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Toxicological Review of Ethyl Tertiary Butyl Ether

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ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ATSDR	Agency for Toxic Substances and Disease Registry
ALP	alkaline phosphatase
ALT	alanine aminotransferase/transaminase
AST	aspartate aminotransferase/transaminase
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
GLP	Good Laboratory Practices
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 erythrocytes
PCNA	proliferating cell nuclear antigen
PND	postnatal day
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
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
U.S.	United States
WT	wild type

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PREFACE

This Toxicological Review critically reviews the publicly available studies on ethyl tertiary butyl ether (ETBE) to identify its adverse health effects and to characterize exposure-response relationships. The assessment examined all effects by oral and inhalation routes of exposure and includes 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 the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) program.

This assessment updates a previous IRIS draft assessment of ETBE that went to peer review in 2010. The previous draft assessment was suspended pending completion of several new 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 overlapping scientific aspects:

- *tert*-Butanol and acetaldehyde are the primary metabolites of ETBE, and some of the toxicological effects of ETBE are likely attributed to *tert*-butanol. Therefore, data on *tert*-butanol are considered informative for the hazard identification and dose-response assessment of ETBE, and vice versa.

- The scientific literature for the two chemicals includes data on α_{2u}-globulin-related nephropathy; therefore, a common approach was used to evaluate the data as they relate to the mode of action for kidney effects.

- A combined physiologically based pharmacokinetic (PBPK) model for ETBE and *tert*-butanol in rats was applied to support the dose-response assessments for these chemicals (Borghoff et al., 2016).

Prior to the development of the IRIS 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. In June 2016, EPA convened a public science meeting to discuss the public comment draft Toxicological Review of tert-Butyl Alcohol (*tert*-butanol) during which time the Agency heard comments on "disentangling mechanisms of kidney toxicity and carcinogenicity," an issue relevant to both *tert*-butanol and ETBE. The complete set of public comments, including the slides presented at the June 2016 public science meeting, is available on the docket at http://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2013-1111). In October 2016, a public science meeting was held to provide the public an opportunity to engage in early discussions on the draft IRIS Toxicological Review of ETBE and the draft charge to the peer review panel prior to release for external peer review. The complete set of

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

Organ-/system-specific reference values are calculated where feasible (in this case only representing kidney toxicity). These reference values could be useful for cumulative risk assessments that consider the combined effect of multiple agents acting on the same biological system.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to IRIS Toxicological Reviews. Appendices for toxicokinetic information, PBPK modeling, genotoxicity study summaries, dose-response modeling, and other information are provided as Supplemental Information to this Toxicological Review. 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.

Uses

ETBE has been used as a fuel oxygenate in the United States 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 United States, and the use of ethanol increased dramatically (Weaver et al., 2010). ETBE is still registered with EPA for use as a fuel additive, but it is not used currently in the United States. The use of ether fuel additives has been banned or limited by several states, largely in response to groundwater contamination concerns.

The United States 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 also is relatively high. Japan's use increased dramatically in 2010 to fulfill its 2010 Kyoto Accord obligations (USDA, 2012).

Fate and Transport

ETBE is expected to be highly mobile in soil due to its high carbon-water partitioning coefficient (HSDB, 2012). ETBE is not predicted to adsorb onto suspended particles and is unlikely to undergo biodegradation in water (HSDB, 2012). ETBE is estimated to have a half-life of 2 days in air (HSDB, 2012).

Occurrence in the Environment

ETBE can be 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 detected ETBE. For instance, a survey of states reported an average detection rate of 18% for ETBE in groundwater samples associated with gasoline contamination (NEIWPCC, 2003). Nontargeted

studies, such as a 2006 U.S. Geological Survey (USGS) study (USGS, 2006) measuring volatile organic compounds (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, which measured several VOCs, was not targeted to sites that would be most vulnerable to ETBE contamination.

Fuel contamination cleanup is done largely by states, and information on the number of private contaminated drinking water wells is not consistently available. The State of California maintains an online database of measurements from contaminated sites (Cal/EPA, 2016). 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 using fewer and less-standardized data. Currently, only 13 states routinely analyze for ETBE at fuel-contaminated sites (NEIWPCC, 2003). Monitoring data associated with leaking storage tanks in Maryland show contamination in groundwater affecting multiple properties (Maryland Department of the Environment, 2016).

General Population Exposure

ETBE exposure can occur in many different settings. Releases from underground storage tanks could result in exposure to individuals who obtain their drinking water from wells. Due to its environmental mobility and resistance to biodegradation, ETBE has the potential to contaminate and persist in groundwater and soil (HSDB, 2012); therefore, exposure through ingestion of contaminated drinking water is possible.

Other human exposure pathways of ETBE include inhalation and, to a lesser extent, dermal contact. ETBE inhalation exposure can occur due the chemical's volatility and release from industrial processes and contaminated sites (HSDB, 2012).

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). The results of this assessment are presented in Appendix A of the Supplemental Information to this Toxicological Review. Of importance to recognize is that earlier assessments could have been prepared for different purposes and might use different methods. In addition, newer studies have been included in the IRIS assessment.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

Note: The Preamble summarizes the objectives and scope of the IRIS program, general principles and systematic review procedures used in developing IRIS assessments, and the overall development process and document structure.

1. Objectives and Scope of the IRIS Program

Soon after EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in support of actions to protect human health and the environment. EPA's IRIS program[1] contributes to this endeavor by reviewing epidemiologic and experimental studies of chemicals in the environment to identify adverse health effects and characterize exposure–response relationships. Health agencies worldwide use IRIS assessments, which are also a scientific resource for researchers and the public.

IRIS assessments cover the hazard identification and dose–response steps of risk assessment. Exposure assessment and risk characterization are outside the scope of IRIS assessments, as are political, economic, and technical aspects of risk management. An IRIS assessment may cover one chemical, a group of structurally or toxicologically related chemicals, or a chemical mixture. Exceptions outside the scope of the IRIS program are radionuclides, chemicals used only as pesticides, and the "criteria air pollutants" (particulate matter, ground-level ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and lead).

Enhancements to the IRIS program are improving its science, transparency, and productivity. To improve the science, the IRIS program is adapting and implementing principles of systematic review (i.e., using explicit methods to identify, evaluate, and synthesize study findings). To increase transparency, the IRIS program discusses key science issues with the scientific community and the public as it begins an assessment. External peer review, independently managed and in public, improves both science and transparency. Increased productivity requires that assessments be concise, focused on EPA's needs, and completed without undue delay.

IRIS assessments follow EPA guidance[2] and standardized practices of systematic review. This Preamble summarizes and does not change IRIS operating procedures or EPA guidance.

Periodically, the IRIS program asks for nomination of agents for future assessment or reassessment. Selection depends on EPA's priorities, relevance to public health, and availability of pertinent studies. The IRIS multiyear agenda[3] lists upcoming assessments. The IRIS program may also assess other agents in anticipation of public health needs.

[1] IRIS program website: http://www.epa.gov/iris/

[2] EPA guidance documents: http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance/

[3] IRIS multiyear agenda: https://www.epa.gov/iris/iris-agenda

2. Planning an Assessment: Scoping, Problem Formulation, and Protocols

Early attention to planning ensures that IRIS assessments meet their objectives and properly frame science issues.

Scoping refers to the first step of planning, where the IRIS program consults with EPA's program and regional offices to ascertain their needs. Scoping specifies the agents an assessment will address, routes and durations of exposure, susceptible populations and lifestages, and other topics of interest.

Problem formulation refers to the science issues an assessment will address and includes input from the scientific community and the public. A preliminary literature survey, beginning with secondary sources (e.g., assessments by national and international health agencies and comprehensive review articles), identifies potential health outcomes and science issues. It also identifies related chemicals (e.g., toxicologically active metabolites and compounds that metabolize to the chemical of interest).

Each IRIS assessment comprises multiple systematic reviews for multiple health outcomes. It also evaluates hypothesized mechanistic pathways and characterizes exposure–response relationships. An assessment may focus on important health outcomes and analyses rather than expand beyond what is necessary to meet its objectives.

Protocols refer to the systematic review procedures planned for use in an assessment. They include strategies for literature searches, criteria for study inclusion or exclusion, considerations for evaluating study methods and quality, and approaches to extracting data. Protocols may evolve as an assessment progresses and new agent-specific insights and issues emerge.

3. Identifying and Selecting Pertinent Studies

IRIS assessments conduct systematic literature searches with criteria for inclusion and exclusion. The objective is to retrieve the pertinent primary studies (i.e., studies with original data on health outcomes or their mechanisms). *PECO statements* (Populations, Exposures, Comparisons, Outcomes) govern the literature searches and screening criteria. "Populations" and animal species generally have no restrictions. "Exposures" refers to the agent and related chemicals identified during scoping and problem formulation and may consider route, duration, or timing of exposure. "Comparisons" means studies that allow comparison of effects across different levels of exposure. "Outcomes" may become more specific (e.g., from "toxicity" to "developmental toxicity" to "hypospadias") as an assessment progresses.

For studies of absorption, distribution, metabolism, and elimination, the first objective is to create an inventory of pertinent studies. Subsequent sorting and analysis facilitates characterization and quantification of these processes.

Studies on mechanistic events can be numerous and diverse. Here, too, the objective is to create an inventory of studies for later sorting to support analyses of related data. The inventory also facilitates generation and evaluation of hypothesized mechanistic pathways.

The IRIS program posts initial protocols for literature searches on its website and adds search results to EPA's HERO database.[4] Then the IRIS program takes extra steps to ensure identification of pertinent studies: by

[4] Health and Environmental Research Online: https://hero.epa.gov/hero/

encouraging the scientific community and the public to identify additional studies and ongoing research; by searching for data submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act; and by considering late-breaking studies that would impact the credibility of the conclusions, even during the review process.[5]

4. Evaluating Study Methods and Quality

IRIS assessments evaluate study methods and quality, using uniform approaches for each group of similar studies. The objective is that subsequent syntheses can weigh study results on their merits. Key concerns are potential *bias* (factors that affect the magnitude or direction of an effect) and *insensitivity* (factors that limit the ability of a study to detect a true effect).

For human and animal studies, the evaluation of study methods and quality considers study design, exposure measures, outcome measures, data analysis, selective reporting, and study sensitivity. For human studies, this evaluation also considers selection of participant and referent groups and potential confounding. Emphasis is on discerning bias that could substantively change an effect estimate, considering also the expected direction of the bias. Low sensitivity is a bias towards the null.

Study-evaluation considerations are specific to each study design, health effect, and agent. Subject-matter experts evaluate each group of studies to identify characteristics that bear on the informativeness of the results. For carcinogenicity, neurotoxicity, reproductive toxicity, and developmental toxicity, there is EPA guidance for study evaluation (U.S. EPA, 2005a, 1998b, 1996, 1991b). As subject-matter experts examine a group of studies, additional agent-specific knowledge or methodologic concerns may emerge and a second pass become necessary.

Assessments use evidence tables to summarize the design and results of pertinent studies. If tables become too numerous or unwieldy, they may focus on effects that are more important or studies that are more informative.

The IRIS program posts initial protocols for study evaluation on its website, then considers public input as it completes this step.

5. Integrating the Evidence of Causation for Each Health Outcome

Synthesis within lines of evidence. For each health outcome, IRIS assessments synthesize the human evidence and the animal evidence, augmenting each with informative subsets of mechanistic data. Each synthesis considers aspects of an association that may suggest causation: consistency, exposure–response relationship, strength of association, temporal relationship, biological plausibility, coherence, and "natural experiments" in humans (U.S. EPA, 1994) (U.S. EPA, 2005a).

Each synthesis seeks to reconcile ostensible inconsistencies between studies, taking into account differences in study methods and quality. This leads to a distinction between *conflicting evidence* (unexplained positive and negative results in similarly exposed human populations or in similar animal models) and *differing results* (mixed results attributable to differences between human populations, animal models, or exposure conditions) (U.S. EPA, 2005a).

Each synthesis of human evidence explores alternative explanations (e.g.,

[5] IRIS "stopping rules": https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf

chance, bias, or confounding) and determines whether they may satisfactorily explain the results. Each synthesis of animal evidence explores the potential for analogous results in humans. Coherent results across multiple species increase confidence that the animal results are relevant to humans.

Mechanistic data are useful to augment the human or animal evidence with information on precursor events, to evaluate the human relevance of animal results, or to identify susceptible populations and lifestages. An agent may operate through multiple mechanistic pathways, even if one hypothesis dominates the literature (U.S. EPA, 2005a).

Integration across lines of evidence. For each health outcome, IRIS assessments integrate the human, animal, and mechanistic evidence to answer the question: *What is the nature of the association between exposure to the agent and the health outcome?*

For cancer, EPA includes a standardized hazard descriptor in characterizing the strength of the evidence of causation. The objective is to promote clarity and consistency of conclusions across assessments (U.S. EPA, 2005a).

Carcinogenic to humans: convincing epidemiologic evidence of a causal association; or strong human evidence of cancer or its key precursors, extensive animal evidence, identification of mode-of-action and its key precursors in animals, and strong evidence that they are anticipated in humans.

Likely to be carcinogenic to humans: evidence that demonstrates a potential hazard to humans. Examples include a plausible association in humans with supporting experimental evidence, multiple positive results in animals, a rare animal response, or a positive study strengthened by other lines of evidence.

Suggestive evidence of carcinogenic potential: evidence that raises a concern for humans. Examples include a positive result in the only study, or a single positive result in an extensive database.

Inadequate information to assess carcinogenic potential: no other descriptors apply. Examples include little or no pertinent information, *conflicting evidence*, or negative results not sufficiently robust for *not likely*.

Not likely to be carcinogenic to humans: robust evidence to conclude that there is no basis for concern. Examples include no effects in well-conducted studies in both sexes of multiple animal species, extensive evidence showing that effects in animals arise through modes-of-action that do not operate in humans, or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

If there is credible evidence of carcinogenicity, there is an evaluation of mutagenicity, because this influences the approach to dose–response assessment and subsequent application of adjustment factors for exposures early in life (U.S. EPA, 2005a), (U.S. EPA, 2005b).

6. Selecting Studies for Derivation of Toxicity Values

The purpose of toxicity values (slope factors, unit risks, reference doses, reference concentrations; see section 7) is to estimate exposure levels likely to be without appreciable risk of adverse health effects. EPA uses these values to support its actions to protect human health.

The health outcomes considered for derivation of toxicity values may depend on the hazard descriptors. For example, IRIS assessments generally derive cancer values for agents that are *carcinogenic* or *likely to be carcinogenic*, and sometimes for agents with *suggestive evidence* (U.S. EPA, 2005a).

Derivation of toxicity values begins with a new evaluation of studies, as some studies used qualitatively for hazard identification may not be useful quantitatively for exposure–response assessment. Quantitative analyses require quantitative measures of exposure and response. An assessment weighs the merits of the human and animal studies, of various animal models, and of

different routes and durations of exposure (U.S. EPA, 1994). Study selection is not reducible to a formula, and each assessment explains its approach.

Other biological determinants of study quality include appropriate measures of exposure and response, investigation of early effects that precede overt toxicity, and appropriate reporting of related effects (e.g., combining effects that comprise a syndrome, or benign and malignant tumors in a specific tissue).

Statistical determinants of study quality include multiple levels of exposure (to characterize the shape of the exposure–response curve) and adequate exposure range and sample sizes (to minimize extrapolation and maximize precision) (U.S. EPA, 2012).

Studies of low sensitivity may be less useful if they fail to detect a true effect or yield toxicity values with wide confidence limits.

7. Deriving Toxicity Values

General approach. EPA guidance describes a two-step approach to dose–response assessment: analysis in the range of observation, then extrapolation to lower levels. Each toxicity value pertains to a route (e.g., oral, inhalation, dermal) and duration or timing of exposure (e.g., chronic, subchronic, gestational) (U.S. EPA, 2002).

