriving reference values. Specifically, effects associated with ETBE exposure in animals included observations of organ weight and histological changes in the kidney reported in several chronic and subchronic studies, mostly in rats.

Kidney Effects

The kidney was identified as the only human hazard of ETBE exposure based on findings of organ weight changes, histopathology (nephropathy, urothelial hyperplasia), and altered serum biomarkers (creatinine, BUN, cholesterol) in rats. The most consistent findings across studies were for kidney weight changes and urothelial hyperplasia. In the case of kidney weight changes, numerous chronic and subchronic studies investigated this endpoint following oral and inhalation exposure (Suzuki et al., 2012; Hagiwara et al., 2011; Fujii et al., 2010; JPEC, 2010b, 2008b, c; Gaoua, 2004b; Medinsky et al., 1999). For urothelial hyperplasia, chronic studies by both inhalation and oral exposure reported this effect to be increased with treatment in male rats.

Hagiwara et al. (2011), with only one dose group, was not considered further given its concordance with several other rat studies that had multiple dose groups. Additionally, as discussed in Section 1.1.1, 2-year organ weight data were not considered suitable due to the prevalence of age-associated confounders. Therefore, only the urothelial hyperplasia data from the (Suzuki et al., 2012; JPEC, 2010a) (Saito et al., 2013; JPEC, 2010b) studies were considered for dose-

response analysis. These and the remaining studies were discussed previously in Section 2.1.1 as part of the derivation of the oral reference dose, so they will not be reviewed here again.

2.2.2. Methods of Analysis

No biologically based dose-response models are available for ETBE. In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, all models available in EPA's Benchmark Dose Software (BMDS) were evaluated. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b), the benchmark concentration (BMC) and the 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) of 10% change from the control mean for organ weight data in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparison across endpoints, studies, and assessments. A benchmark response (BMR) of 10% extra risk was considered appropriate for the quantal data on incidences of slight urothelial hyperplasia. The estimated BMCLs were used as points of departure (PODs). Further details including the modeling output and graphical results for the best fit model for each endpoint can be found in Appendix C of the Supplemental Information.

In general, absolute and relative kidney weight data may both be considered appropriate endpoints for analysis. Body weight, which may impact interpretation of relative organ weights, was not significantly affected in the studies chosen as discussed in Section 2.1.2.

PODs from Inhalation Studies

Because the RfC is applicable to a continuous lifetime human exposure but is derived from animal studies featuring intermittent exposure, EPA guidance (U.S. EPA, 1994) provides mechanisms for: (1) adjusting experimental exposure concentrations to a value reflecting continuous exposure duration and (2) determining a human equivalent concentration (HEC) from the animal exposure data. The former employs an inverse concentration-time relationship to derive a health-protective duration adjustment to time-weight the intermittent exposures used in the studies. The animal exposures in both inhalation studies (JPEC, 2008b; Medinsky et al., 1999) were adjusted to reflect a continuous exposure by multiplying concentration by (6 hours/day)/(24 hours/day) and (5 days/week)/(7 days/week) as follows:

$$BMCL_{ADJ} = BMCL\ (mg/m^3) \times (6 \div 24) \times (5 \div 7)$$
$$= BMCL\ (mg/m^3) \times (0.1786)$$

The RfC methodology provides a mechanism for deriving a human equivalent concentration from the duration-adjusted POD ($BMCL_{ADJ}$) determined from the animal data. The approach takes into account the extra-respiratory nature of the toxicological responses and accommodates species differences by considering blood:air partition coefficients for ETBE in the laboratory animal (rat or

mouse) and humans. According to the RfC guidelines (U.S. EPA, 1994), ETBE is a Category 3 gas because it is largely inactive in the respiratory tract, is rapidly transferred between the lungs and blood, and the toxicological effects observed are extra-respiratory. Therefore, the duration-adjusted $BMCL_{ADJ}$ is multiplied by the ratio of animal/human blood:air partition coefficients (L_A/L_H). As detailed in Appendix B.2.2 of the Supplementary Information, the values reported in the literature for these parameters include an L_A of 11.6 for Wistar rats (Kaneko et al., 2000) and an L_H in humans of 11.7 (Nihlén et al., 1995). This allowed a $BMCL_{HEC}$ to be derived as follows:

$$BMCL_{HEC} = BMCL_{ADJ}\ (mg/m^3) \times (L_A \div L_H)\ \text{(interspecies conversion)}$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (11.6 \div 11.7)$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (0.992)$$

Table 2-5 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD for each inhalation data set discussed above.

Table 2-5. Summary of derivation of PODs following inhalation exposure

Endpoint and Reference	Species/ Sex	Model[a]	BMR	BMC (mg/m^3)	BMCL (mg/m^3)	POD_{ADJ}[b] (mg/m^3)	POD_{HEC}[c] (mg/m^3)
Kidney							
Increased urothelial hyperplasia (Saito et al., 2013; JPEC, 2010b)	Male F344 rats	Gamma	10% RD	2734	1498	268	265
Increased absolute kidney weight JPEC (2008b)	Male Sprague-Dawley rats	Hill	10% RD	911	68	12	11.9
Increased relative kidney weight JPEC (2008b)	Male Sprague-Dawley rats	Hill	10% RD	1965	556	99	98
Increased absolute kidney weight JPEC (2008b)	Female Sprague-Dawley rats	Linear	10% RD	28,591	16,628	2969	2945
Increased relative kidney weight JPEC (2008b)	Female Sprague-Dawley rats	Hill	10% RD	5559	1321	236	234
Increased absolute kidney weight Medinsky et al. (1999)	Male F344 rats	Hill	10% RD	6968	2521	450	446

Endpoint and Reference	Species/ Sex	Model[a]	BMR	BMC (mg/m^3)	BMCL (mg/m^3)	POD_{ADJ}[b] (mg/m^3)	POD_{HEC}[c] (mg/m^3)
Increased absolute kidney weight Medinsky et al. (1999)	Female F344rats	Exponential (M4)	10% RD	5610	3411	609	604

[a]For modeling details, see Appendix C of the Supplemental Information.
[b]PODs were adjusted for continuous daily exposure: POD_{ADJ} = POD × (hours exposed per day / 24 hrs) × (days exposed per week / 7 days).
[c]POD_{HEC} calculated by adjusting the POD_{ADJ} by the DAF for a Category 3 gas (U.S. EPA, 1994).

PODs from Oral Studies – Use of PBPK Model for Route-to-route Extrapolation

Since *tert*-butanol is the primary metabolite of ETBE and the evidence suggests it is involved in kidney toxicity, a PBPK model for ETBE and its metabolite *tert*-butanol in rats was developed, as described in Appendix B. Using this model, route-to-route extrapolation of the oral BMDLs to derive inhalation PODs was performed as follows. First, the internal dose in the rat at each oral BMDL (assuming continuous exposure) was estimated using the PBPK model to derive an "internal dose BMDL." Then, the inhalation air concentration (again assuming continuous exposure) that led to the same internal dose in the rat was estimated using the PBPK model. The resulting BMCL already reflects a continuous exposure so it is equivalent to a $BMCL_{ADJ}$, described above. This value was then converted to a human equivalent dose POD using the formula previously described in "PODs from inhalation studies":

$$BMCL_{HEC} = BMCL_{ADJ}\ (mg/m^3) \times (L_A \div L_H)\ \text{(interspecies conversion)}$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (11.6 \div 11.7)$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (0.992)$$

A critical decision in the route-to-route extrapolation is the selection of the internal dose metric to use that established "equivalent" oral and inhalation exposures. For ETBE-induced kidney effects, the four options are the concentration of *tert*-butanol in blood, the rate of *tert*-butanol metabolism, the rate of ETBE metabolism, and the concentration of ETBE in blood. Note that using a kidney concentration for ETBE or *tert*-butanol will lead to the same route-to-route extrapolation relationship as using blood concentration of ETBE or *tert*-butanol, respectively, because the distribution from blood to kidney is independent of route. The major systemically available metabolite of ETBE is *tert*-butanol, which has also been shown to cause kidney toxicity, so *tert*-butanol is a plausible dose metric. There are no data to suggest that metabolites of *tert*-butanol mediate its renal toxicity, so the rate of *tert*-butanol metabolism is not a supported dose metric. The other metabolite of ETBE is acetaldehyde, but it is largely produced in the liver, and its systemic availability is limited due to its rapid clearance. Therefore, the rate of metabolism of ETBE is not supported as a dose metric. The final dose metric option is ETBE blood concentration. It is clear that

ETBE alone cannot fully account for the kidney effects, given the presence of systemically available *tert*-butanol following ETBE exposure and the relatively small concentrations of ETBE measured in the urine. Therefore, *tert*-butanol in blood was selected as the best available dose metric for route-to-route extrapolation, while recognizing that some uncertainty remains as to whether it can fully account for the kidney effects of ETBE.

Table 2-6 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD for each inhalation data set discussed above.

Table 2-6. Summary of derivation of inhalation PODs derived from route-to-route extrapolation from oral exposures

Endpoint and reference	Species/sex	BMR	BMDL (mg/kg-d)	Internal dose[a] (mg/L)	Equivalent POD_{HEC}[b] (mg/m^3)
Kidney					
Increased urothelial hyperplasia (Suzuki et al., 2012; JPEC, 2010a)	Male F344 rats	10%	60.5	2.11	171
Increased absolute kidney weight JPEC (2008c); Miyata et al. (2013)	Male Sprague-Dawley rats	10%	115	4.25	326
Increased relative kidney weight JPEC (2008c); Miyata et al. (2013)	Male Sprague-Dawley rats	NA	25[c]	1.99	70
Increased absolute kidney weight JPEC (2008c); Miyata et al. (2013)	Female Sprague-Dawley rats	10%	57	1.99	161
Increased relative kidney weight JPEC (2008c); Miyata et al. (2013)	Female Sprague-Dawley rats	10%	20	0.670	56
Increased absolute kidney weight (P0 generation) Gaoua (2004b)	Male Sprague-Dawley rats	10%	94	3.41	266
Increased relative kidney weight (P0 generation) Gaoua (2004b)	Male Sprague-Dawley rats	10%	137	5.17	388
Increased absolute kidney weight (P0 generation) Gaoua (2004b)	Female Sprague-Dawley rats	10%	1030	90.2	2770
Increased relative kidney weight (P0 generation) Gaoua (2004b)	Female Sprague-Dawley rats	NA	1000[c]	85.5	2700
Increased absolute kidney weight (F1 generation) Gaoua (2004b)	Male Sprague-Dawley rats	10%	235	9.7	667
Increased relative kidney weight (F1 generation) Gaoua (2004b)	Male Sprague-Dawley rats	NA	250[c]	10.4	710
Increased absolute kidney weight (F1 generation) Gaoua (2004b)	Female Sprague-Dawley rats	10%	670	42.4	1900
Increased relative kidney weight (F1 generation) Gaoua (2004b)	Female Sprague-Dawley rats	NA	500[c]	26.7	1440

Endpoint and reference	Species/sex	BMR	BMDL (mg/kg-d)	Internal dose[a] (mg/L)	Equivalent POD_{HEC}[b] (mg/m^3)
Increased absolute kidney weight (P0 generation) Fujii et al. (2010)	Male Sprague-Dawley rats	10%	139	5.25	394
Increased relative kidney weight (P0 generation) Fujii et al. (2010)	Male Sprague-Dawley rats	10%	129	4.83	365
Increased absolute kidney weight (P0 generation) Fujii et al. (2010)	Female Sprague-Dawley rats	10%	905	71.5	2480
Increased relative kidney weight (P0 generation) Fujii et al. (2010)	Female Sprague-Dawley rats	10%	1254	127	3230

[a]Average blood concentration of *tert*-butanol under continuous oral exposure to ETBE at the BMDL (from Table 2-1).
[b]Continuous ETBE inhalation human equivalent concentration that leads to the same average blood concentration of *tert*-butanol as continuous oral exposure to ETBE at the BMDL (see text for details).
[c]BMD modeling failed to successfully calculate a BMD value (see Appendix C of the Supplemental Information). NOAEL or LOAEL was used for route-to-route extrapolation.
NA = not applicable

2.2.3. Derivation of Candidate Values

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), also described in the Preamble, five possible areas of uncertainty and variability were considered. An explanation follows:

An intraspecies uncertainty factor, UF_H, of 10 was applied to all PODs to account for potential differences in toxicokinetics and toxicodynamics in the absence of information on the variability of response in the human population following inhalation exposure to ETBE.