IRIS assessments derive a candidate value from each suitable data set. Consideration of candidate values yields a toxicity value for each organ or system. Consideration of the organ/system-specific values results in the selection of an overall toxicity value to cover all health outcomes. The organ/system-specific values are useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common anatomical site.

Analysis in the range of observation. Within the observed range, the preferred approach is modeling to incorporate a wide range of data. Toxicokinetic modeling has become increasingly common for its ability to support target-dose estimation, cross-species adjustment, or exposure-route conversion. If data are too limited to support toxicokinetic modeling, there are standardized approaches to estimate daily exposures and scale them from animals to humans (U.S. EPA, 1994), (U.S. EPA, 2005a), (U.S. EPA, 2011, 2006).

For human studies, an assessment may develop exposure–response models that reflect the structure of the available data (U.S. EPA, 2005a). For animal studies, EPA has developed a set of empirical ("curve-fitting") models[6] that can fit typical data sets (U.S. EPA, 2005a). Such modeling yields a *point of departure*, defined as a dose near the lower end of the observed range, without significant extrapolation to lower levels (e.g., the estimated dose associated with an extra risk of 10% for animal data or 1% for human data, or their 95% lower confidence limits) (U.S. EPA, 2005a), (U.S. EPA, 2012).

When justified by the scope of the assessment, toxicodynamic ("biologically based") modeling is possible if data are sufficient to ascertain the key events of a mode-of-action and to estimate their parameters. Analysis of model uncertainty can determine the range of lower doses where data support further use of the model (U.S. EPA, 2005a).

For a group of agents that act at a common site or through common mechanisms, an assessment may derive relative potency factors based on relative toxicity, rates of absorption or metabolism, quantitative structure–activity relationships, or receptor-binding characteristics (U.S. EPA, 2005a).

Extrapolation: slope factors and unit risks. An *oral slope factor* or an *inhalation*

[6] Benchmark Dose Software: http://www.epa.gov/bmds/

unit risk facilitates subsequent estimation of human cancer risks. Extrapolation proceeds linearly (i.e., risk proportional to dose) from the point of departure to the levels of interest. This is appropriate for agents with direct mutagenic activity. It is also the default if there is no established mode-of-action (U.S. EPA, 2005a).

Differences in susceptibility may warrant derivation of multiple slope factors or unit risks. For early-life exposure to carcinogens with a mutagenic mode-of-action, EPA has developed default *age-dependent adjustment factors* for agents without chemical-specific susceptibility data (U.S. EPA, 2005a), (U.S. EPA, 2005b).

If data are sufficient to ascertain the mode-of-action and to conclude that it is not linear at low levels, extrapolation may use the reference-value approach (U.S. EPA, 2005a).

Extrapolation: reference values. An *oral reference dose* or an *inhalation reference concentration* is an estimate of human exposure (including in susceptible populations) likely to be without appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002). Reference values generally cover effects other than cancer. They are also appropriate for carcinogens with a nonlinear mode-of-action.

Calculation of reference values involves dividing the point of departure by a set of *uncertainty factors* (each typically 1, 3, or 10, unless there are adequate chemical-specific data) to account for different sources of uncertainty and variability (U.S. EPA, 2002), (U.S. EPA, 2014).

Human variation: An uncertainty factor covers susceptible populations and lifestages that may respond at lower levels, unless the data originate from a susceptible study population.

Animal-to-human extrapolation: For reference values based on animal results, an uncertainty factor reflects cross-species differences, which may cause humans to respond at lower levels.

Subchronic-to-chronic exposure: For chronic reference values based on subchronic studies, an uncertainty factor reflects the likelihood that a lower level over a longer duration may induce a similar response. This factor may not be necessary for reference values of shorter duration.

Adverse-effect level to no-observed-adverse-effect level: For reference values based on a lowest-observed-adverse-effect level, an uncertainty factor reflects a level judged to have no observable adverse effects.

Database deficiencies: If there is concern that future studies may identify a more sensitive effect, target organ, population, or lifestage, a *database uncertainty factor* reflects the nature of the database deficiency.

8. Process for Developing and Peer-Reviewing IRIS Assessments

The IRIS process (revised in 2009 and enhanced in 2013) involves extensive public engagement and multiple levels of scientific review and comment. IRIS program scientists consider all comments. Materials released, comments received from outside EPA, and disposition of major comments (steps 3, 4, and 6 below) become part of the public record.

Step 1: Draft development. As outlined in section 2 of this Preamble, IRIS program scientists specify the scope of an assessment and formulate science issues for discussion with the scientific community and the public. Next, they release initial protocols for the systematic review procedures planned for use in the assessment. IRIS program scientists then develop a first draft, using structured approaches to identify pertinent studies, evaluate study methods and quality, integrate the evidence of causation for each health outcome, select studies for derivation of toxicity values, and derive toxicity values, as outlined in Preamble sections 3–7.

Step 2: Agency review. Health scientists across EPA review the draft assessment.

Step 3: Interagency science consultation. Other federal agencies and the Executive Office of the President review the draft assessment.

Step 4: Public comment, followed by external peer review. The public reviews the draft assessment. IRIS program scientists release a revised draft for independent external peer review. The peer reviewers consider whether the draft assessment assembled and evaluated the evidence according to EPA guidance and whether the evidence justifies the conclusions.

Step 5: Revise assessment. IRIS program scientists revise the assessment to address the comments from the peer review.

Step 6: Final agency review and interagency science discussion. The IRIS program discusses the revised assessment with EPA's program and regional offices and with other federal agencies and the Executive Office of the President.

Step 7: Post final assessment. The IRIS program posts the completed assessment and a summary on its website.

9. General Structure of IRIS Assessments

Main text. IRIS assessments generally comprise two major sections: (1) Hazard Identification and (2) Dose–Response Assessment. Section 1.1 briefly reviews chemical properties and toxicokinetics to describe the disposition of the agent in the body. This section identifies related chemicals and summarizes their health outcomes, citing authoritative reviews. If an assessment covers a chemical mixture, this section discusses environmental processes that alter the mixtures humans encounter and compares them to mixtures studied experimentally.

Section 1.2 includes a subsection for each major health outcome. Each subsection discusses the respective literature searches and study considerations, as outlined in Preamble sections 3 and 4, unless covered in the front matter. Each subsection concludes with evidence synthesis and integration, as outlined in Preamble section 5.

Section 1.3 links health hazard information to dose–response analyses for each health outcome. One subsection identifies susceptible populations and lifestages, as observed in human or animal studies or inferred from mechanistic data. These may warrant further analysis to quantify differences in susceptibility. Another subsection identifies biological considerations for selecting health outcomes, studies, or data sets, as outlined in Preamble section 6.

Section 2 includes a subsection for each toxicity value. Each subsection discusses study selection, methods of analysis, and derivation of a toxicity value, as outlined in Preamble sections 6 and 7.

Front matter. The Executive Summary provides information historically included in IRIS summaries on the IRIS program website. Its structure reflects the needs and expectations of EPA's program and regional offices.

A section on systematic review methods summarizes key elements of the protocols, including methods to identify and evaluate pertinent studies. The final protocols appear as an appendix.

The Preface specifies the scope of an assessment and its relation to prior assessments. It discusses issues that arose during assessment development and emerging areas of concern.

This Preamble summarizes general procedures for assessments begun after the date below. The Preface identifies assessment-specific approaches that differ from these general procedures.

August 2016

10. Preamble References

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EXECUTIVE SUMMARY

Summation of Occurrence and Health Effects

Ethyl tert-butyl ether (ETBE) does not occur naturally; it is an ether oxygenate produced by humans and primarily used as a gasoline additive. It was used until 2006 in the United States and is still used in Japan and the European Union. ETBE is released into the environment because of gasoline leaks, evaporation, and spills. Exposure to ETBE can occur by drinking contaminated groundwater or by inhaling off-gases 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 noncancer kidney effects following oral and inhalation exposure. Evidence is suggestive of carcinogenic potential for ETBE based on liver tumors in rats following inhalation exposure.

Effects Other Than Cancer Observed Following Oral Exposure

Kidney effects were identified as a potential human hazard of ETBE exposure. Although no human studies are available to evaluate the effects of ETBE, oral exposure studies in animals have consistently reported increased kidney weight in male and female rats accompanied by increased chronic progressive nephropathy (CPN), urothelial hyperplasia of the renal pelvis (in males), and increased blood concentrations of total cholesterol, blood urea nitrogen (BUN), and creatinine. Overall, there was consistency across multiple measures of potential kidney toxicity, including organ weight increases, exacerbated CPN, urothelial hyperplasia of the renal pelvis, and increases in serum markers of kidney function. Additionally, effects were also observed across routes of exposure, and sex (with the exception of urothelial hyperplasia of the renal pelvis which was observed only in male rats).

The relevance of the kidney findings to humans was evaluated with respect to α2u-globulin nephropathy, a disease process that occurs exclusively in the male rat kidney {Capen, 1999, 699905; U.S. EPA, 1991, 635839}. While ETBE binds to α_{2u}-globulin and meets some criteria of the α_{2u}-globulin EPA and IARC frameworks {U.S. EPA, 1991, 635839; Capen, 1999, 699905}, it does not meet all. With respect to male rats, US EPA 1991 notes that "[i]f a compound induces α_{2u}-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially occuring in humans." However, as α2u-globulin nephropathy is strictly a male rat phenomenon, the dose-related kidney effectsin female rats are not confounded by α2u-globulin nephropathy.

It has been observed that chemicals that bind to α_{2u}-globulin also exacerbate the incidence and/or severity of background chronic progressive nephropathy (CPN) in male rats {Travlos, 2011, 1239901}{U.S. EPA, 1991, 635839}{Frazier, 2012, 2919046}. While the etiology of CPN is unknown {Hard, 2004, 782757}{NIEHS, 2019, 5098230}{Peter, 1986, 194755} and it has no known analog in the aging human kidney {Hard, 2009, 667590}{NIEHS, 2019, 5098230}, it cannot be ruled out that a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. chronic kidney disease, diabetic nephropathy, glomerulonephritis, interstitial nephritis, etc){NIEHS, 2019, 5098230}. Therefore, increased incidence of kidney effects with ETBE exposure in the female rat (but not the male rat) are considered appropriate for identifying a hazard to the kidney.

Evidence is suggestive that liver toxicity follows oral ETBE exposure. The strongest supporting evidence is the increased liver weights and centrilobular hypertrophy in exposed male and female rats consistently reported across studies evaluating oral exposures. No additional histopathological findings were observed, however, and only one serum marker potentially indicative 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 were minimal 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 inadequate evidence exists to establish a relationship between receptor activation and liver toxicity resulting from ETBE exposure. In addition, mechanistic data suggest possibly greater susceptibility of toxic effects related to reduced clearance of acetaldehyde, a metabolite of ETBE. Thus, even with the consistently observed increases in rat liver weight and centrilobular hypertrophy, the evidence remains suggestive that liver toxicity follows ETBE exposure because of the relatively small magnitude of effects and lack of consistent dose response relationships.

Inadequate information exists to draw conclusions regarding reproductive effects, developmental effects, or immune system effects.

Oral Reference Dose (RfD) for Effects Other Than Cancer

Kidney toxicity, represented by increased absolute kidney weight in female rats, was chosen as the basis for the overall oral reference dose (RfD) (See Table ES-1). The chronic study by (JPEC, 2010a) [with selected data published as Suzuki et al. (2012)] and the observed kidney effects were used to derive the RfD. The endpoint of increased kidney weight was selected as the critical effect because it is a specific and sensitive indicator of kidney toxicity and was induced in a dose-responsive manner. Benchmark dose (BMD) modeling was used to derive the benchmark dose lower confidence limit ($BMDL_{10\%}$) of 120 mg/kg-day. The BMDL was converted to a human equivalent dose (HED) of 28.8 mg/kg-day using body weight$^{3/4}$ scaling (U.S. EPA, 2011), and this value was used as the point of departure (POD) for RfD derivation.

The 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 (3) and interindividual differences in human susceptibility (10).

Table ES-1. Organ-/system-specific RfDs and overall RfD for ETBE

Hazard	Basis	Point of departure* (mg/kg-day)	UF	Chronic RfD (mg/kg-day)	Study exposure description	Confidence
Kidney	Increased absolute kidney weight	28.8	30	1×10^0	Chronic	High
Overall RfD	**Kidney**	**28.8**	**30**	**1×10^0**	**Chronic**	**High**

* Human equivalent dose (HED) PODs were calculated using body weight to the ¾ power ($BW^{3/4}$) scaling (U.S. EPA, 2011).

Effects Other Than Cancer Observed Following Inhalation Exposure

Kidney effects are a potential human hazard of inhalation exposure to ETBE. While no human studies are available to evaluate the effects of exposure, studies in animals have observed increases in kidney weight, altered kidney histopathology, as well as alterations in clinical chemistry including serum cholesterol, BUN, and creatinine. While the histological lesion of urothelial hyperplasia of the renal pelvis was a sensitive endpoint in male rats, it was not observed in female rats or mice of either sex, whereas increased kidney weights were observed in multiple studies in rats of both sexes and in mice. Changes in kidney weight in female rats, were dose-dependant, consistent across multiple studies, and are not confounded by α_{2u}-globulin nephropathy, and therefore considered appropriate for identifying a hazard to the kidney.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

Kidney toxicity, represented by increased absolute kidney weight, was chosen as the basis for the overall inhalation reference concentration (RfC) (See Table ES-2). The chronic study by JPEC (2010b) [selected data published as Saito et al. (2013)] and the observed kidney effects were used to derive the RfC. The endpoint, increased absolute kidney weight, was selected as the critical effect because it is a specific and sensitive indicator of kidney toxicity and was induced in a dose-responsive manner. BMD modeling was attempted, but an adequate fit was not achieved. Therefore, a NOAEL was used to derive the POD of 6270 mg/m^3. The NOAEL was adjusted for continuous exposure and converted to a human equivalent concentration (HEC) of 1110 mg/m^3.

The overall RfC was calculated by dividing the POD for increased absolute kidney weight 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. Organ-/system-specific RfCs and overall RfC for ETBE

Hazard	Basis	Point of departure* (mg/m^3)	UF	Chronic RfC (mg/m^3)	Study exposure description	Confidence
Kidney	Increased absolute kidney weight	1110	30	4×10^{1}	Chronic	Medium
Overall RfC	**Kidney**	**1110**	**30**	**4×10^{1}**	**Chronic**	**Medium**

*Continuous inhalation HEC was adjusted for continuous daily exposure and calculated by adjusting the duration-adjusted POD (POD_{ADJ}) by the dosimetric adjustment factor (DAF = 0.992) for a Category 3 gas.

Evidence of Human Carcinogenicity

Under EPA's cancer guidelines (U.S. EPA, 2005a), the evidence of carcinogenic potential for ETBE is *suggestive* for inhalation exposure and *inadequate* for oral exposure. ETBE induced liver tumors in male (but not female) rats in a 2-year inhalation exposure study. No significant effects were observed in two chronic oral studies in male and female rats {JPEC, 2010, 1517477}{Maltoni, 1999, 87642} (see Section 1.2.5). Data on tumorigenicity in mice following ETBE oral or inhalation exposure was not available. However, supplementary evidence from 2-stage initiation-promotion oral carcinogenesis bioassays indicate increased mutagen-initiated liver, as well as increased tumor incidence in the thyroid, colon, urinary bladder.

Quantitative Estimate of Carcinogenic Risk from Oral Exposure

A quantitative estimate of carcinogenic potential from oral exposure to ETBE was not derived as an increase in tumors was not observed in the two available chronic oral cancer bioassays. A route to route extrapolation of cancer risk from the inhalation to oral route was not carried out because there was no consistent dose-response relationship observed for liver tumors when compared across oral and inhalation studies on the basis of PBPK modeled internal dose.