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to all PODs to account for residual uncertainty in the extrapolation from laboratory animals to humans in the absence of information to characterize toxicodynamic differences between rodents and humans after inhalation exposure to ETBE. This value is adopted by convention where an adjustment from animal to a human equivalent concentration has been performed as described in EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

A subchronic to chronic uncertainty factor, UF_S, differs depending on the exposure duration. For rodent studies, exposure durations of 90 days (or 13 weeks) are generally considered subchronic, so a UF_S of 10 was applied for studies of 13 weeks. In the case of the studies of 16–26 week duration, the magnitude of change observed in kidney weights was similar to the effect observed at 104 weeks. This suggests a maximum effect may have been reached by 16-26 weeks.

However, the 104 week kidney data are confounded due to age-associated factors, so this comparison may not be completely reliable. Additionally, some, but not all markers of kidney toxicity appear to be more severely affected by ETBE at 2 years (e.g., BUN). Thus a UF_S of 3 was applied for studies of 16-26 week duration to account for this uncertainty and a UF_S of 1 was applied to 2 year studies.

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because either the POD was a NOAEL or a BMCL. When the POD is a BMCL, the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, BMRs of a 10% change in absolute or relative kidney weight and a 10% extra risk of urothelial hyperplasia were selected under an assumption that they represent minimal biologically significant changes. When the POD was a LOAEL, a UF_L of 10 was applied.

A database uncertainty factor, UF_D, of 1 was applied to all PODs. The ETBE toxicity database includes two chronic toxicity studies in rats (Suzuki et al., 2012; JPEC, 2010a)(Saito et al., 2013; JPEC, 2010b), several 13-26 week toxicity studies in mice and rats (Miyata et al., 2013; Medinsky et al., 1999; JPEC, 2008b), prenatal developmental toxicity studies in rats and rabbits (Aso et al., 2014; Asano et al., 2011), and both single- and multi-generation reproductive studies and developmental studies in rats (Fujii et al., 2010; Gaoua, 2004a; Gaoua, 2004b). Additionally, the available mouse study observed effects that were less severe than those in rats, suggesting that mice are not more sensitive than rats. Although most of the studies are in rats, the ETBE database adequately covers all major systemic effects, including reproductive and developmental effects, and does not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD. Therefore, a database UF_D of 1 was applied.

Table 2-7 is a continuation of Tables 2-5 and 2-6, and summarizes the application of UFs to each POD to derive a candidate value for each data set. The candidate values presented in the table below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative inhalation reference value for a specific hazard and subsequent overall RfC for ETBE.

Figure 2-2 presents graphically the candidate values, UFs, and PODs, with each bar corresponding to one data set described in Table 2-7.

Table 2-7. Effects and corresponding derivation of candidate values

Endpoint (Sex and species) and Reference	POD_{HEC}[a] (mg/m^3)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/m^3)
Kidney									
Increased urothelial hyperplasia; male rat Suzuki et al. (2012); JPEC (2010a)	171	$BMCL_{10\%}$	3	10	1	1	1	30	6×10^0
Increased urothelial hyperplasia; male rat Saito et al. (2013); JPEC (2010b)	265	$BMCL_{10\%}$	3	10	1	1	1	30	9×10^0
Increased absolute kidney weight; male rat JPEC (2008c); Miyata et al. (2013)	326	$BMCL_{10\%}$	3	10	1	3	1	100	3×10^0
Increased relative kidney weight; male rat JPEC (2008c); Miyata et al. (2013)	70	NOAEL	3	10	1	3	1	100	7×10^{-1}
Increased absolute kidney weight; female rat JPEC (2008c); Miyata et al. (2013)	161	$BMCL_{10\%}$	3	10	1	3	1	100	2×10^0
Increased relative kidney weight; female rat JPEC (2008c); Miyata et al. (2013)	56	$BMCL_{10\%}$	3	10	1	3	1	100	6×10^{-1}
Increased absolute kidney weight; P0 male rat Gaoua (2004b)	266	$BMCL_{10\%}$	3	10	1	3	1	100	3×10^0
Increased relative kidney weight; P0 male rat Gaoua (2004b)	388	$BMCL_{10\%}$	3	10	1	3	1	100	4×10^0
Increased absolute kidney weight; P0 female rat Gaoua (2004b)	2770	$BMCL_{10\%}$	3	10	1	3	1	100	3×10^1
Increased relative kidney weight; P0 female rat Gaoua (2004b)	2700	NOAEL	3	10	1	3	1	100	3×10^1
Increased absolute kidney weight; F1 male rat Gaoua (2004b)	667	$BMCL_{10\%}$	3	10	1	3	1	100	7×10^0

Endpoint (Sex and species) and Reference	POD_{HEC}[a] (mg/m^3)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/m^3)
Increased relative kidney weight; F1 male rat Gaoua (2004b)	710	LOAEL	3	10	10	3	1	1000	7×10^{-1}
Increased absolute kidney weight; F1 female rat Gaoua (2004b)	1900	$BMCL_{10\%}$	3	10	1	3	1	100	2×10^{1}
Increased relative kidney weight; F1 female rat Gaoua (2004b)	1440	NOAEL	3	10	1	3	1	100	1×10^{1}
Increased absolute kidney weight; P0 male rat Fujii et al. (2010)	394	$BMCL_{10\%}$	3	10	1	3	1	100	4×10^{0}
Increased relative kidney weight; P0 male rat Fujii et al. (2010)	365	$BMCL_{10\%}$	3	10	1	3	1	100	4×10^{0}
Increased absolute kidney weight; P0 female rat Fujii et al. (2010)	2480	$BMCL_{10\%}$	3	10	1	3	1	100	2×10^{1}
Increased relative kidney weight; P0 female rat Fujii et al. (2010)	3230	$BMCL_{10\%}$	3	10	1	3	1	100	3×10^{1}
Increased absolute kidney weight; male rat JPEC (2008b)	11.9	$BMCL_{10\%}$	3	10	1	10	1	300	4×10^{-2}
Increased relative kidney weight; male rat JPEC (2008b)	98	$BMCL_{10\%}$	3	10	1	10	1	300	3×10^{-1}
Increased absolute kidney weight; female rat JPEC (2008b)	2945	$BMCL_{10\%}$	3	10	1	10	1	300	1×10^{1}
Increased relative kidney weight; female rate JPEC (2008b)	234	$BMCL_{10\%}$	3	10	1	10	1	300	8×10^{-1}
Increased absolute kidney weight; male rat Medinsky et al. (1999)	446	$BMCL_{10\%}$	3	10	1	10	1	300	1×10^{0}

Endpoint (Sex and species) and Reference	POD_{HEC}[a] (mg/m^3)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/m^3)
Increased absolute kidney weight; female rat Medinsky et al. (1999)	604	$BMCL_{10\%}$	3	10	1	10	1	300	2×10^0

[a] POD_{HECs} from JPEC (2008c), Gaoua (2004b), and Fujii et al. (2010) derived from route-to-route extrapolation using a dose metric of average blood concentration of *tert*-butanol under continuous oral exposure to ETBE at the BMDL.

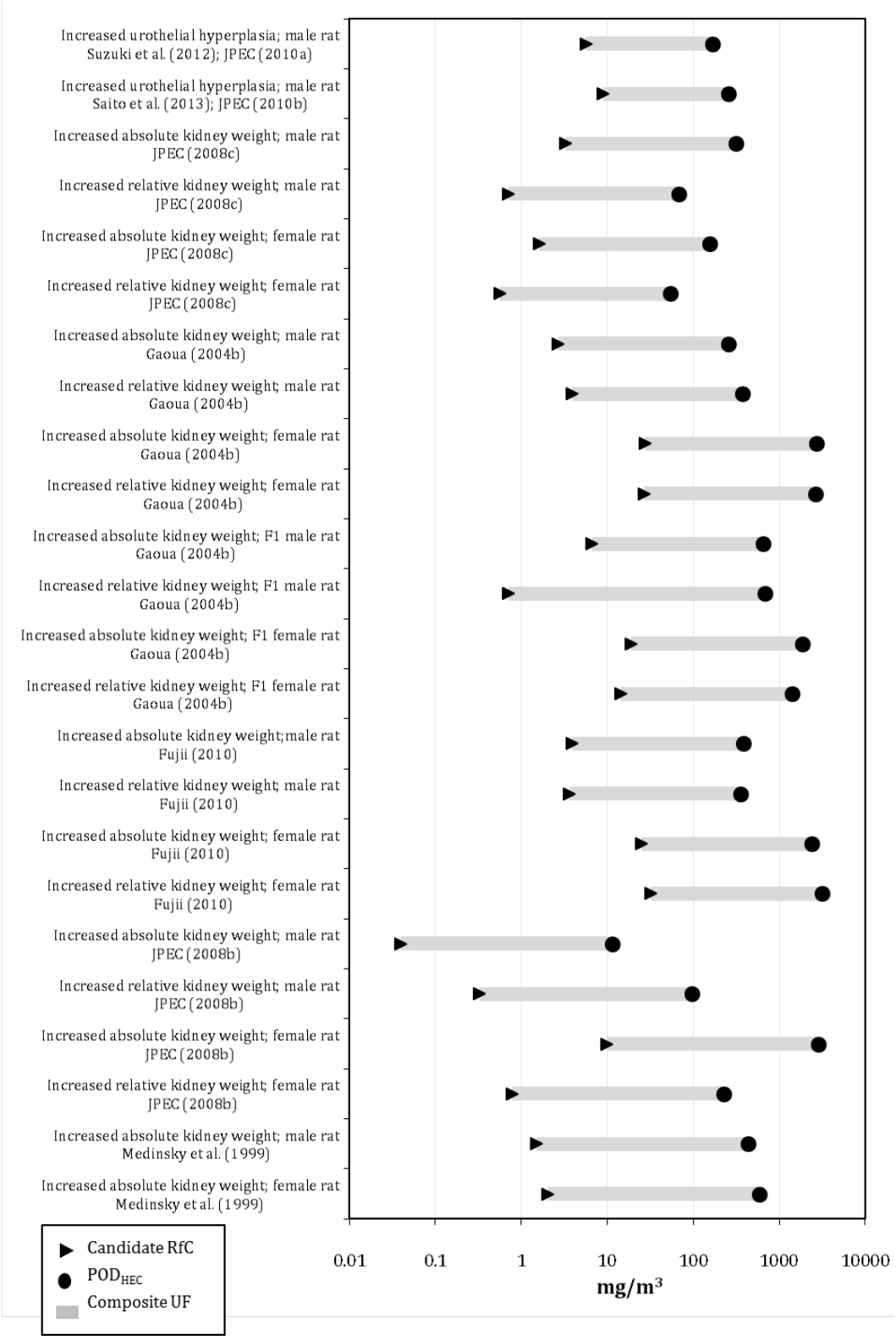


Figure 2-2. Candidate values with corresponding POD and composite UF

2.2.4. Derivation of Organ/System-Specific Reference Concentrations

Table 2-7 distills the candidate values from Table 2-6 into a single value for the kidney. Organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Kidney Toxicity