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

A quantitative estimate of carcinogenic potential from inhalation exposure to ETBE was based on the increased incidence of hepatocellular adenomas and carcinomas in male F344 rats following 2-year inhalation exposure (Saito et al., 2013; JPEC, 2010b). 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.

Although ETBE was considered to have "suggestive evidence of carcinogenic potential," the main study (Saito et al., 2013; JPEC, 2010b) was conducted according to well-established guidelines for examining potential carcinogenicity and considered suitable for quantitative analyses. An inhalation unit risk was derived for liver tumors in male F344 rats. The modeled ETBE POD was scaled to an HEC according to EPA guidance based on inhalation dosimetry for a Category 3 gas (U.S. EPA, 1994). Using linear extrapolation from the $BMCL_{10}$, a human equivalent inhalation unit risk was derived (inhalation unit risk = $0.1/BMCL_{10}$). The inhalation unit risk is **8×10^{-5} per mg/m^3**.

Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

ETBE is metabolized to *tert*-butanol and acetaldehyde. Evidence is suggestive that genetic polymorphism of aldehyde dehydrogenase (ALDH)—the enzyme that oxidizes acetaldehyde to acetic acid—could affect ETBE toxicity. The virtually inactive form, ALDH2*2, is found in about one-half of all East Asians (and by extension people of East Asian ancestry) (Brennan et al., 2004). Evidence is strong in humans that heterozygous *ALDH2* increases the internal dose and the cancer risks from acetaldehyde, especially in the development of alcohol-related cancers in the esophagus and upper aerodigestive tract, but relevance of this finding in regards to liver tumorigenesis is less clear (IARC, 2010). Several in vivo and in vitro genotoxicity assays in *Aldh2* knockout (KO) and heterozygous mice reported that genotoxicity was significantly increased compared with wild-type controls following ETBE exposure to similar doses associated with cancer and noncancer effects in rodents {Weng, 2019, 5343910}(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* KO mice compared with wild type {Weng, 2013, 2279880}. 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.

Collectively, these data present evidence that people with diminished ALDH2 activity could be considered a susceptible population that could be more sensitive to ETBE exposure.

Key Issues Addressed in Assessment

The human relevance of the kidney effects observed in male and female rats was analyzed in the assessment, particularly as they relate to α2u-globulin nephropathy and the exacerbation of chronic progressive nephropathy. An evaluation of whether ETBE caused α_{2u}-globulin-associated nephropathy was performed using the EPA 1991 and IARC 1999 α_{2u}-globulin frameworks {Capen, 1999, 699905}{U.S. EPA, 1991, 635839}. ETBE induced an increase in hyaline droplet accumulation and increased α_{2u}-globulin deposition in male rats; however, most of the subsequent steps in the pathological sequence were not observed. Although the conditions were not fully met with either α_{2u}-globulin framework, {U.S. EPA, 1991, 635839@@author-year} states that "[i]f a compound induces α_{2u}-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially

occuring in humans." However, as α2u-globulin nephropathy is strictly a male rat phenomenon, dose-related kidney effects in female rats are not confounded by α2u-globulin nephropathy. CPN also plays a role in the exacerbation of nephropathy in rats, however the MOA of CPN is unknown, and therefore, its potential relevance to humans cannot be ruled out {NIEHS, 2019, 5098230}. Dose-related changes in several indicators of kidney toxicity were observed, including increased absolute kidney weight, histological changes, and increased blood biomarkers (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). These specific effects are considered relevant to humans, particularly the endpoints observed in female rats, as they are not confounded by α_{2u}-globulin related processes.

In addition, the human relevance of the observed liver tumors was discussed in the assessment (see Sections 1.2.2 and 1.3.2). Briefly, a well conducted inhalation study demonstrated a significant, positive exposure-response for hepatocellular adenomas and carcinomas in male rats (Saito et al., 2013; JPEC, 2010b). While the majority of liver tumors occurred at the highest exposure, statistical tests conducted by the study authors found significant dose-response trend by both the Peto test (incidental tumor test) and the Cochran-Armitage test. However, two chronic oral exposure studies (one with survival issues) were negative for liver tumors {Maltoni, 1999, 87642}{JPEC, 2010, 1517477}.

The potential MOA of for the observed liver tumors was evaluated in the assessement (see Section 1.2.2). The available evidence base for the nuclear hormone receptor MOAs (i.e., PPARα, PXR, and CAR) was inadequate to determine the role these pathways play, if any, in ETBE-induced liver carcinogenesis. Acetaldehyde-mediated genotoxicity also was evaluated as a possible MOA, and although evidence suggests that *ALDH2* deficiency enhanced ETBE-induced genotoxicity in exposed mice, the available database was inadequate to establish acetaldehyde-mediated mutagenicity as an MOA for ETBE-induced liver tumors. No other MOAs for liver carcinogenesis were identified, and the rat liver tumors observed following inhalation exposure are considered relevant to humans (U.S. EPA, 2005a).

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

A literature search and screening strategy consisted of a broad search of online scientific databases and other sources 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. The original chemical-specific search was conducted in four online scientific databases, PubMed, Toxline, Web of Science, and TSCATS, through December 2016, 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, 847 unique citations were identified. The resulting 847 citations were screened for pertinence and separated into categories as presented in Figure LS-1

Figure LS-1 using the title and either abstract or full text, or both, to examine the health effects of ETBE exposure. The inclusion and exclusion criteria used to screen the references and identify sources of health effects data are provided in Table LS-3.

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

- 70 references were identified as "Supporting Studies." These included 31 studies describing physiologically based pharmacokinetic (PBPK) models and other toxicokinetic information; 25 studies providing genotoxicity and other mechanistic information; 9 acute, short-term, or preliminary toxicity studies; and 5 direct administration (e.g., dermal) studies of ETBE. Although 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 supplementary health effects information.

- 29 references were identified as "Secondary Literature and Sources of Contextual Information" (e.g., reviews and other agency assessments); these references were retained as additional resources for development of the Toxicological Review.

- 715 references were identified as being not pertinent (not on topic) to an evaluation of health effects for ETBE and were excluded from further consideration (see Figure LS-1) for exclusion categories and Table LS-3 for exclusion criteria). For example, 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. Retrieving numerous references that are not on topic is a consequence of applying an

initial search strategy designed to cast a wide net and to minimize the possibility of missing potentially relevant health effects data.

Figure LS-1The complete list of references as sorted above can be found on the ETBE project page of the HERO website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/1376.

Post-Peer-Review Literature Search Update

A post-peer-review literature search update was conducted in PubMed, Toxline, TSCATS, and DTIC for the period Dec 2016 to July 2019 using a search strategy consistent with previous literature searches (see Table LS-1). Consistent with the IRIS Stopping Rules (https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf), manual screening of the literature search update focused on identifying new studies that might change a major conclusion of the assessment. No animal bioassays or epideminological studies were identified in the post-peer-review literature search which would change any major conclusions in the assessment.

The documentation and results for the literature search and screen, including the specific references identified using each search strategy and tags assigned to each reference based on the manual screen, can be found on the HERO website on the ETBE project page at: (https://hero.epa.gov/hero/index.cfm/project/page/project_id/1376).

Selection of Studies for Inclusion in Evidence Tables

To summarize the important information systematically from the primary health effects studies in the ETBE evidence base, evidence tables were constructed in a standardized tabular format as recommended by NRC (2011). Studies were arranged in evidence tables by route of exposure and then alphabetized by author. Of the studies retained after the literature search and screen, 33 were identified as "Sources of Health Effects Data" and considered for extraction into evidence tables for the hazard identification in Section 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 32 studies were extracted into evidence tables.

Supplementary 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, and pilot studies, were not included in the evidence tables. One controlled human exposure toxicokinetic study was identified, which is discussed in Appendix B.2 (Toxicokinetics). Short-term and acute studies did not differ qualitatively from the results of the longer-term studies (i.e., ≥90-day exposure studies). These were grouped as supplementary studies, however, because the evidence base of chronic and

subchronic rodent studies was considered sufficient for evaluating chronic health effects of ETBE exposure. Additionally, studies of effects from chronic exposure are most pertinent to lifetime human exposure (i.e., the primary characterization provided by IRIS assessments) and are the focus of this assessment. Such supplementary studies can be discussed in the narrative sections of Section 1 and are described in sections such as *Mode of action analysis* to augment the discussion or presented in appendices, if they provide additional information.

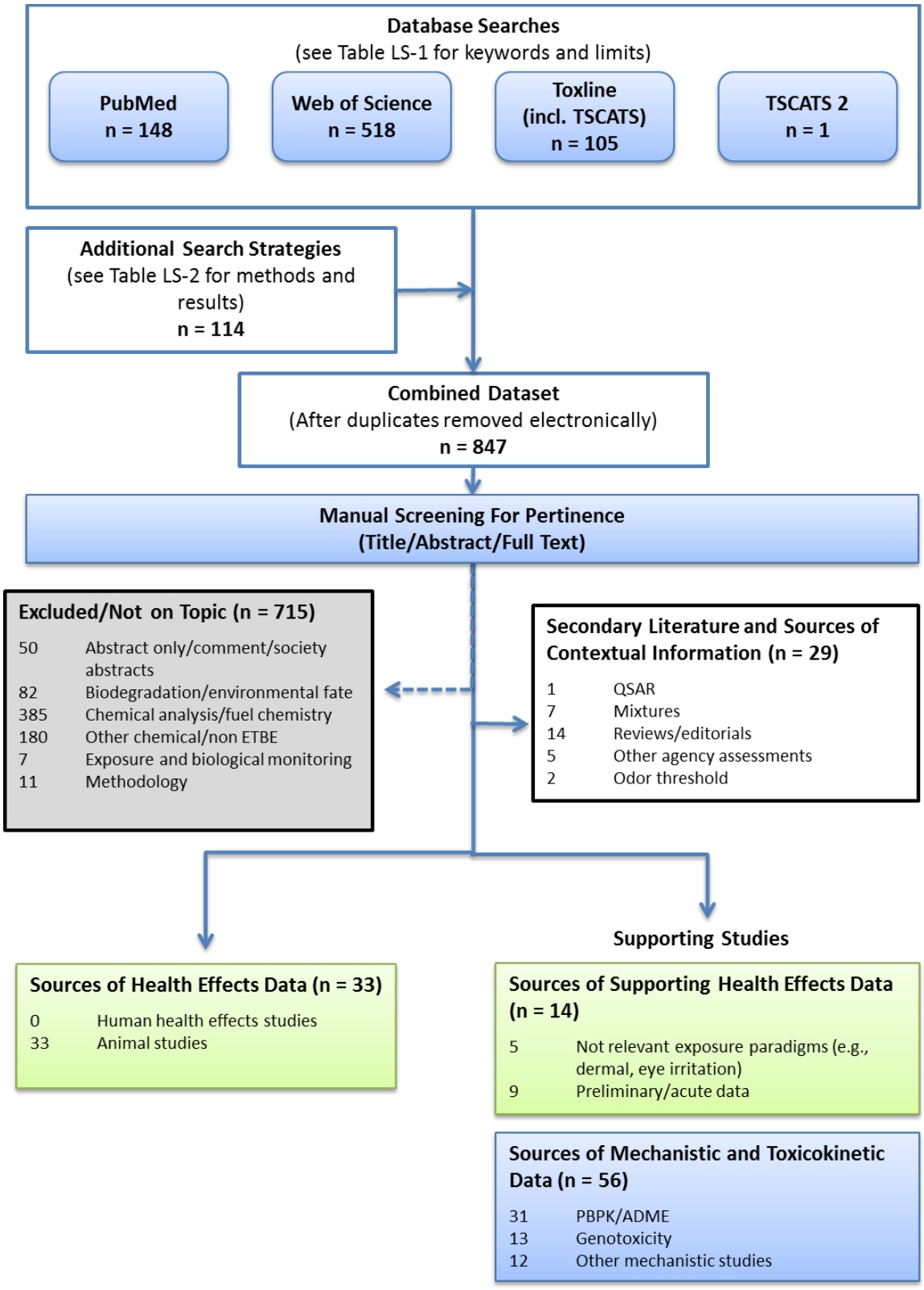


Figure LS-1. Summary of literature search and screening process for ETBE.

Table LS-1. Details of the search strategy employed for ETBE

Database (Search date)	Keywords	Limits
PubMed (03/31/2014) Updated (12/2016)	*"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) Updated (12/2016)	*"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 (e.g. the search term is reduced to its lexical root)
Toxline (includes TSCATS) (03/31/2014) Updated (12/2016)	*"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) Updated (12/2016)	637-92-3	01/2004 to 7/2019

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	Japan Petroleum Energy Center	3/2014 Updated (12/2016)	21 references

Table LS-3. Inclusion-exclusion criteria

	Inclusion criteria	Exclusion criteria/Supplemental material*
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, monkey, dog	• Ecological species* • Nonmammalian species*
Exposure	• Exposure is to ETBE • Exposure is measured in an environmental medium (e.g., air, water, diet) • Exposure via oral or inhalation routes; for supporting health effect studies, exposure via oral or inhalation routes	• Study population is not exposed to ETBE • Exposure to a mixture only (e.g., gasoline containing ETBE) • Exposure via injection (e.g., intravenous) • Exposure paradigm not relevant (e.g., acute, dermal, or ocular)
Outcome	• Study includes a measure of one or more health effect endpoints, including effects on the nervous, kidney/urogenital, musculoskeletal, cardiovascular, immune, and gastrointestinal systems; reproduction; development; liver; eyes; and cancer	• Odor threshold studies*

	Inclusion criteria	Exclusion criteria/Supplemental material*
Other		Not on topic, including: • Abstract only, editorial comments, policy papers, were not considered further because study was not potentially relevant • Bioremediation, biodegradation, or environmental fate of ETBE, including evaluation of wastewater treatment technologies and methods for remediation of contaminated water and soil • Chemical, physical, or fuel chemistry studies • Analytical methods for measuring/detecting/ remotely sensing ETBE • Not chemical specific: Studies that do not involve testing of ETBE • Quantitative structure activity relationship studies • Exposure studies without health effect evaluation

*Studies that met this exclusion criterion were considered supplemental, e.g. not considered a primary source of health effects data, but were retained as potential sources of contextual information.

Evidence base Evaluation

For this draft assessment, 33 experimental animal studies comprised the primary sources of health effects data; no studies were identified that evaluated humans exposed to ETBE (e.g., cohort studies, case reports, ecological studies). The animal studies were evaluated considering aspects of design, conduct, or reporting that could affect the interpretation of results, overall contribution to the synthesis of evidence, and determination of hazard potential as noted in various EPA guidance documents (U.S. EPA, 2005a, 1998b, 1996, 1991b). The objective was to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design. Studies were evaluated to identify their suitability based on:

- Study design
- Nature of the assay and validity for its intended purpose
- Characterization of the nature and extent of impurities and contaminants of ETBE administered, if applicable
- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects
- Sample sizes 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

Additionally, several general considerations, presented in Table LS-4, were used in evaluating the animal studies (Table LS-5). 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.

EPA considered statistical tests to evaluate whether the observations might 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. Studies that did not report statistical testing were identified and, when appropriate, statistical tests were conducted by EPA.

Information on study features related to this evaluation is reported in evidence tables and documented in the synthesis of evidence. Discussions of study strengths and limitations are included in the text where relevant. If EPA's interpretation of a study differs from that of the study authors, the draft assessment discusses the basis for the difference.

Experimental Animal Studies

The 33 experimental animal studies, all of which were performed on rats, mice, and rabbits, were associated with drinking water, oral gavage, or inhalation exposures to ETBE. Many of these studies were conducted according to Organisation for Economic Co-operation and Development Good Laboratory Practice (GLP) guidelines and used well established methods, were well-reported, and evaluated an extensive range of endpoints and histopathological data. For the body of available studies, detailed discussion of any identified methodological concerns precedes each endpoint evaluated in the hazard identification section. Overall, the experimental animal studies of ETBE involving repeated oral or inhalation exposure were considered acceptable quality, and whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to ETBE.