For ETBE, candidate reference values were for increased kidney weight in both sexes, spanning a range from 4×10^{-2} to 3×10^{1} mg/m^3, for an overall 750-fold range. Selection of a point estimate considered multiple aspects, including study design and consistency across estimates. The only data from a chronic study are for urothelial hyperplasia in male rats, exposed via inhalation or oral routes (Suzuki et al., 2012; JPEC, 2010a)(Saito et al., 2013; JPEC, 2010b). This is a specific indicator of kidney toxicity and is synonymous with the transitional epithelial hyperplasia observed after chronic *tert*-butanol exposure NTP (1995). Additionally, estimated benchmark doses are consistent between the two chronic ETBE studies, with the benchmark dose estimated from the oral study within less than twofold of the benchmark dose derived by PBPK model-based route-to-route extrapolation from the inhalation study. On the other hand, data on kidney weight changes are limited to studies of 13–26 week duration, and the estimated benchmark doses are highly variable across studies. Based on the previous discussion in Section 2.1.4, the results in male rats from the chronic studies (Suzuki et al., 2012; JPEC, 2010a)(Saito et al., 2013; JPEC, 2010b). For the RfC, the results from the inhalation study (Saito et al., 2013; JPEC, 2010b) are preferred, though it is notable that the two candidate values are very similar.

Therefore, to estimate an exposure level below which kidney toxicity from ETBE exposure is not expected to occur, the candidate RfC of **9 mg/m^3** for increased incidence of urothelial hyperplasia in male rats from (Saito et al., 2013; JPEC, 2010b) is proposed as the kidney-specific reference concentration for ETBE. Confidence in this kidney-specific RfC is high. The POD is based on modeled benchmark dose estimates, and the candidate value is derived from a well-conducted GLP study, involving a sufficient number of animals per group, and assessing a wide range of kidney endpoints. A candidate RfC for the same endpoint of urothelial hyperplasia based on route-to-route extrapolation from the oral study (Suzuki et al., 2012; JPEC, 2010a) is 6 mg/kg-day, differing from the recommended kidney-specific RfC by less than twofold.

Table 2-8. Organ/system-specific RfCs and proposed overall RfC for ETBE

Effect	Basis	RfC (mg/m^3)	Exposure description	Confidence
Kidney toxicity	Increased urothelial hyperplasia	9×10^{0}	Chronic	HIGH
Proposed overall RfC	**Increased urothelial hyperplasia**	**9×10^{0}**	**Chronic**	**HIGH**

2.2.5. Selection of the Proposed Overall Reference Concentration

For ETBE, only kidney effects were identified as a hazard; thus a single organ/system-specific reference concentration was derived. Therefore, the kidney-specific RfC of **9 mg/m^3** is proposed as an estimated exposure level below which deleterious effects from ETBE exposure are not expected to occur. The overall reference concentration is derived to be protective for all types of effects for a given duration of exposure and is intended to protect the population as a whole including potentially susceptible subgroups (U.S. EPA, 2002).

2.2.6. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The overall confidence in this RfC is high. Confidence in the principal study JPEC (2008c); Miyata et al. (2013) is high. The study was well conducted following OECD GLP Guideline 452 that involved a sufficient number of animals per group (including both sexes) and assessed a wide range of tissues and endpoints. Confidence in the database is high; the available studies evaluated a comprehensive array of endpoints and there is no indication that additional studies would lead to identification of a more sensitive endpoint. Reflecting high confidence in the principal studies and high confidence in the database, confidence in the overall RfC is high.

2.2.7. Previous IRIS Assessment

An RfC for ETBE was not previously available on IRIS.

2.2.8. Uncertainties in the Derivation of the Reference Dose and Reference Concentration

The following discussion identifies uncertainties associated with the RfD and RfC values derived for ETBE. To derive the RfD and RfC, the UF approach (U.S. EPA, 2000a, 1994) was applied to a POD based on renal changes in rats treated chronically. UFs were applied to the PODs to account for extrapolating from an animal bioassay to human exposure, the likely existence of a diverse population of varying susceptibilities, and database deficiencies. These extrapolations are carried out with default approaches given the lack of data to inform individual steps.

The database for ETBE contains no human data on adverse health effects from subchronic or chronic exposure. Data on the effects of ETBE are derived from a small, but high-quality database of studies in animal models, primarily rats. The database for ETBE exposure includes three lifetime bioassays in rats, several reproductive/developmental studies in rats and rabbits, and several subchronic studies in rats and mice.

Although the database is adequate for reference value derivation, there is uncertainty associated with the database, including the lack of chronic studies in a species other than rats, such

as mice. Additionally, there are no available developmental/reproductive inhalation studies. Finally, the database lacks adequate studies that examine the effect on kidney or liver in animals with deficient Aldh2.

The toxicokinetic and toxicodynamic differences between the animal species from which the POD was derived and humans are unknown for ETBE. Although sufficient information is available to develop a PBPK model in rats to evaluate differences across routes of exposure, the ETBE database lacks an adequate model that would inform potential interspecies differences. Generally, it was found that males appear more susceptible than females to ETBE toxicity. However, the underlying mechanistic basis of this apparent difference is not understood. Most importantly, it is unknown which animal species and/or sexes may be more comparable to humans.

2.3. ORAL SLOPE FACTOR FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question, and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure.

2.3.1. Analysis of Carcinogenicity Data

As noted in Section 1.2.2, EPA concluded that there is "suggestive evidence of carcinogenic potential" for ETBE. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state:

> When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however when the evidence includes a well-conducted study, quantitative analysis may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.

In this case, the carcinogenicity of ETBE has been evaluated in three oral and inhalation cancer bioassays in rats (Saito et al., 2013; Suzuki et al., 2012; Malarkey and Bucher, 2011; JPEC, 2010a, b). The strongest evidence of carcinogenicity is the increased incidence of liver tumors in male F344 rats (Saito et al., 2013; JPEC, 2010b). Mechanistic data on liver tumor promotion and enhanced genotoxicity in the absence of Aldh2 provide some biological plausibility for liver carcinogenicity. Considering these data along with the uncertainty associated with the suggestive nature of the weight of evidence, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. Because the data are from an inhalation study and ETBE induces systemic toxicity independent of exposure route, a PBPK model is used to conduct route-to-route extrapolation to the oral route. Description of analysis of carcinogenicity data is contained in the section on the inhalation unit risk, Section 2.4.1.