Table LS-4. Considerations for evaluation of experimental animal studies

Methodological feature	Considerations (relevant information extracted into evidence tables)
Test animal	Suitability of species, strain, sex, and source of test animals
Experimental design	Suitability of animal age/lifestage at exposure and endpoint testing; periodicity and duration of exposure (e.g., hr/day, day/week); timing of endpoint evaluations; and sample size and experimental unit (e.g., animals, dams, litters)
Exposure	Characterization of test article source, composition, purity, and stability; suitability of control (e.g., vehicle control); documentation of exposure techniques (e.g., route,

	chamber type, gavage volume); verification of exposure levels (e.g., consideration of homogeneity, stability, analytical methods)
Endpoint evaluation	Suitability of specific methods for assessing endpoint(s) of interest
Results presentation	Data presentation for endpoint(s) of interest (including measures of variability) and for other relevant endpoints needed for results interpretation (e.g., maternal toxicity, decrements in body weight relative to organ weight)

Table LS-5. Summary of experimental animal evidence base

Study Category	Study duration, species/strain, and administration method
Chronic	2-year study in F344 rats (drinking water) JPEC (2010a)*; Suzuki et al. (2012) 2-year study in F344 rats (inhalation) JPEC (2010b)*; Saito et al. (2013) 2-year study in Sprague-Dawley rats (gavage) Maltoni et al. (1999)
Subchronic	13-week study in F344 rats (inhalation) Medinsky et al. (1999); Bond et al. (1996b) 26-week study in Sprague-Dawley rats (gavage) JPEC (2008c)*; Miyata et al. (2013) Fujii et al. (2010); JPEC (2008e) 13-week study in Sprague-Dawley rats (inhalation) JPEC (2008b)* 23-week study in F344 rats (gavage) Hagiwara et al. (2011); JPEC (2008d) 13-week study in CD-1 mice (inhalation) Medinsky et al. (1999); Bond et al. (1996a) 23-week study in Wistar rats (gavage) Hagiwara et al. (2015) 31-week study in F344/DuCrlCrlj rats (drinking water) Hagiwara et al. (2013) 13-week study in C57BL/6 mice (inhalation) Weng et al. (2012)
Reproductive	Two-generation reproductive toxicity study on Sprague-Dawley rats (gavage) Gaoua (2004b)* One-generation reproductive toxicity study on Sprague-Dawley rats (gavage) Fujii et al. (2010); JPEC (2008e) 2-week study on Simonson albino rats (drinking water) Berger and Horner (2003) 9-week study on C57BL/6 mice (inhalation) Weng et al. (2014) 14-day study on F344 rats (gavage) de Peyster et al. (2009) Two-generation reproductive toxicity study in Sprague-Dawley rats (gavage) Gaoua (2004b)*
Developmental	Developmental study (GD6–27) on New Zealand rabbits (gavage) Asano et al. (2011); JPEC (2008i) Developmental study (GD5–19) on Sprague-Dawley rats (gavage) Aso et al. (2014); JPEC (2008h) Developmental study (GD5–19) on Sprague-Dawley rats (gavage) Gaoua (2004b)* Developmental study (GD5–19) on Sprague-Dawley rats (gavage) Gaoua (2004a)*
Pharmacokinetic	Single-dose study on Sprague-Dawley rats (gavage) JPEC (2008g) 14-day study on Sprague-Dawley rats (gavage) JPEC (2008f) Single-dose study on Sprague-Dawley rats (gavage) JPEC (2008g)* 14-day study on Sprague-Dawley rats (gavage) JPEC (2008f)*

*The IRIS program had this study peer reviewed.

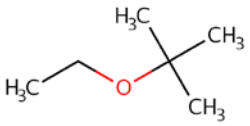
1. HAZARD IDENTIFICATION

1.1. OVERVIEW OF CHEMICAL PROPERTIES AND TOXICOKINETICS

1.1.1. Chemical Properties

ETBE is a liquid at a temperature range of −94 to 72.6°C. It is soluble in ethanol, ethyl ether, and water (Drogos and Diaz, 2001). ETBE has a strong, highly objectionable odor and taste at relatively low concentrations. The chemical is highly flammable and reacts with strong oxidizing agents. ETBE is stable when stored at room temperature in tightly closed containers (Drogos and Diaz, 2001). Information on physiochemical properties for ETBE is available at U.S. Environmental Protection Agency (EPA)'s Comptox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) and is summarized in Table 1-1.

Table 1-1. Chemical identity and physicochemical properties of ethyl tert-butyl ether (ETBE) from EPA's CompTox Chemicals Dashboard

Characteristic or property	Value	
Chemical structure		
CASRN	637-92-3	
Synonyms	Ethyl T-butyl ether; 2-Ethoxy-2-methylpropane; Propane, 2-ethoxy-2-methyl; Ethyl tert-butyl ether; 2-Methyl-2-ethoxypropane (see https://comptox.epa.gov/dashboard for additional synonyms)	
Molecular formula	$C_6H_{14}O$	
Molecular weight	102.177	
	Average experimental value[a]	**Average predicted value[a]**
Flash point (°C)	—	-10.9
Boiling point (°C)	72.4	74.3
Melting point (°C)	-94	-90.8
Log K_{OW}	—	1.72
Density (g/cm^3)	—	0.768

Henry's law constant (atm-m^3/mole)	1.64×10^{-3}	1.66×10^{-3}
Vapor pressure (mm Hg at 20°C)	124	124

atm = atmosphere; CASRN = Chemical Abstracts Service registry number.
[a]Median values and ranges for physical chemical properties of ETBE are also provided on the CompTox Chemicals Dashboard at https://comptox.epa.gov/dashboard/.

1.1.2. Toxicokinetics

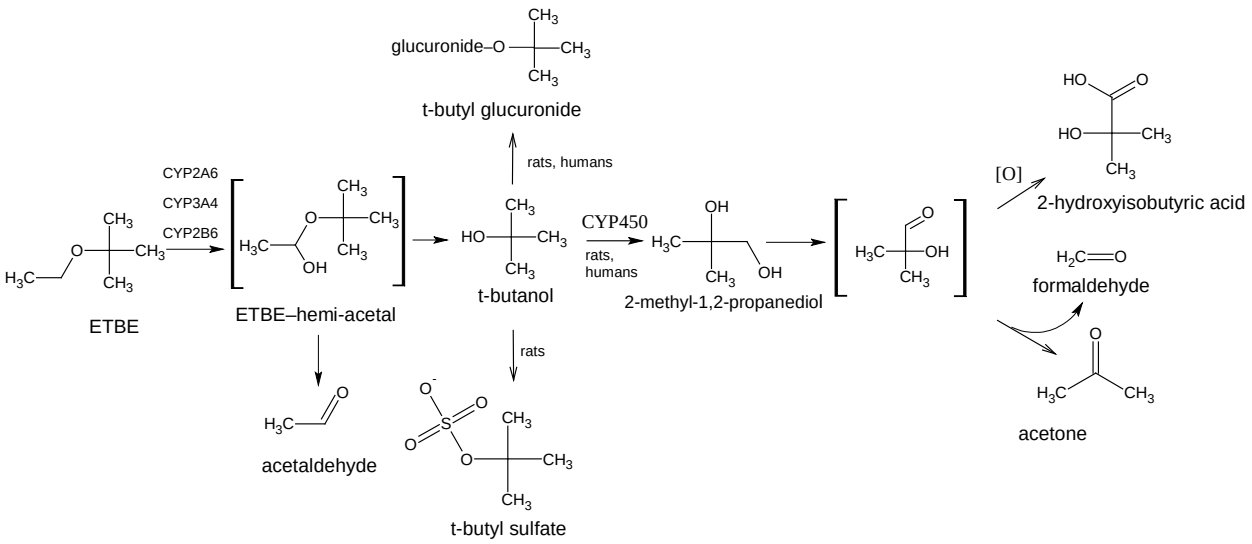
ETBE is rapidly absorbed following exposure by oral and inhalation routes (see Appendix B.1.1). Studies in experimental animals indicate that >90% of the compound was absorbed after oral administration within 6–10 hours (JPEC, 2008d, e). No data are available for oral absorption in humans. ETBE is moderately absorbed following inhalation exposure in both rats and humans; human blood levels of ETBE approached—but did not reach—steady-state concentrations within 2 hours, and a net respiratory uptake of ETBE was estimated to be 26% in a short term exposure study conducted at light activity levels (Nihlén et al., 1998b).

ETBE and its metabolite, *tert*-butanol, are distributed throughout the body following oral, inhalation, and i.v. exposures (JPEC, 2008d, e; Poet et al., 1997; Faulkner et al., 1989; ARCO, 1983). Following exposure to ETBE in rats, ETBE was found in kidney, liver, and blood. Comparison of ETBE distribution in rats and mice demonstrated that concentrations of ETBE in the rat kidney and mouse liver are proportional to the blood concentration.

A general metabolic scheme for ETBE, illustrating the biotransformation in rats and humans, is shown in Figure 1-1 (see Appendix B.1.3).

Human data on the excretion of ETBE was measured in several studies (Nihlén et al., 1998a, c). The half-life of ETBE in urine was biphasic with half-lives of 8 minutes and 8.6 hours (Johanson et al., 1995). These studies showed urinary excretion of ETBE to be less than 0.2% of the uptake or absorption of ETBE (Nihlén et al., 1998a, c). Amberg et al. (2000) observed a similar half-life of 1–6 hours after human exposure to ETBE of 170 mg/m^3; however, the elimination for ETBE in rat urine was considerably faster than in humans, and ETBE itself was undetectable in rat urine.

A more detailed summary of ETBE toxicokinetics is provided in Appendix B.1.



Source: Adapted from Dekant et al. (2001), NSF International (2003), ATSDR (1996), Bernauer et al. (1998), Amberg et al. (1999), and Cederbaum and Cohen (1980).

Figure 1-1. Proposed metabolism of ETBE.

1.1.3. Description of Toxicokinetic Models

Two physiologically based pharmacokinetic (PBPK) models have been developed specifically for administration of ETBE in rats (Borghoff et al., 2016; Salazar et al., 2015). The previously available models have studied *tert*-butanol as the primary metabolite after oral or inhalation exposure to MTBE in rats and humans or ETBE in humans. Models for MTBE oral and inhalation exposure include a component for the binding of *tert*-butanol to α_{2u}-globulin (Borghoff et al., 2010; Leavens and Borghoff, 2009). A PBPK model for inhalation exposure of humans to ETBE has also been reported (Nihlén and Johanson, 1999).

All available PBPK models of ETBE and its principal metabolite *tert*-butanol were evaluated for potential use in this assessment (for extrapolation from animals to humans and for extrapolation between routes of exposure). Regarding the extrapolation from animals to humans, the existing human PBPK model was not considered adequate (see Appendix B.1.7), therefore, default methodologies were applied to extrapolate toxicologically equivalent exposures from adult laboratory animals to adult humans. Regarding the extrapolation between routes of exposure, extrapolation from inhalation to oral routes of exposure was not supported due to the lack of a consistent dose-response relationship for liver tumors combined across oral and inhalation studies.

A detailed summary of the toxicokinetic models is provided in Appendix B.1.5 (U.S. EPA, 2017).

1.1.4. Related Chemicals that Provide Supporting Information

ETBE is metabolized to acetaldehyde and t*ert*-butanol, and effects induced by these metabolites can inform the evaluation of ETBE-induced effects. Some of the non-cancer kidney

effects observed in ETBE have been attributed to *tert*-butanol (Salazar et al., 2015). Animal studies demonstrate that chronic exposure to *tert*-butanol is associated with noncancer kidney effects, including increased kidney weights in male and female rats accompanied by increased chronic progressive nephropathy (CPN), urothelial hyperplasia/transitional epithelial hyperplasia (in males and females), and increased suppurative inflammation in females (NTP, 1997, 1995b).

Inhalation exposures to acetaldehyde were concluded to cause carcinomas of the nasal mucosa in rats and carcinomas of the larynx in hamsters (IARC, 1999b). In addition, acetaldehyde was concluded to be the key metabolite in cancer of the esophagus and aerodigestive tract associated with ethanol consumption (IARC, 2010).

MTBE is a structurally related compound that is metabolized to formaldehyde and *tert*-butanol. In 1996, the U.S. Agency for Toxic Substances and Disease Registry's (ATSDR) *Toxicological Profile for MTBE* (ATSDR, 1996) identified cancer effect levels of MTBE based on carcinogenicity data in animals. ATSDR reported that inhalation exposure resulted in kidney cancer in rats and liver cancer in mice. ATSDR concluded that oral exposure to MTBE might cause liver and kidney damage and nervous system effects in rats and mice. The chronic inhalation minimal risk level was derived based on incidence and severity of chronic progressive nephropathy in female rats (ATSDR, 1996). In 1998, the International Agency for Research on Cancer (IARC) found "limited" evidence of MTBE carcinogenicity in animals and classified MTBE in Group 3 (i.e., not classifiable as to carcinogenicity in humans) (IARC, 1999d). Although some similar effects are seen with *tert*-butanol and ETBE, the evidence from MTBE is confounded by its metabolite formaldehyde, a known human carcinogen (as classified by IARC and NTP).

1.2. PRESENTATION AND SYNTHESIS OF EVIDENCE BY ORGAN/SYSTEM

1.2.1. Kidney Effects

Synthesis of Effects in Kidney

This section reviews the studies that investigated whether subchronic or chronic exposure to ETBE can cause kidney toxicity in humans or animals. The evidence base examining kidney effects following ETBE exposure contains no human data and 10 animal studies, predominantly in rats. Exposures ranged from 13 weeks to 2 years and both inhalation and oral exposure routes are well represented. Studies using 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 inform mode of action (MOA) or hazard identification. Four unpublished technical reports relevant to the kidney were externally peer reviewed at the request of EPA in August 2012 (Table LS-5): JPEC (2010a), JPEC (2010b), JPEC (2008c), JPEC (2008b), some of which were subsequently published. These are JPEC (2010a) [published as Suzuki et al. (2012)], JPEC (2010b) [published as Saito et al. (2013)], and JPEC (2008c) [published as Miyata et al. (2013)]. Gaoua (2004b) was

externally peer reviewed at the request of EPA in November 2008. Studies are arranged in evidence tables by effect and alphabetical order by author.

The unpublished report by Cohen et al. (2011) was not peer reviewed externally. In Cohen et al. (2011), a pathology working group reexamined kidney histopathology from the JPEC (2010a) [subsequently published as Suzuki et al. (2012)] and JPEC (2007a) studies. Cohen et al. (2011) did not report incidences of carcinomas that differed from those in the original study (Suzuki et al., 2012; JPEC, 2010a); thus, these data have been presented only once and it was not considered necessary to have the Cohen report peer reviewed externally. Histopathological interpretations from both Cohen et al. (2011) and JPEC (2007b) are considered for hazard identification.

The design, conduct, and reporting of each study were reviewed, and each study was considered adequate to provide information pertinent to this assessment. Interpretation of non-neoplastic kidney endpoints in rats, however, is complicated by the common occurrence of age-related spontaneous lesions characteristic of CPN (NTP, 2015; Hard et al., 2013; Melnick et al., 2012; U.S. EPA, 1991a); http://ntp.niehs.nih.gov/nnl/urinary/kidney/necp/index.htm). CPN is more severe in male rats than in females and is particularly common in the Sprague-Dawley and Fischer 344 strains. Dietary and hormonal factors play a role in modifying CPN, although the etiology is unknown (see further discussion below).