2.3.2. Dose-Response Analysis—Adjustments and Extrapolations Methods

Details of the modeling and the model selection process can be found in Appendix C of the Supplemental Information. A POD for estimating low-dose risk was identified at doses at the lower end of the observed data corresponding to 10% extra risk.

A PBPK model for ETBE in rats has been developed as described in Appendix B of the Supplemental Information. Using this model, route-to-route extrapolation of the inhalation BMCL to derive an oral POD was performed as follows. First, the internal dose in the rat at the inhalation $BMCL_{ADJ}$ (i.e., adjusted to continuous exposure) was estimated using the PBPK model to derive an "internal dose BMDL." Then, the oral dose (again assuming continuous exposure) that led to the same internal dose in the rat was estimated using the PBPK model, resulting in a route-to-route extrapolated BMDL.

A critical decision in the route-to-route extrapolation is the selection of the internal dose metric for establishing "equivalent" oral and inhalation exposures. For ETBE-induced liver tumors, the four options are the concentration of *tert*-butanol in blood, the rate of *tert*-butanol metabolism, the concentration of ETBE in blood, and the rate of ETBE metabolism. The major systemically available metabolite of ETBE is *tert*-butanol, which has not been shown to cause liver toxicity, so *tert*-butanol and ETBE metabolism to *tert*-butanol are not plausible dose metrics. ETBE in the blood is not supported as a dose metric either because liver concentrations of ETBE are more proximal to the site of interest. However, liver concentration for ETBE will lead to the same route-to-route extrapolation relationship as using metabolism of ETBE because the metabolism is proportional to the liver concentration in a manner independent of route. Therefore, the rate of metabolism of ETBE is a plausible dose metric based on the possibility that ETBE itself is responsible for potential liver carcinogenicity in addition to acetaldehyde, the other metabolite of ETBE produced in the liver, and a genotoxic carcinogen. Therefore, the rate of metabolism of ETBE was selected as the best available basis for route-to-route extrapolation.

The route-to-route extrapolated ETBE BMDL is scaled to HED according to EPA guidance (U.S. EPA, 2011, 2005a). In particular, the BMDL was converted to an HED assuming that doses in animals and humans are toxicologically equivalent when scaled by body weight raised to the $^3/_4$ power. Standard body weights of 0.25 kg for rats and 70 kg for humans were used (U.S. EPA, 1988). The following formula was used for the conversion of oral BMDL to oral HED:

$$\text{Scaled HED in mg/kg-d} = (\text{BMDL in mg/kg-d}) \times (0.25/70)^{1/4}$$
$$= (\text{BMDL in mg/kg-d}) \times 0.24$$

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the MOA of carcinogenicity has not been established (U.S. EPA,

2005a). In the case of ETBE, the mode of carcinogenic action for liver tumors is not understood (see Section 1.2.2). Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with ETBE exposure.

2.3.3. Derivation of the Oral Slope Factor

The results from route-to-route extrapolation of the male rat liver tumor data (Saito et al., 2013; JPEC, 2010b) are summarized in Table 2-9. The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (slope factor = $0.1/BMDL_{10}$). This slope, a 95% upper confidence limit, represents a plausible upper bound on the true risk. Using linear extrapolation from the $BMDL_{10}$, a human equivalent oral slope factor was derived as presented in Table 2-9.

A single oral slope factor was derived. The recommended oral slope factor for providing a sense of the magnitude of potential carcinogenic risk associated with lifetime oral exposure to ETBE is **9×10^{-4} per mg/kg-day** based on the liver tumor response in male F344 rats (Saito et al., 2013; JPEC, 2010b).

Table 2-9. Summary of the oral slope factor derivation

Tumor	Species/Sex	BMR	$BMCL_{ADJ}$ (mg/m^3)	Internal Dose[a] (mg/h)	BMDL[b] (mg/kg-d)	POD= $BMDL_{HED}$[c] (mg/kg-d)	Slope Factor[d] $(mg/kg\text{-}d)^{-1}$
Hepatocellular adenomas and carcinomas	Male F344 rat	10%	1,271	4.00	455	111	9×10^{-4}

[a]Average rate of ETBE metabolism in rats under continuous inhalation exposure at the $BMCL_{ADJ}$.
[b]Continuous oral exposure in rats that leads to the same average rate of ETBE metabolism as continuous inhalation exposure in rats at the BMCL.
[c]Continuous oral exposure human equivalent dose = BMDL × $(0.25/70)^{¼}$.
[d]Human equivalent oral slope factor = $0.1/BMDL_{HED}$.

2.3.4. Uncertainties in the Derivation of the Oral Slope Factor

There is uncertainty when extrapolating data from animals to estimate potential cancer risks to human populations from exposure to ETBE (see Table 2-10). There are no data in humans to support the tumors observed in animals. Although changing the methods used to derive the oral slope factor could change the results, standard practices were used due to the lack of a human PBPK model or specific MOA to indicate other methods would be preferable. Additionally, considering the uncertainty associated with the suggestive nature of the weight of evidence, the oral slope factor is recommended only for providing a sense of the magnitude of potential carcinogenic risk.

Table 2-10. Summary of uncertainties in the derivation of cancer risk values for ETBE

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of target organ ↓ oral slope factor by unknown amount if liver not selected.	The liver was selected as the target organ.	The liver was the best supported target site based on a single bioassay result in male rats, one data set on tumor promotion, and mechanistic data providing biological plausibility. However, the overall evidence for carcinogenicity was considered "suggestive."
Selection of data set ↓ oral slope factor by unknown amount if different data set selected.	Saito et al. (2013),JPEC (2010b) was selected.	Saito et al. (2013), JPEC (2010b) was a well-conducted study. It was also the only bioassay that reported increased liver tumors. Additional bioassays might add support to the findings or provide results for different (possibly lower) doses, which may affect the oral slope factor.
Selection of extrapolation approach Different PBPK model could ↓ or ↑ oral slope factor.	PBPK model-based extrapolation of inhalation data was used for oral slope factor.	PBPK model accurately predicted ETBE toxicokinetics. Data and model predictions were within twofold of each other.
Selection of dose metric Alternatives could ↓ or ↑ oral slope factor.	ETBE metabolism rate as the dose metric for route-to-route extrapolation was converted to HED.	ETBE metabolized is the best supported dose metric. It is consistent with a hypothesis of acetaldehyde playing a role in liver carcinogenesis of ETBE. It is also consistent with ETBE concentration in the liver being the mediator of carcinogenesis (metabolism is proportional to ETBE liver concentration). Alternative dose metrics of ETBE concentration, *tert*-butanol concentration, or *tert*-butanol metabolism would result in a range of 2.4-fold decrease to 1.04-fold increase in the oral slope factor.
Interspecies extrapolation of dosimetry and risk Alternatives could ↓ or ↑ slope factor (e.g., 3.5-fold ↓ [scaling by body weight] or ↑ 2-fold [scaling by $BW^{2/3}$]).	The default approach of body weight$^{3/4}$ was used.	There are no data to suggest an alternative approach. Because the dose metric was not an area under the curve, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks. While the true human correspondence is unknown, this overall approach is expected to neither over- nor underestimate human equivalent risks.
Dose-response modeling Alternatives could ↓ or ↑ slope factor.	Used multistage dose-response model to derive a BMD and BMDL.	No biologically based models for ETBE were available. The multistage model has biological support and is the model most consistently used in EPA cancer assessments.

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Low-dose extrapolation ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation.	Linear extrapolation of risk in low-dose region used.	Linear low-dose extrapolation for agents without a known MOA is supported.
Statistical uncertainty at POD ↓ oral slope factor 1.5-fold if BMD used as the POD rather than BMDL.	BMDL (preferred approach for calculating plausible upper bound slope factor).	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure at 10% extra risk of liver.
Sensitive subpopulations ↑ oral slope factor to unknown extent.	Individuals deficient in ALDH2 are potentially more sensitive.	Experiments showed enhanced liver toxicity and genotoxicity in mice when Aldh2 was absent. Human subpopulations deficient in ALDH2 are known to be at enhanced risk of ethanol-induced cancer mediated by acetaldehyde. However, no chemical-specific data are available to determine the extent of enhanced susceptibility due to ETBE-induced carcinogenicity. Because determination of a mutagenic MOA has not been made, an age-specific adjustment factor is not applied.

2.3.5. Previous IRIS Assessment: Oral Slope Factor

A cancer assessment for ETBE was not previously available on IRIS.

2.4. INHALATION UNIT RISK FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question, and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the inhalation unit risk is a plausible upper bound on the estimate of risk per μg/m^3 air breathed.

2.4.1. Analysis of Carcinogenicity Data

As noted in Section 1.2.2, EPA concluded that there is "suggestive evidence of carcinogenic potential" for ETBE. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state:

> When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analysis may be useful for some purposes. For example, it could provide a sense of the magnitude and uncertainty of potential risks, rank potential hazards, or set research priorities.

In this case, the carcinogenicity of ETBE has been evaluated in three cancer bioassays in rats (Saito et al., 2013; Suzuki et al., 2012; Malarkey and Bucher, 2011; JPEC, 2010a, b). Considering these data and uncertainty associated with the suggestive nature of the weight of evidence, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk.

The most robust evidence of carcinogenicity is the increased incidences of liver tumors in male F344 rats (Saito et al., 2013; JPEC, 2010b). These data have additional support due to the biological plausibility of mechanistic data on tumor promotion and genotoxicity in the absence of Aldh2, and analogy to the human carcinogenicity of acetaldehyde after consumption of ethanol. The Saito et al. (2013), (JPEC, 2010b) study was considered suitable for dose-response analysis. It was conducted in accordance with GLP (OECD Guideline 451), and all aspects were subjected to retrospective quality assurance audits. The study included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting of methods and results. With respect to hepatocellular adenomas and carcinomas, statistical tests conducted by the study authors found significant dose-response trends by both the Peto test (incidental tumor test) and the Cochran-Armitage test; a significant increase in the 20,894-mg/m^3 group compared with controls was calculated by Fisher's exact test. In females, no exposure-related neoplastic lesions were observed. Therefore, the hepatocellular adenomas and carcinomas in male rats were considered suitable for quantitative analysis.

2.4.2. Dose-Response Analysis—Adjustments and Extrapolations Methods

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the MOA of carcinogenicity has not been established (U.S. EPA, 2005a). In the case of ETBE, the modes of carcinogenic action for liver tumors are not fully understood (see Section 1.2.2). Therefore, a linear low-dose extrapolation approach was used to estimate potential human carcinogenic risk associated with ETBE exposure. Details of the modeling and the model selection process can be found in Appendix C of the Supplemental Information. A POD for estimating low-dose risk was identified at a dose at the lower end of the observed data, generally corresponding to 10% extra risk.

Because the inhalation unit risk is applicable to a continuous lifetime human exposure but derived from animal studies featuring intermittent exposure, EPA guidance (U.S. EPA, 1994) provides mechanisms for: (1) adjusting experimental exposure concentrations to a value reflecting continuous exposure duration and (2) determining a human equivalent concentration (HEC) from the animal exposure data. The former employs an inverse concentration-time relationship to derive a health-protective duration adjustment to time-weight the intermittent exposures used in the

study. The animal BMCL estimated from the inhalation study Saito et al. (2013), (JPEC, 2010b) was adjusted to reflect a continuous exposure by multiplying it by (6 hours/day)/(24 hours/day) and (5 days/week)/(7 days/week) as follows:

$$BMCL_{ADJ} = BMCL\ (mg/m^3) \times 6/24 \times 5/7$$
$$= 7{,}118\ mg/m^3 \times 0.25 \times 0.71$$
$$= 1{,}271\ mg/m^3$$