Kidney weight. Kidney weights (see Figure 1-2) exhibited strong dose-related increases with estimates of the primary ETBE metabolite, tert-butanol, in blood in male rats following oral exposures (Spearman's rank coefficient = 0.86, $p < 0.01$) of 16 weeks or longer (Miyata et al., 2013; Suzuki et al., 2012; Fujii et al., 2010; JPEC, 2010a, 2008c; Gaoua, 2004b), and following inhalation exposures (Spearman's rank coefficient = 0.71, $p = 0.05$) of 13 weeks or longer (Saito et al., 2013; JPEC, 2010b, 2008b; Medinsky et al., 1999). Kidney weight also showed strong dose-related increases following inhalation exposure (Spearman's rank coefficient = 0.82, $p = 0.01$) and moderate dose-related increases following oral exposure (Spearman's rank coefficient = 0.42, $p = 0.2$). Short-term studies in rats also observed increased kidney weight (JPEC, 2008a). In utero ETBE exposure induced greater increases in kidney weights in F1 male and female rats compared to parental exposure in one unpublished study (independently peer reviewed via EPA contract, see Table LS-5) but the magnitude of increases were comparable to those observed in other adult oral studies (Gaoua, 2004b). A 13-week mouse inhalation study observed small increases in kidney weight in both sexes, with a greater magnitude of effect in males, up to 10% as compared to 4-6% increases in females, see Figure 1-3 {Bond, 1996, 74002}{Medinsky, 1999, 10740}.

In most of the studies with data available for relative and absolute organ weight comparisons, both relative and absolute kidney weights are increased (Miyata et al., 2013; Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010b, 2008b, c; Gaoua, 2004b). Measures of relative, as opposed to absolute, organ weight can account for the influence of body weight on some organ weights (Bailey et al., 2004). For ETBE, body weight in exposed animals was consistently decreased at several doses relative to controls in the oral and inhalation studies. Thus, use of relative organ

weight change would not be a reliable measure of the kidney weight increase for this assessment. Additionally, a recent analysis indicates that absolute, but not relative, subchronic kidney weights are significantly correlated with chemically induced histopathological findings in the kidney in chronic and subchronic studies (Craig et al., 2014). Therefore, absolute weight was used as the more appropriate measure of kidney weight change for determining ETBE hazard potential. Absolute and relative kidney weight data are presented in Appendix B of the Supplemental Information.

Interpretation of 2-year kidney weight data in male rats treated by inhalation is complicated by increased mortality attributed to CPN which would be expected to bias) the analysis of kidney weight (toward the null), as organ weight was not assessed in animals that did not survive to study termination (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). 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), thus animals severely affected by CPN, including those that died due to CPN, would be expected to have enlarged kidneys. Although mortality in female rats in the 2-year inhalation study was also significantly increased, the study authors attributed these deaths to pituitary tumors, which would not be expected to bias measurement of kidney weight {JPEC, 2010, 1517421}. Mortality of male and female rats in the 2 year drinking water studies was not significantly different from controls {JPEC, 2010, 1517477}.

Kidney histopathology. Kidney lesions also were observed in several studies. The incidence of nephropathy, which was characterized as CPN due to sclerosis of glomeruli, thickening of the renal tubular basement membranes, inflammatory cell infiltration, and interstitial fibrosis, was not increased in any chronic study because of ETBE exposure. However, the severity of CPN was exacerbated by ETBE in male and female rats in a 2-year inhalation study, and the number of CPN foci was increased in male rats in a 13-week drinking water study (see Table 1-2) (Cohen et al., 2011; JPEC, 2010b, 2007a). Increases in CPN graded as marked or severe were dose-related when compared on an internal dose basis across routes of exposure in male and female rats (Salazar et al., 2015).

CPN is a common and well-established constellation of age-related lesions in the kidney of rats, although the mode of action of CPN is not known. In addition, no known counterpart to CPN has been identified in the aging human kidney. However, several individual lesions noted in CPN (e.g. tubule atrophy, tubule dilation, thickening of tubular basement membranes, glomerulosclerosis) also occur in the human kidney {Frazier, 2012, 2919046}{Lusco, 2016, 5926047}{Zoja, 2015, 5926046}{Satirapoj, 2012, 5926045}{NTP, 2019, 5926049}. Therefore, exacerbation of one or more of these lesions following ETBE exposure may reflect some type of cell injury or inflammatory process, which is relevant to the human kidney.

Increased incidence of urothelial hyperplasia (also known as transitional epithelial hyperplasia) of the renal pelvis (graded as slight or minimal) was observed in male rats in 2-year studies by both inhalation and oral exposure (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b).

However, urothelial hyperplasia was not observed in female rats following 2 years of oral or inhalation exposure (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). The histological finding of urothelial hyperplasia represents in an increase in the layers of urothelium and is typically associated with inflammation or neoplasia {Peterson, 2019, 5374688}{NTP, 2019, 5934030}. The increase in urothelial hyperplasia in male rats appeared to be dose related on an internal dose basis across routes of exposure (Salazar et al., 2015). Cohen et al. (2011), however, attributed this effect to CPN rather than the "direct" result of ETBE treatment. In addition, there is some confusion regarding the terminology of "urothelial hyperplasia of the renal pelvis" as reported in the JPEC studies {JPEC, 2010, 1517477;JPEC, 2010, 1517421}. Specifically, based on the pathological description of this lesion, it may represent proliferation of the papillary lining epithelium and not true "urothelial hyperplasia" {NIEHS, 2019, 5098230}. Hyperplasia of the epithelial lining of the renal papilla has been associated with advanced CPN {NTP, 2019, 5926049}.

To determine if the severity of the hyperplasia was positively associated with the severity of CPN, contingency tables comparing the occurrence of urothelial hyperplasia with CPN in individual rats were arranged by severity and analyzed with Spearman's rank correlation tests to determine strength of associations for each comparison (Table 1-25, 1-6). Urothelial hyperplasia and severity of CPN were weakly correlated (Spearman's rank coefficient = 0.36) in males following oral and inhalation exposure to ETBE. The biological significance of urothelial hyperplasia and any relationship with CPN is discussed in *Mode of Action Analysis* (see below).

The number and size of hyaline droplets were increased in the proximal tubules of male rats, but not in 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 finding, along with other potentially related histopathological effects, such as necrosis, linear tubule mineralization, and tubular hyperplasia, are discussed in *Mode of action analysis* (see below).

Serum and urinary biomarkers. The increased kidney weight and CPN in male rats is associated with several changes in urinary and serum biomarkers of renal function (see Table 1-2, Table 1-3). CPN is proposed to be associated with several changes in urinary and serum measures such as proteinuria, blood urea nitrogen (BUN), creatinine, and hypercholesterolemia (Hard et al., 2009). In general, ETBE exposure, increased serum measures at lower doses and in more studies than were associated with increased CPN severity. Considering male rat blood concentrations in both chronic and subchronic studies, total cholesterol was elevated in 3 of 4 studies, BUN was elevated in 2 of 4 studies, and creatinine was elevated 1 of 4 studies (Miyata et al., 2013; Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b, 2008c). In F344 female rats, cholesterol and BUN were elevated at the highest dose in one chronic inhalation study, which corresponded with an elevated severity of CPN in females (Saito et al., 2013; JPEC, 2010b). The single reported instance of elevated proteinuria occurred in female rats following chronic inhalation exposure; thus, no correlation of elevated proteinuria with CPN in males was observed (Saito et al., 2013; JPEC, 2010b).

Kidney tumors. No increase in kidney tumor incidence was observed following chronic oral or inhalation exposure in either F344 rats (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b) or SD rats {Maltoni, 1999, 87642}(see Table 1-4). However, animals in the {Maltoni, 1999, 87642@@author-year} study had extremely depressed survival in the control and treated groups (approximately 25%-30%) after 104 weeks, which is much lower than anticipated for a 2-year study, ultimately limiting the ability of this study to predict potential carcinogenicity (see discussion in Section 1.2.5).

In two-stage ("initiation, promotion") cancer bioassays, 23 weeks of daily gavage ETBE exposure did not increase kidney tumor incidence following 4 weeks of treatment with a 5-mutagens mixture (DMBDD) in male F344 rats (Hagiwara et al., 2011; JPEC, 2008d); however, a moderate, but statistically significant dose-response trend in the incidence of renal tubular adenoma or carcinoma incidence was observed with 19 weeks of daily gavage ETBE exposure following 2 weeks of the mutagen (N-ethyl-N-hydroxyethylnitrosamine [EHEN]) administration in male Wistar rats (Hagiwara et al., 2015). In Hagiwara et al. (2011), kidney tumors were not observed following 23 weeks of ETBE exposure without mutagen exposure (n=11). An ETBE-only exposure group was not evaluated in the later study in Wistar rats (Hagiwara et al., 2015).

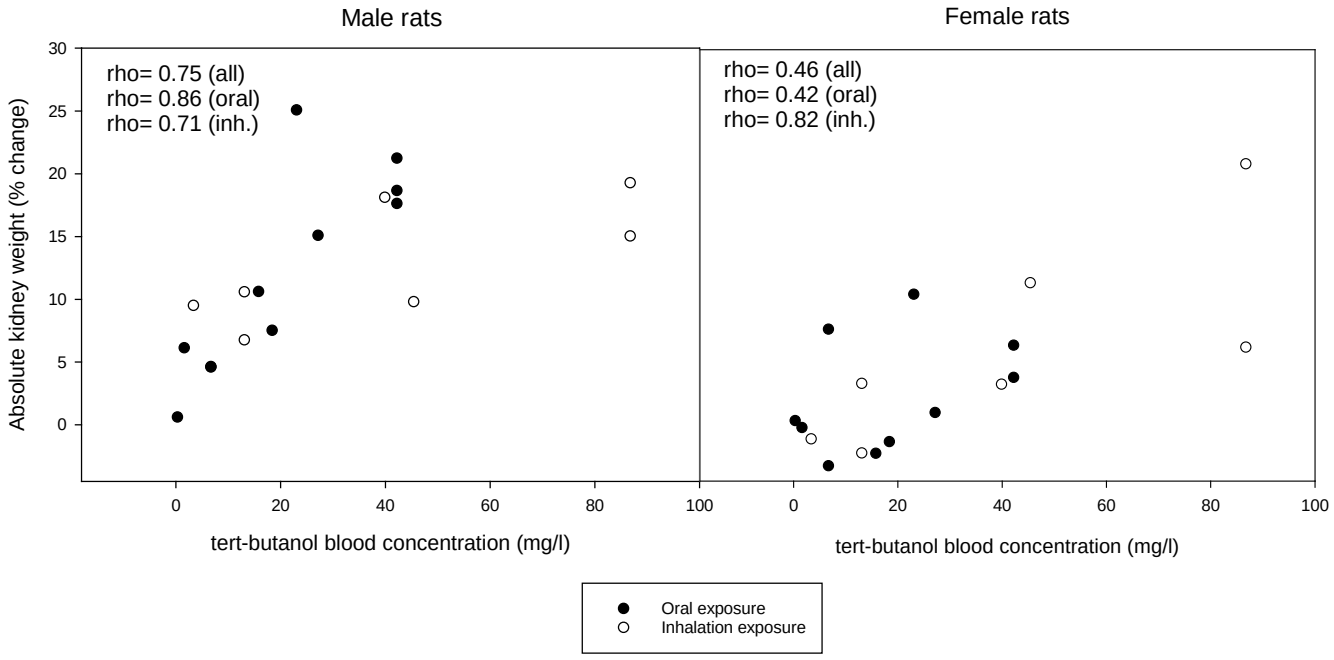


Figure 1-2. Comparison of absolute kidney weight change in male and female rats across oral and inhalation exposure based on metabolite internal blood concentration. Spearman rank coefficient (rho) was calculated to evaluate the direction of a monotonic association (e.g., positive value = positive association) and the strength of association.

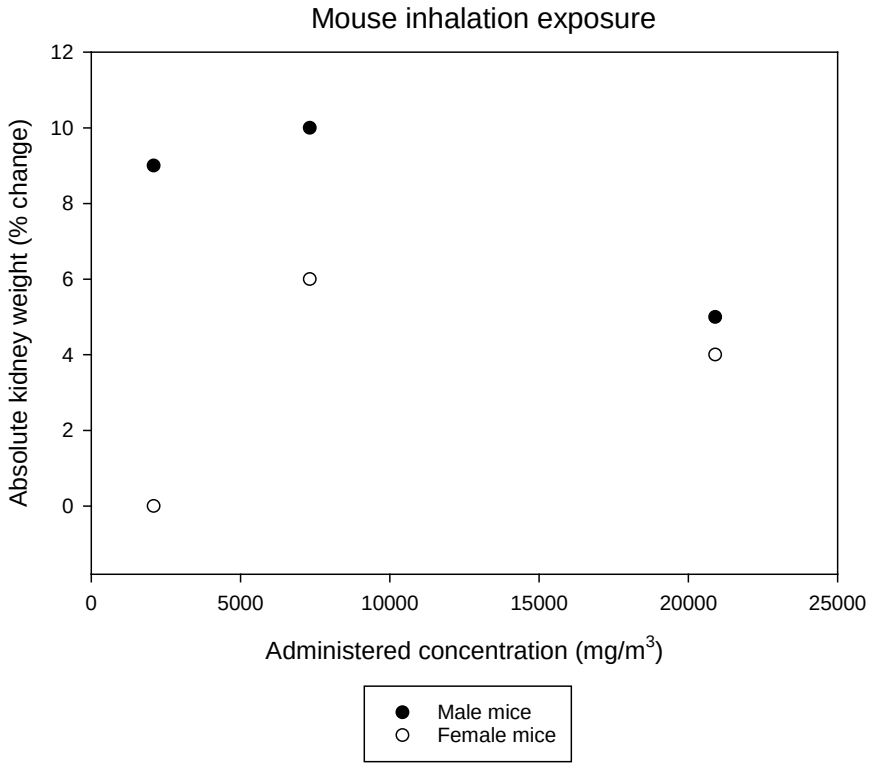


Figure 1-3. Comparison of absolute kidney weight change in male and female mice following 13 week inhalation exposure. Source: {Medinsky, 1999, 10740@@author-year}{Bond, 1996, 74002@@author-year}. No significant relationships were calculated.