The approach to determine the HEC takes into account the extra-respiratory nature of the toxicological responses and accommodates species differences by considering blood:air partition coefficients for ETBE in the laboratory animal (rat) and humans. According to the RfC guidelines (U.S. EPA, 1994), ETBE is a Category 3 gas because extra-respiratory effects were observed. The values reported in the literature for these parameters include an L_A of 11.6 for rats (Kaneko et al., 2000), and an L_H in humans of 11.7 (Nihlén et al., 1995). This allowed a $BMCL_{HEC}$ to be derived as follows:

$$BMCL_{HEC} = BMCL_{ADJ}\ (mg/m^3) \times (L_A/L_H)\ (interspecies\ conversion)$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (11.6/11.7)$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (0.992)$$
$$= 1{,}271\ mg/m^3 \times (0.992)$$
$$= 1{,}261\ mg/m^3$$

2.4.3. Inhalation Unit Risk Derivation

The POD estimate based on the male liver tumor data (Saito et al., 2013; JPEC, 2010b) is summarized in Table 2-11. The lifetime inhalation unit risk for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (inhalation unit risk = $0.1/BMCL_{10}$). This slope, a 95% upper confidence limit, represents a plausible upper bound on the true risk. Using linear extrapolation from the $BMCL_{10}$, a human equivalent inhalation unit risk was derived as presented in Table 2-11

A single inhalation unit risk was derived. Therefore, the recommended inhalation unit risk for providing a sense of the magnitude of potential carcinogenic risk associated with lifetime inhalation exposure to ETBE is **8×10^{-5} per mg/m³**, based on the liver tumor response in male F344 rats (Saito et al., 2013; JPEC, 2010b).

Table 2-11. Summary of the inhalation unit risk derivation

Tumor	Species/Sex	Selected Model	BMR	BMC (mg/m^3)	POD= BMCL (mg/m^3)	Slope factor[a] $(mg/m^3)^{-1}$
Hepatocellular adenomas and carcinomas	Male F344 rat	1° Multistage	10%	1928	1261	8×10^{-5}

[a]Human equivalent slope factor = $0.1/BMCL_{10HEC}$; see Appendix C of the Supplemental Information for details of modeling results.

2.4.4. Uncertainties in the Derivation of the Inhalation Unit Risk

There is uncertainty when extrapolating data from animals to estimate potential cancer risks to human populations from exposure to ETBE. There are no data in humans to support the tumors observed in animals. Although changing the methods used to derive the inhalation unit risk could change the results, standard practices were used due to the lack of a human PBPK model or specific MOA to indicate other methods would be preferable. Additionally, considering the uncertainty associated with the suggestive nature of the weight of evidence, the inhalation unit risk is recommended only for providing a sense of the magnitude of potential carcinogenic risk.

Table 2-12. Summary of uncertainties in the derivation of cancer risk values for ETBE

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of target organ ↓ inhalation unit risk by unknown amount if liver not selected.	The liver was selected as the target organ.	The liver was the best supported target site, based on a single bioassay result in male rats, one data set on tumor promotion, and mechanistic data providing biological plausibility. However, the overall evidence for carcinogenicity was considered "suggestive."
Selection of data set ↓ or ↑ inhalation unit risk by unknown amount if different data set selected.	Saito et al. (2013), JPEC (2010b) was selected.	Saito et al. (2013), JPEC (2010b) was a well-conducted study, and it was also the only bioassay that reported increased liver tumors. Using other bioassays (and hence other target organs) would decrease the inhalation unit risk. Additional bioassays (e.g., in mice) might add support to the findings or provide results for different (possibly lower) doses, which may affect the inhalation unit risk.
Selection of extrapolation approach	Inhalation data used for inhalation unit risk.	No extrapolation methods were used.

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of dose metric Alternatives could ↓ or ↑ inhalation unit risk.	Administered concentration was used.	Modeling based on the best supported PBPK model-based internal dose metric of ETBE metabolism decreased the BMCL by 2.1-fold.
Interspecies extrapolation of dosimetry and risk Alternatives could ↓ or ↑ inhalation unit risk.	The default approach for a Category 3 gas was used.	There are no data to suggest an alternative approach. While the true human correspondence is unknown, this overall approach is expected to neither over- or underestimate human equivalent risks.
Dose-response modeling Alternatives could ↓ or ↑ slope factor.	Multistage dose-response model to derive a BMC and BMCL was used.	No biologically based models for ETBE were available. The multistage model has biological support and is the model most consistently used in EPA cancer assessments.
Low-dose extrapolation ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation.	Linear extrapolation of risk in low-dose region was used.	Linear low-dose extrapolation for agents without a known MOA is supported.
Statistical uncertainty at POD ↓ oral slope factor 1.5-fold if BMC used as the POD rather than BMCL.	BMCL (preferred approach for calculating plausible upper bound slope factor) was used.	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure at 10% extra risk of liver tumors.
Sensitive subpopulations ↑ oral slope factor to unknown extent.	Individuals deficient in ALDH2 are potentially more sensitive.	Experiments showed enhanced liver toxicity and genotoxicity in mice when ALDH2 was absent. Human subpopulations deficient in ALDH2 are known to be at enhanced risk of ethanol-induced cancer mediated by acetaldehyde. However, no chemical-specific data are available to determine the extent of enhanced sensitivity due to ETBE-induced carcinogenicity. Because determination of a mutagenic MOA has not been made, an age-specific adjustment factor is not applied.

2.4.5. Previous IRIS Assessment: Inhalation Unit Risk

A cancer assessment for ETBE was not previously available on IRIS.

2.5. APPLICATION OF AGE-DEPENDENT ADJUSTMENT FACTORS

As discussed in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005c), either default or chemical-specific age-dependent adjustment factors (ADAFs) are applied to account for early-life exposure to carcinogens that act through a mutagenic mode of action. Because chemical-specific life-stage susceptibility data for

cancer are not available, and because the mode of action for ETBE carcinogenicity is not known (see Section 1.1.4), ADAFs were not applied.

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