Table 1-2. Evidence pertaining to kidney histopathology effects in animals following exposure to ETBE

Reference and study design	Results					
Cohen et al. (2011) rat, F344/DuCrlCrlj oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a] reanalysis of histopathology data from JPEC (2010a) study, for which animals were dosed daily for 104 wk	**Male**			**Female**		
	Dose (mg/kg-d)	Average severity of CPN	Incidence of CPN	Dose (mg/kg-d)	Average severity of CPN	Incidence of CPN
	0	2.08	49/50	0	1.14	45/50
	28	-	-	46	0.98	41/50
	121	-	-	171	1.2	46/50
	542	2.72*	50/50	560	1.36	46/50
Cohen et al. (2011) rat, F344/DuCrlCrlj oral – water male (10/group): 0, 250, 1,600, 4,000, 10,000 ppm (0, 17, 40, 101, 259, 626 mg/kg-d)[a] reanalysis of histopathology data from JPEC 2007 (study No. 0665) study, for which animals were dosed daily for 13 wk	**Male**					
	Dose (mg/kg-d)	Number of CPN foci/rat		Number of granular casts/rat		
	0	1.2		0		
	17	-		-		
	40	-		-		
	101	-		-		
	259	-		-		
	626	27.2		8.2		
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral – gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 d	**Male**		**Female**			
	Dose (mg/kg-d)	Incidence of papillary mineralization	Dose (mg/kg-d)	Incidence of papillary mineralization		
	0	0/15	0	0/15		
	5	0/15	5	-		
	25	0/15	25	-		
	100	1/15	100	-		
	400	0/15	400	0/15		

Reference and study design	Results
Saito et al. (2013); JPEC (2010b) rat, F344 inhalation – vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration reported	**Male** Dose (mg/m^3) / Average severity of CPN as calculated by EPA[c] / Incidence of CPN / Incidence of papillary mineralization / Incidence of urothelial hyperplasia of the renal pelvis 0 / 2.4 / 49/50 / 0/50 / 2/50 2,090 / 2.6 / 50/50 / 0/50 / 5/50 6,270 / 2.7 / 49/49 / 1/49 / 16/49* 20,900 / 3.1* / 50/50 / 6/50* / 41/50* **Female** Dose (mg/m^3) / Average severity of CPN as calculated by EPA[c] / Incidence of CPN 0 / 0.9 / 32/50 2,090 / 1.3 / 38/50 6,270 / 1.3 / 41/50 20,900 / 1.6* / 40/50 Atypical tubule hyperplasia not observed in males or females. Papillary mineralization and urothelial hyperplasia of the renal pelvis not observed in females.
Suzuki et al. (2012); JPEC (2010a) rat, F344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a] daily for 104 wk	**Male** Dose (mg/kg-d) / Average severity of CPN / Average severity of CPN as calculated by EPA[c] / Incidence of atypical tubule hyperplasia / Incidence of CPN 0 / 2.1 / 2.1 / 0/50 / 49/50 28 / 2.0 / 1.7 / 0/50 / 43/50 121 / 2.0 / 1.8 / 0/50 / 45/50 542 / 2.4* / 2.3 / 1/50 / 48/50 Dose (mg/kg-d) / Incidence of papillary necrosis / Incidence of papillary mineralization / Incidence of urothelial hyperplasia of the renal pelvis 0 / 0/50 / 0/50 / 0/50 28 / 1/50 / 0/50 / 0/50 121 / 0/50 / 16/50* / 10/50* 542 / 2/50 / 42/50* / 25/50*

Reference and study design	Results
	Female Dose (mg/kg-d) / Average severity of CPN / Average severity of CPN as calculated by EPA[c] / Incidence of atypical tubule hyperplasia / Incidence of CPN 0 / 1.2 / 1.0 / 0/50 / 41/50 46 / 1.2 / 0.9 / 0/50 / 37/50 171 / 1.5 / 1.1 / 0/50 / 37/50 560 / 1.5* / 1.2 / 2/50 / 39/50 Dose (mg/kg-d) / Incidence of papillary necrosis / Incidence of papillary mineralization / Incidence of urothelial hyperplasia of the renal pelvis 0 / 0/50 / 0/50 / 0/50 46 / 1/50 / 0/50 / 0/50 171 / 1/50 / 1/50 / 0/50 560 / 2/50 / 3/50 / 0/50

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
[c]Average severity calculated as (grade × number of affected animals) ÷ total number of animals exposed.
*: 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].

1

Table 1-3. Evidence pertaining to kidney biochemistry and urine effects in animals following exposure to ETBE

Reference and study design	Results			
JPEC (2008b) rat, CRL:CD(SD) inhalation – vapor male (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a]; female (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	**Male**			
	Dose (mg/m^3)	Blood urea nitrogen (BUN)	Cholesterol	Creatinine
	0	-	-	-
	627	-9%	8%	-13%
	2,090	-5%	9%	-6%
	6,270	4%	26%	-6%
	20,900	4%	15%	-3%
	Dose (mg/m^3)	Proteinuria severity[b]	Proteinuria incidence	Urinary casts
	0	0.5	3/6	0/6
	627	1.2	5/6	0/6
	2,090	1.2	5/6	0/6
	6,270	1.3	6/6	0/6
	20,900	1.0	4/6	0/6
	Female			
	Dose (mg/m^3)	Blood urea nitrogen (BUN)	Cholesterol	Creatinine
	0	-	-	-
	627	-5%	7%	0%
	2,090	3%	9%	3%
	6,270	-8%	11%	-9%
	20,900	-4%	21%	-9%
	Dose (mg/m^3)	Proteinuria severity[b]	Proteinuria incidence	Urinary casts
	0	0.2	1/6	0/6
	627	0.3	1/6	0/6
	2,090	0.2	1/6	0/6
	6,270	0.5	2/6	0/6
	20,900	0.3	2/6	0/6

Reference and study design	Results			
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral – gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for approximately 26 wk	**Male**			
	Dose (mg/kg-d)	Blood urea nitrogen (BUN)	Cholesterol	Creatinine
	0	-	-	-
	5	12%	-5%	0%
	25	1%	21%	-10%
	100	4%	12%	-3%
	400	8%	53%*	0%
	Dose (mg/kg-d)	Proteinuria incidence	Proteinuria severity[b]	Urinary casts
	0	10/10	1.5	0/10
	5	10/10	1.6	-
	25	10/10	1.6	-
	100	10/10	1.3	-
	400	10/10	1.5	0/10
	Female			
	Dose (mg/kg-d)	Blood urea nitrogen (BUN)	Cholesterol	Creatinine
	0	-	-	-
	5	-5%	-7%	-19%
	25	-7%	-7%	-12%
	100	-1%	-2%	-16%
	400	4%	3%	-16%
	Dose (mg/kg-d)	Proteinuria incidence	Proteinuria severity[b]	Urinary casts
	0	8/10	1.2	0/10
	5	9/10	1.3	-
	25	7/10	1.0	-
	100	9/10	1.3	-
	400	7/10	1.0	0/10

Reference and study design	Results
Saito et al. (2013); JPEC (2010b) rat, F344 inhalation – vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method reported	Response relative to control **Male** Dose (mg/m^3) / Blood urea nitrogen (BUN) / Cholesterol / Creatinine / Proteinuria incidence / Proteinuria severity[b] 0 / - / - / - / 44/44 / 3.7 2,090 / 41%* / 10% / 14%* / 38/38 / 3.5 6,270 / 45%* / 29%* / 29%* / 40/40 / 3.6 20,900 / 179%* / 52%* / 71%* / 31/31 / 3.6 **Female** Dose (mg/m^3) / Blood urea nitrogen (BUN) / Cholesterol / Creatinine / Proteinuria incidence / Proteinuria severity[b] 0 / - / - / - / 33/38 / 2.8 2,090 / 10% / -3% / 0% / 39/39 / 3.1 6,270 / 4% / -4% / 0% / 30/30 / 3.3 20,900 / 30%* / 53%* / 0% / 30/30 / 3.4*

Reference and study design	Results
Suzuki et al. (2012); JPEC (2010a) rat, F344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[c]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[c] daily for 104 wk	Response relative to control **Male** Dose (mg/kg-d) / Blood urea nitrogen (BUN) / Cholesterol / Creatinine / Proteinuria incidence / Proteinuria severity[b] 0 / - / - / - / 39/39 / 3.0 28 / 3% / -11% / 0% / 37/37 / 3.1 121 / 20%* / 10% / 17% / 34/34 / 3.1 542 / 43%* / 31%* / 17% / 35/35 / 3.1 **Female** Dose (mg/kg-d) / Blood urea nitrogen (BUN) / Cholesterol / Creatinine / Proteinuria incidence / Proteinuria severity[b] 0 / - / - / - / 37/37 / 2.8 46 / -8% / -2% / 0% / 37/37 / 3.0 171 / -5% / 12% / -17% / 38/38 / 3.0 560 / -5% / 8% / 0% / 38/38 / 3.1

[a]4.18 mg/m^3 = 1 ppm.
[b]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.
[c]Conversion performed by study authors.
*: 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 1-4. Evidence pertaining to kidney tumor effects in animals following exposure to ETBE

Reference and study design	Results			
{Saito, 2013, 2321101@@author-year}{JPEC, 2010, 1517421@@author-year} rat, F344 inhalation – vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[c]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[c]	**Male**		**Female**	
	Dose (mg/m^3)	Renal cell carcinoma	Dose (mg/m^3)	Renal cell carcinoma
	0	0/50	0	0/50
	2,090	1/50	2,090	0/50
	6,270	0/49	6,270	0/50
	20,900	0/50	20,900	0/50

Reference and study design	Results			
{JPEC, 2010, 1517477@@author-year}{Suzuki, 2012, 1433129@@author-year} rat, F344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[b]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[d] daily for 104 wk	**Male**		**Female**	
	Dose (mg/kg-d)	Renal cell carcinoma	Dose (mg/kg-d)	Renal cell carcinoma
	0	0/50	0	0/50
	28	0/50	46	0/50
	121	0/50	171	0/50
	542	1/50	560	1/50
{Hagiwara, 2011, 1248019@@author-year}{JPEC, 2008, 1517752@@author-year} rat, F344 oral – gavage male (12/group): 0, 1,000 mg/kg-d daily for 23 wk	**Male**			
	Dose (mg/kg-d)	Renal transitional cell carcinoma	Renal tubular adenoma or carcinoma	
	0	0/12	0/12	
	1,000	0/12	0/12	
Initiation Promotion Studies				
{Hagiwara, 2011, 1248019@@author-year }{JPEC, 2008, 1517752@@author-year} rat, Fischer 344 oral – gavage male (30/group): 0, 300, 1,000 mg/kg-d daily for 23 wk following a 4-wk tumor initiation by DMBDD[c]	**Male**			
	Dose (mg/kg-d)	Renal tubular adenoma or carcinoma	Renal transitional cell carcinoma	
	0	11/30	1/30	
	300	6/30	0/30	
	1,000	13/30	2/30	
{Hagiwara, 2015, 3046107@@author-year} rat, Wistar oral – gavage male (30/group): 0,100, 300, 500, 1,000 mg/kg-d daily for 19 wk following a 2-wk tumor initiation by N-ethyl-N-hydroxyethylnitrosamine (EHEN)	**Male**			
	Dose (mg/kg-d)	Renal tubular adenoma or carcinoma[d]		
	0	18/30		
	100	23/30		
	300	25/30		
	500	26/30		
	1,000	26/30		

[a]4.18 mg/m^3 = 1 ppm.
[b]Conversion performed by study authors.
[c]Diethylnitrosamine (DEN), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), N-methyl-N-nitrosourea (MNU), 1,2-dimethylhydrazine dihydrochloride (DMH), and N-bis(2-hydroxypropyl)nitrosamine (DHPN).
[d]Authors report significant trend.

Table 1-5. Comparison of nephropathy and urothelial hyperplasia in individual male rats from 2-year oral exposure (JPEC, 2010a)

Urothelial hyperplasia	CPN				
	None	Minimal	Mild	Moderate	Marked
None	15	21	105	23	1
Minimal	0	0	17	16	2
Mild	0	0	0	0	0
Moderate	0	0	0	0	0
Marked	0	0	0	0	0

Spearman's rank correlation test (1-sided), $p < 0.0001$, $r_s = 0.36$

Table 1-6. Comparison of nephropathy and urothelial hyperplasia in individual male rats from 2-year inhalation exposure (JPEC, 2010b)

Urothelial hyperplasia	CPN				
	None	Minimal	Mild	Moderate	Marked
None	1	3	59	68	4
Minimal	0	0	14	29	21
Mild	0	0	0	0	0
Moderate	0	0	0	0	0
Marked	0	0	0	0	0

Spearman's rank correlation test (1-sided), $p < 0.0001$, $r_s = 0.36$

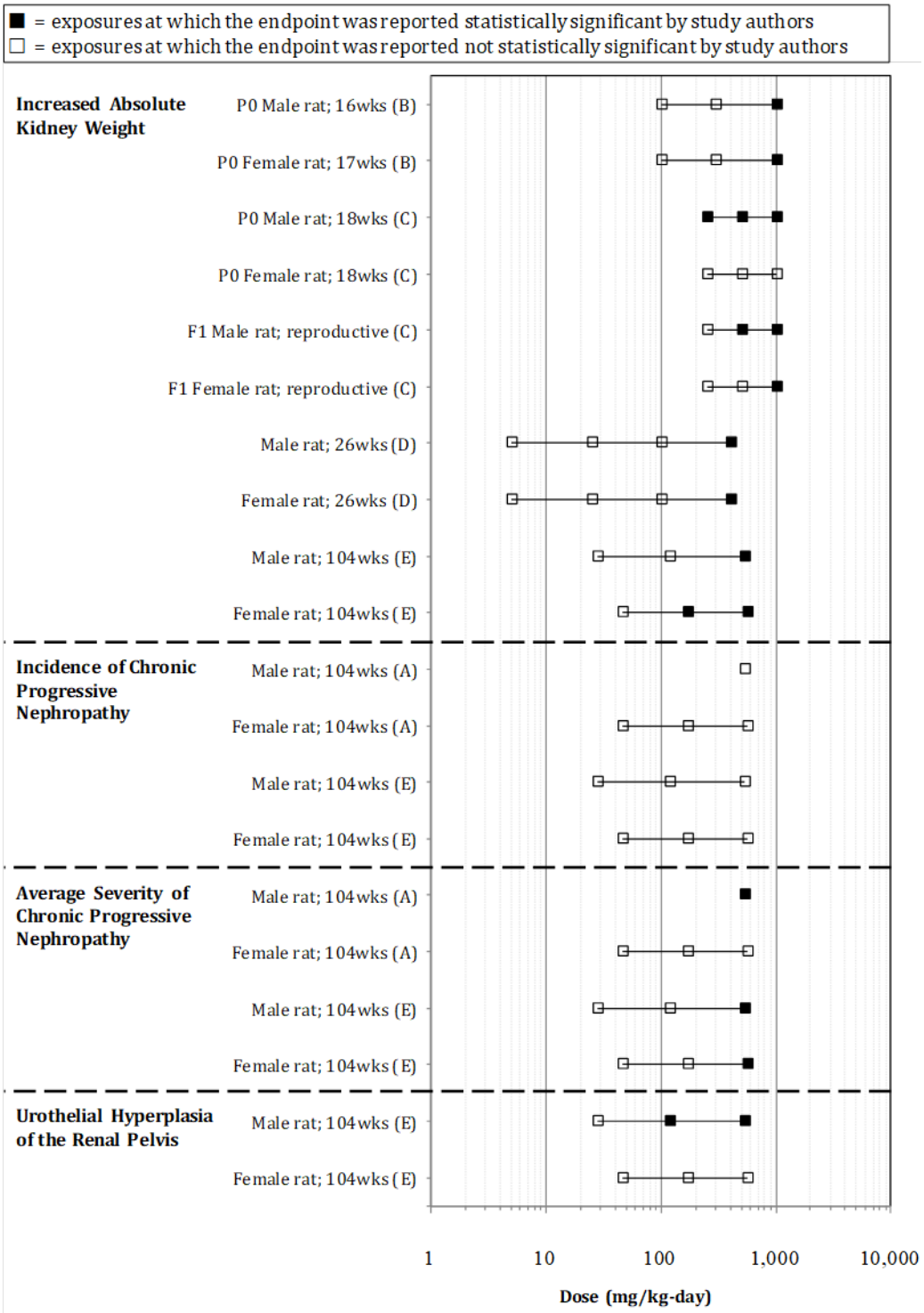


Figure 1-4. Exposure-response array of kidney effects following oral exposure to ETBE.

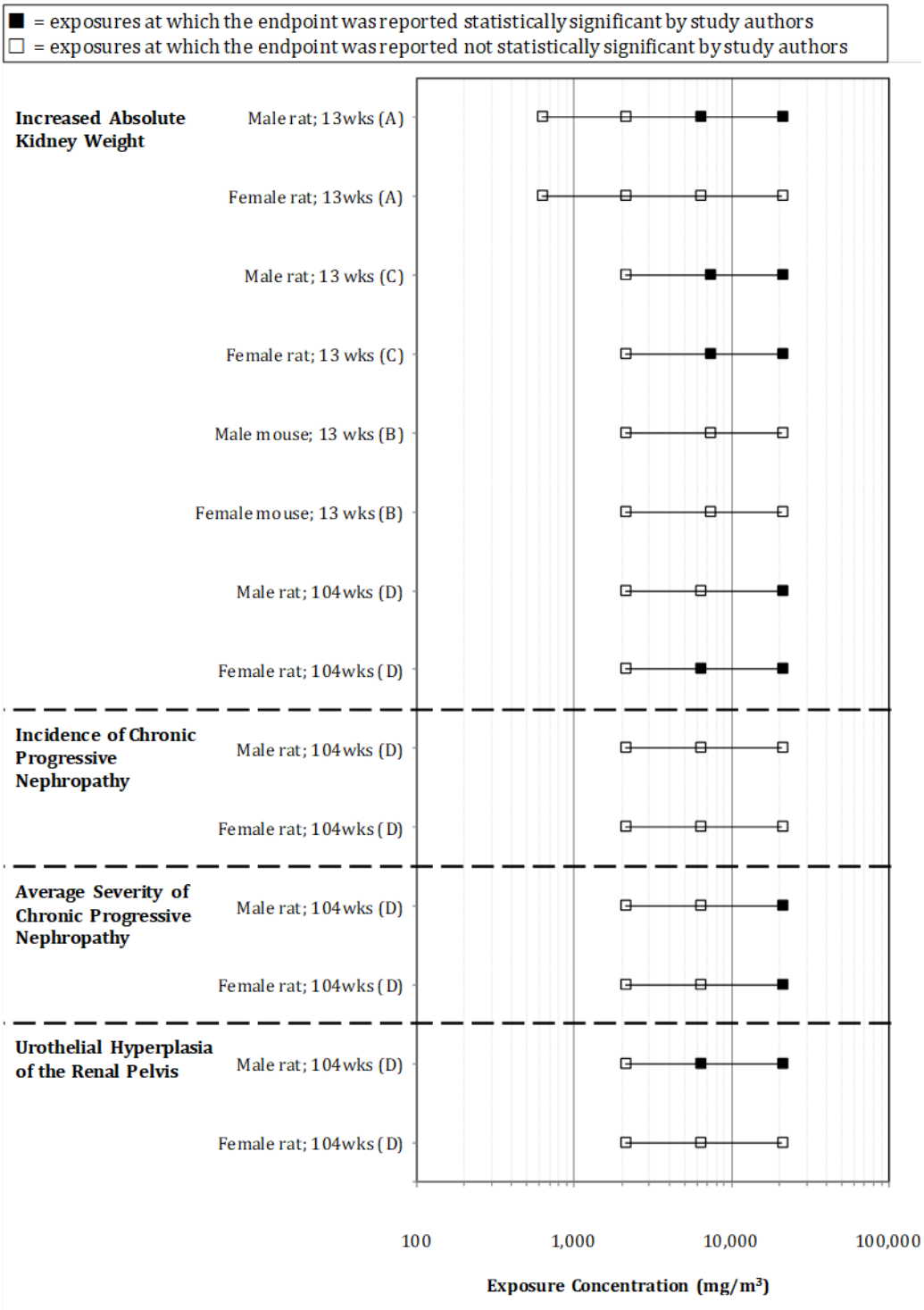


Figure 1-5. Exposure-response array of kidney effects following inhalation exposure to ETBE.

Mode of Action Analysis—Kidney Effects

a) 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 metabolized further in the liver. The main circulating breakdown product of ETBE metabolism 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 more detail in the draft IRIS assessment of *tert*-butanol) also are relevant to evaluating the kidney effects observed after ETBE exposure. In particular, similar to ETBE, *tert*-butanol has been reported to cause nephrotoxicity in rats, including effects associated with α_{2u}-globulin nephropathy (https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=262086). Unlike ETBE, however, increased renal tumors, in the absence of an initiator, were reported following chronic drinking water exposure to *tert*-butanol.

b) α_{2u}-Globulin-Associated Renal Tubule Nephropathy

One disease process to consider when interpreting kidney effects in rats is related to the accumulation of α_{2u}-globulin protein. α_{2u}-globulin, a member of a large superfamily of low-molecular-weight proteins, was first characterized in male rat urine. Such proteins have been detected in various tissues and fluids of most mammals (including humans), but the particular isoform of α_{2u}-globulin commonly detected in male rat urine and associated with renal tubule nephropathy (?) is considered specific to that sex and species. Exposure to chemicals that induce α_{2u}-globulin accumulation can initiate a sequence of histopathological events leading to kidney tumorigenesis. Because α_{2u}-globulin-related renal tubule nephropathy and carcinogenicity occurring in male rats are presumed not relevant for assessing human health hazards (U.S. EPA, 1991a), evaluating the data to determine whether α_{2u}-globulin plays a role is important. The role of α_{2u}-globulin accumulation in the development of renal tubule nephropathy and carcinogenicity observed following ETBE exposure was evaluated using the U.S. EPA (1991b) Risk Assessment Forum Technical panel report, *Alpha$_{2u}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat* as well as the IARC α_{2u}-globulin criteria {Capen, 1999, 699905}. These frameworks provide specific guidance for evaluating renal tubule tumors that are related to chemical exposure for the purpose of risk assessment, based on an examination of the potential involvement of α_{2u}-globulin accumulation.

The hypothesized sequence of α_{2u}-globulin renal tubule nephropathy, as described by U.S. EPA (1991a), is as follows. Chemicals that induce α_{2u}-globulin accumulation do so rapidly. α_{2u}-Globulin accumulating in hyaline droplets is deposited in the S2 (P2) segment of the proximal tubule within 24 hours of exposure. Hyaline droplets are a normal constitutive feature of the mature male rat kidney; they are particularly evident in the S2 (P2) segment of the proximal tubule

and contain α_{2u}-globulin (U.S. EPA, 1991a). Abnormal increases in hyaline droplets have more than one etiology and can be associated with the accumulation of different proteins. As hyaline droplet deposition continues, single-cell necrosis occurs in the S2 (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 occurs in the S2 (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 S3 (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 can result in the induction of renal tubule adenomas and carcinomas (Figure **1-6**).

U.S. EPA (1991a) identified two questions that must be addressed to determine the extent to which α_{2u}-globulin-mediated processes induce renal tubule nephropathy and carcinogenicity. First, whether the α_{2u}-globulin process occurs in male rats and influences renal tubule tumor development must be determined. Second, whether the renal effects in male rats exposed to ETBE are due solely to the α_{2u}-globulin process must be determined.

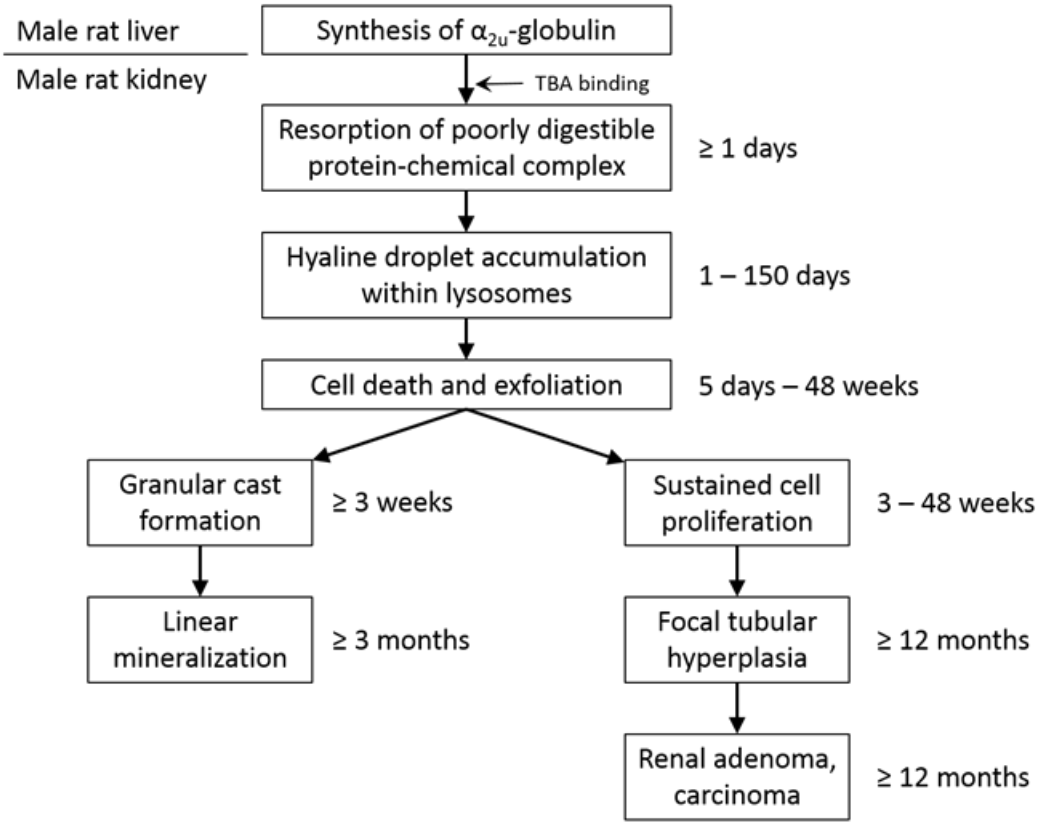
U.S. EPA (1991a) stated that the criteria for answering the first question in the affirmative are as follows:

1) hyaline droplets are larger and more numerous in treated male rats,

2) the protein is present in the hyaline droplets in treated male rats is α_{2u}-globulin (i.e., immunohistochemical evidence), and

3) several (but not necessarily all) additional steps in the pathological sequence appear in treated male rats as a function of time, dose, and progressively increasing severity consistent with the understanding of the underlying biology, as described above, and illustrated in Figure 1-6.

The available data relevant to this first question are summarized in Table 1-7, Table 1-8, Figure 1-7, and Table 1-10, and are evaluated below.



Source: Adapted from Swenberg and Lehman-McKeeman (1999); U.S. EPA (1991a).

Figure 1-6. Temporal pathogenesis of α_{2u}-globulin-associated nephropathy in male rats. α_{2u}-Globulin synthesized in the livers of male rats is delivered to the kidney, where it can accumulate in hyaline droplets and be retained by epithelial cells lining the S2 (P2) segment of the proximal tubules. Renal pathogenesis following continued exposure and increasing droplet accumulation can progress stepwise from increasing epithelial cell damage, death, and dysfunction, leading to the formation of granular casts in the corticomedullary junction, and linear mineralization of the renal papilla, in parallel with carcinogenesis of the renal tubular epithelium.

Table 1-7. Additional kidney effects potentially relevant to mode of action in animals exposed to ETBE

Reference and study design	Results
JPEC (2008b) rat, CRL:CD(SD) inhalation – vapor male (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a]; female (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	**Male** Dose (mg/m^3) / Incidence of hyaline droplets in the proximal tube epithelium 0 / 0/10 627 / 3/10 2,090 / 8/10* 6,270 / 8/10* 20,900 / 8/10* Unspecified representative samples reported as "weakly positive" for α_{2u}-globulin in males; no hyaline droplets observed in proximal tubule of females; hyaline droplets positive for α_{2u}-globulin not examined in females.
JPEC (2008c); Miyata et al. (2013) rat, CRL:CD(SD) oral – gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 d	**Male** / **Female** Dose (mg/kg-d) / Incidence of hyaline droplets / Incidence of hyaline droplets positive for α_{2u}-globulin / Dose (mg/kg-d) / Incidence of hyaline droplets 0 / 0/15 / 0/1 / 0 / 0/15 5 / 0/15 / - / 5 / - 25 / 0/15 / - / 25 / - 100 / 4/15 / 2/2 / 100 / - 400 / 10/15* / 1/1 / 400 / 0/15
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation – vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	**Male** Dose (mg/m^3) / Hyaline droplet severity / Proximal tubule proliferation: 1 week / 4 weeks / 13 weeks 0 / 1.8 / - / - / - 2,090 / 3.0 / 39% / 24% / 137%* 7,320 / 3.2 / 23% / -14% / 274%* 20,900 / 3.8 / 102%* / 175%* / 171%*

Reference and study design	Results
	Female Proximal tubule proliferation Dose (mg/m^3) / 1 week / 4 weeks / 13 weeks 0 / - / - / - 2,090 / 60%* / 3% / 73% 7,320 / 88%* / 15% / 64% 20,900 / 49%* / 31%* / 47%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation – vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[a] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method reported	**Male** No hyaline droplets observed. **Female** No hyaline droplets observed.
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[b]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[b] daily for 104 wk	**Male** No hyaline droplets observed. **Female** No hyaline droplets observed.

[a]4.18 mg/m^3 = 1 ppm.
[b]Conversion performed by study authors.
*: 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.

1

Table 1-8. 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	(+)[a]	Medinsky et al. (1999)
	4 wk	(+)[a]	Medinsky et al. (1999)
	13 wk	(+)[a]	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	(+)[b]	JPEC (2008b)
	4 wk	(+)[b]	Medinsky et al. (1999)
	13 wk	(+)[b]	Medinsky et al. (1999)
	13 wk	(+)[b]	JPEC (2008b)
	26 wk	(+)[c]	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); JPEC 2007a
	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 of tubules in the renal papilla	13 wk	–	JPEC (2008b)
	13 wk	–	Medinsky et al. (1999)

Criterion	Duration	Results	Reference
	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) Proliferation and foci of tubular hyperplasia	13 wk	–	JPEC (2008b)
	13 wk	+/–[d]	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 reported in one or more treated groups, but statistics not reported.
– = No statistically significant change reported in any of the treated groups.
[a]Droplet severity.
[b]Unspecified "representative samples" examined.
[c]Three samples from highest two dose groups examined.
[d]Labeling index statistically significantly increased, but no hyperplasia reported.

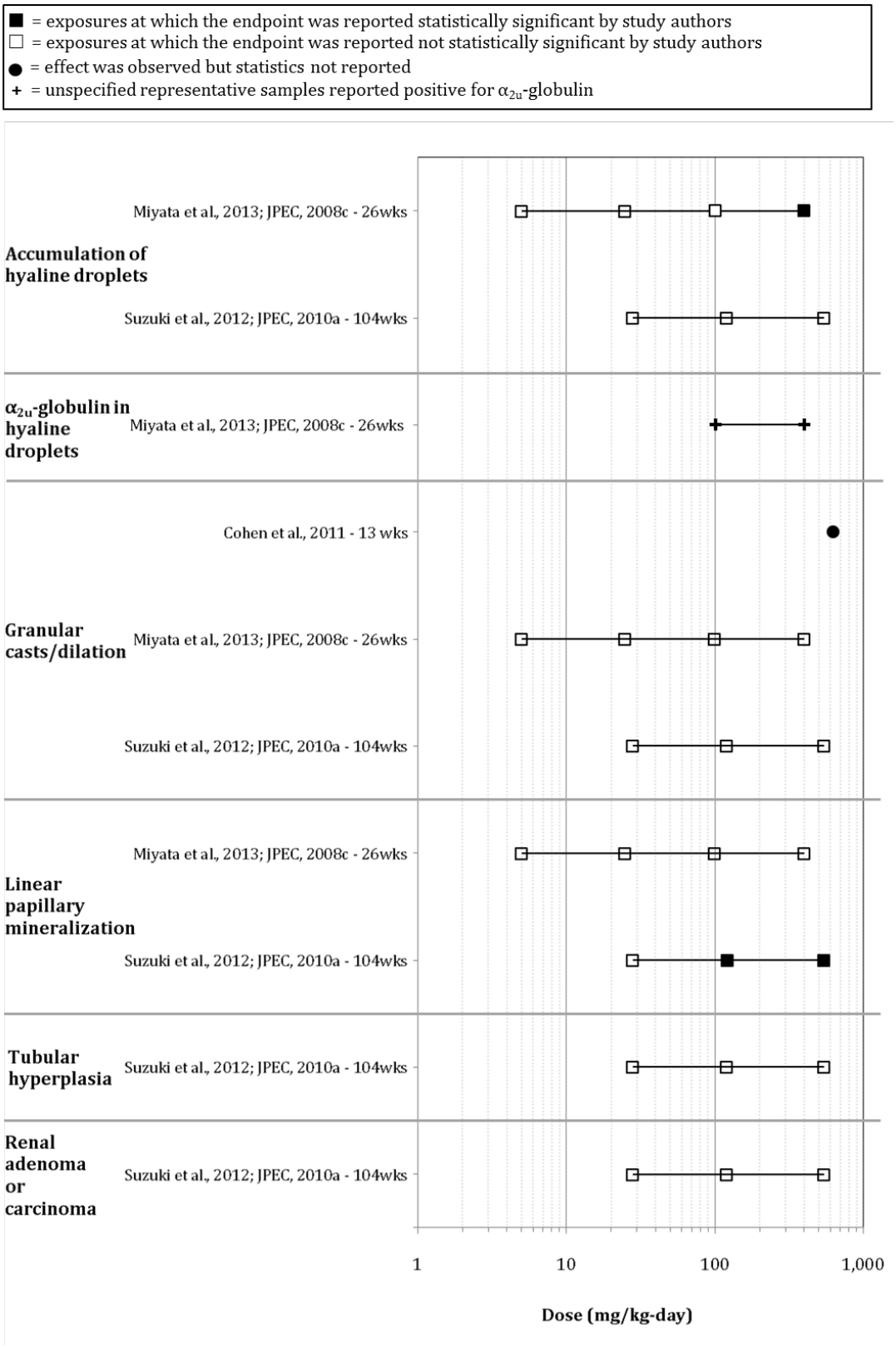


Figure 1-7. ETBE oral exposure array of α_{2u}-globulin data in male rats.

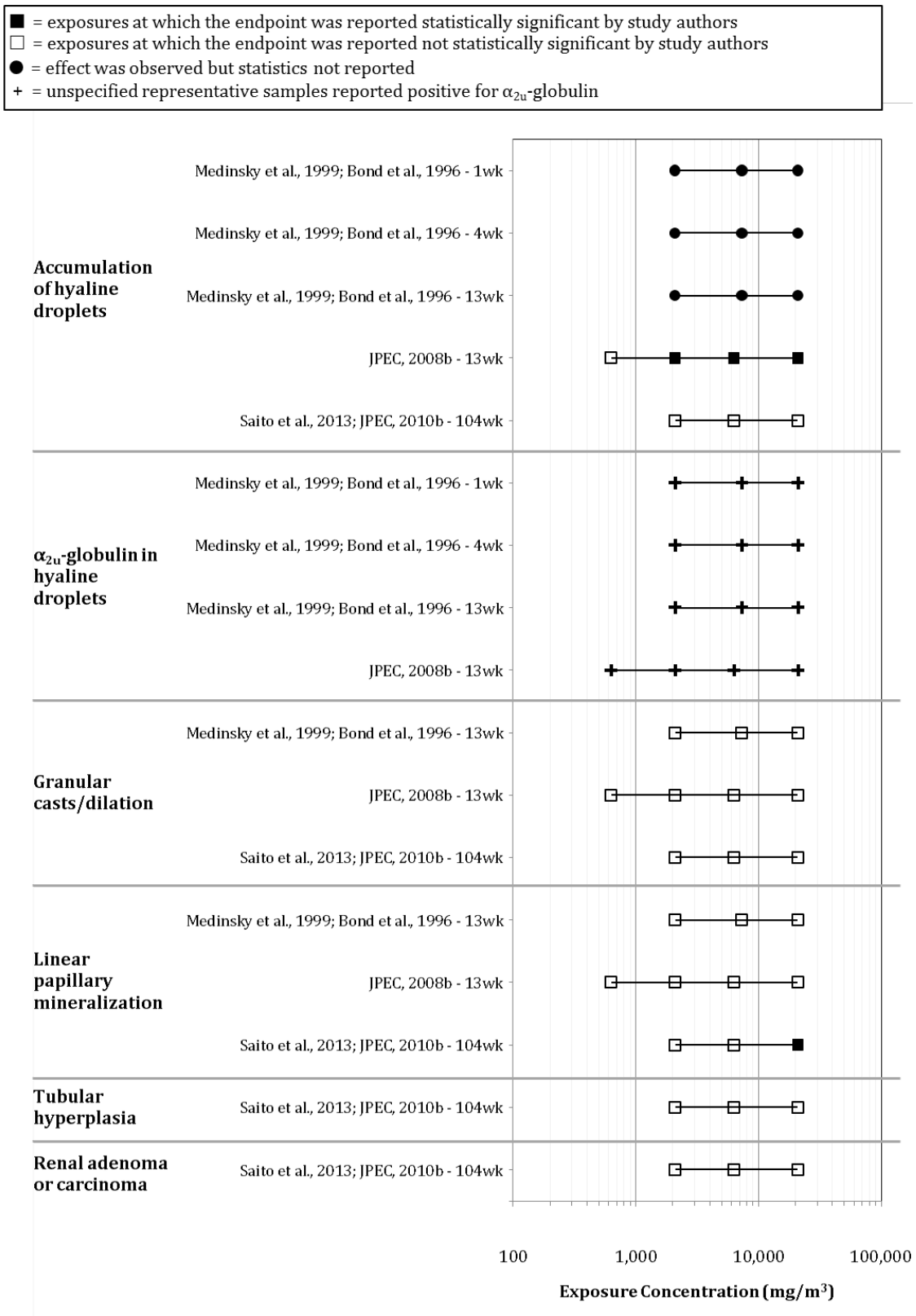


Figure 1-8. ETBE inhalation exposure array of α_{2u}-globulin data in male rats.

Question One: Is the α_{2u}-globulin process occurring in male rats exposed to ETBE?[7]

(1) The first criterion to consider is whether hyaline droplets are larger and more numerous 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 1-7 and Table 1-8). 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 (U.S. EPA, 1991a). Accumulation of hyaline droplets in the proximal tubular epithelium of the kidney was observed in male rats following 90-day inhalation exposure to 627, 2,090, 6,270, and 20,900 mg ETBE/m^3 (JPEC, 2008b). The increases at the three highest concentrations were statistically significant; however, none of the animals had hyaline droplet grades over 1 (JPEC, 2008b). 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, 2,090, 7,320, and 20,900 mg ETBE/m^3, respectively, and 90 days of ETBE inhalation exposure increased the severity grades of hyaline droplets from 1.8 in the control to 3.0, 3.2, and 3.8 (Medinsky et al., 1999). In addition, the incidence of hyaline droplets statistically significantly increased in a dose-related manner after 26 weeks of gavage exposure to 100 and 400 mg ETBE/kg-day (Miyata et al., 2013; JPEC, 2008c). These data indicate consistent evidence of hyaline droplets increasing both in a dose-responsive manner and within the expected timeframe. Therefore, the available data are sufficient to fulfill the first criterion that hyaline droplets are increased in size and number in male rats.

(2) The second criterion to consider 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 performed only 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 observed only in male rats. No statistical tests were performed on these results. The available studies that tested for α_{2u}-globulin in 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. Therefore, the available data are minimally sufficient to fulfill the second criterion for α_{2u}-globulin present in the hyaline droplets, but suggest weak induction of α_{2u}-globulin by ETBE.

(3) The third criterion considered is whether several (but not necessarily all) additional steps in the histopathological sequence associated with α_{2u}-globulin nephropathy appear in male

[7] If the chemical meets the criteria for question one, then a second question is asked: *Are the renal effects in male rats exposed to this chemical due solely to the α2u globulin process?*

rats in a manner consistent with the understanding of α_{2u}-globulin pathogenesis (refer to Table 1-8). Of the remaining five endpoints in the pathological sequence, only linear papillary mineralization and granular casts were observed. Papillary mineralization typically appears at chronic time points, occurring after exposures of 3 months up to 2 years (U.S. EPA, 1991a). The incidence of papillary mineralization was increased statistically significantly in both 2-year studies. Papillary mineralization increased in a dose-related manner following oral ETBE exposure in male rats at concentrations of 0, 28, 121, and 542 mg/kg-day, respectively (Suzuki et al., 2012; JPEC, 2010a), and in males at ETBE inhalation concentrations of 0, 2,090, 6,270, and 20,900 mg/m^3 (Saito et al., 2013; JPEC, 2010b). Hyaline droplet deposition was observed at a similar frequency as mineralization following oral ETBE exposure (Miyata et al., 2013; Suzuki et al., 2012; JPEC, 2010a, 2008c); however, hyaline droplet deposition was observed in 80% of animals at all three inhalation exposure concentrations (JPEC, 2008b) compared with mineralization rates of 0, 2, and 12% (lowest to highest exposure concentration) (Saito et al., 2013; JPEC, 2010b). A detailed evaluation and analysis of all the evidence relevant to this criterion follows.

Detailed evaluation of the available evidence supporting the third criterion

a) Single cell death, exfoliation into the renal tubules, and necrosis were not observed in any study (JPEC, 2008b, c; Medinsky et al., 1999). This observation might not be inconsistent with the hypothesized MOA because cell death and exfoliation has been observed to occur as early as 5 days post exposure, peak at 3 weeks, and then decline to near background levels by 4–5 weeks (Kanerva et al., 1987); this endpoint was not examined in any study evaluating ETBE exposures less than 13 weeks. Thus, the lack of exfoliation observations could be the result of both weak induction of α_{2u}-globulin and a lack of appropriately timed examinations.

b) Granular cast formation was observed in one study. The JPEC (2007a) study 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 at similar time points did not report the presence of granular casts (JPEC, 2008b, c; Medinsky et al., 1999) despite using similar exposure concentrations. Granular cast formation, however, might not occur with weak inducers of α_{2u}-globulin (Short et al., 1986), which is consistent with the weak staining of α_{2u}-globulin, as discussed above (JPEC, 2008b).

c) Linear mineralization of tubules within the renal papilla was consistently observed in male rats after 2 years (Saito et al., 2013; Suzuki et al., 2012). This lesion typically appears at chronic time points, occurring after exposures of 3 months up to 2 years (U.S. EPA, 1991a).

d) Cellular proliferation was increased after 1, 4, and 13 weeks in males and females; however, the magnitude of effect was reduced in females compared to males. Observation of proliferation in both sexes suggests that this effect is not male specific,

and thus not solely due to α_{2u}-globulin. Furthermore, renal tubule hyperplasia was not observed in any 2-year study, suggesting that ETBE does not induce sustained proliferation (Saito et al., 2013; Suzuki et al., 2012). Renal tubule hyperplasia is the preneoplastic lesion associated with α_{2u}-globulin nephropathy in chronic exposures that leads to renal tubule tumors (U.S. EPA, 1991a).

The progression of histopathological lesions for α_{2u}-globulin nephropathy is predicated on the initial response of excessive hyaline droplet accumulation (containing α_{2u}-globulin) leading to cell necrosis and cytotoxicity, which in turn cause the accumulation of granular casts, linear mineralization, and tubular hyperplasia resulting from sustained cellular proliferation. Therefore, observations of temporal and dose-response concordance for these effects are informative for drawing conclusions on causation.

As mentioned above (see Table 1-8), some steps in the sequence of α_{2u}-globulin nephropathy are observed at the expected time points following exposure to ETBE. Accumulation of hyaline droplet severity was observed early, at 1 week following inhalation exposure (Medinsky et al., 1999), and increased incidence was subsequently observed at 90 days (JPEC, 2008b) or 26 weeks (JPEC, 2008c); α_{2u}-globulin was identified as the protein in these droplets (Borghoff et al., 2001; Williams and Borghoff, 2001). Observations of the subsequent linear mineralization of tubules fall within the expected timeframe of the appearance of these lesions. Granular cast formation was reported in one oral study (Cohen et al., 2011), while three other oral and inhalation studies reported none (JPEC, 2008b, c; Medinsky et al., 1999), which also could indicate weak α_{2u}-globulin induction. Neither α_{2u}-globulin-mediated regenerative cell proliferation nor atypical renal tubule hyperplasia were observed. Lack of necrosis and exfoliation might be due to the weak induction of α_{2u}-globulin and a lack of appropriately timed examinations..

Hyaline droplets were weakly induced in all male rats in the 13-week inhalation studies (JPEC, 2008b; Medinsky et al., 1999), which did not result in increased linear mineralization at the corresponding doses. The lack of increased linear mineralization at low doses also is consistent with weak induction of hyaline droplets.

Overall, the histopathological sequence has numerous data gaps, such as the lack of observable necrosis, cytotoxicity, and tubule hyperplasia at stages plausibly within the timeframe of detectability. Furthermore, no explicit inconsistencies are present in the temporal appearance of the histopathological lesions associated with the α_{2u}-globulin nephropathy induced following ETBE exposure; however, the data set would be bolstered by measurements at additional time points to lend strength to the MOA evaluation. Therefore, the number of histopathological steps observed was insufficient to fulfill the third criterion.

Summary and conclusions for question one, Is the α_{2u}-globulin process occurring in male rats exposed to ETBE?

The evidence suggests that ETBE causes hyaline droplets to increase in size and number. The documentation of α_{2u}-globulin staining is poor and provides weak evidence of α_{2u}-globulin in the hyaline droplets. Only one of the additional steps in the pathological sequence was consistently observed (linear papillary mineralization), and the ETBE database lacks evidence of renal tubule hyperplasia and adenomas or carcinomas, despite multiple chronic bioassays, exposure routes, and durations ranging from 13 weeks to 2 years. Overall, the available data were insufficient to conclude that the α_{2u}-globulin process is exclusively operative.

Consideration of additional IARC 1999 Criteria

An α_{2u}-globulin framework was published by IARC in 1999 {Capen, 1999, 699905}. See below for the criterion laid out in the IARC consensus document.

IARC criteria for an agent causing kidney tumors through an α_{2u}-globulin associated response in male rats:

- Lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of in-vitro and in-vivo data
- Male rat specificity for nephropathy and renal tumorigenicity
- Induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory
- Identification of the protein accumulating in tubule cells as α_{2u}-globulin -
- Reversible binding of the chemical or metabolite to α_{2u}-globulin
- Induction of sustained increased cell proliferation in the renal cortex
- Similarities in dose—response relationship of the tumor outcome with the histopathological end-points (protein droplets, α_{2u}-globulin accumulation, cell proliferation)

A few minor differences exist between the EPA and IARC criteria. The EPA framework requires the observation of several (but not necessarily all) additional steps in the histopathological sequence associated with α2u globulin nephropathy, whereas IARC requires the "induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory", but doesn't specify which or how many of the additional histopathological changes must be observed to consider this criteria met. In addition, the IARC criteria have specific criteria pertaining to lack of genotoxicity of parent compound/metabolite and male rat specificity for nephropathy and renal tumorigenicity whereas the EPA framework considers these data as supplemental information (see *Part 4, XVII B. Additional Information Useful for the Analysis*). These additional criteria required by IARC (1999) are discussed below:

Lack of genotoxic action

As discussed in Appendix B.2.2, limited data are available help inform the genotoxic potential of ETBE. Most studies indicate that ETBE does not induce genotoxicity in the systems tested, although several key types of assays are missing (e.g. studies investigating chromosomal aberrations and DNA adducts). Regarding *tert*-butanol, the major metabolite of ETBE, while some data suggest tert-butanol could be genotoxic, the overall evidence is inadequate to establish a conclusion. Regarding the ETBE metabolite acetaldehyde, acetaldehyde has induced sister chromatid exchanges in Chinese hamster ovary cells, gene mutations in mouse lymphomas, and DNA strand breaks in human lymphocytes {IARC, 1999, 2342650}. In addition, increased genotoxicity of ETBE is noted when tested in animals with polymorphisms in the acetaldehyde dehydrogenase gene ALDH2, which decreases the ability to metabolize acetaldehyde {Wang, 2012, 1249293;Weng, 2014, 2321096;Weng, 2019, 5343910;Weng, 2013, 2279880;Weng, 2012, 1248016;Weng, 2011, 1062385}. Approximately 8% of the world's population carries this variant {Gross, 2015, 5353621}. Overall, this criterion has been weakly met for non-susceptible populations but not met for the subset of the population which can't efficiently detoxify acetaldehyde.

Male rat specificity for nephropathy

There is limited information to evaluate the potential for ETBE mediated kidney effects in other species. Only one subchronic study in WT mice {Bond, 1996, 74002}{Medinsky, 1999, 10740} and no chronic studies are available which evaluated kidney effects in mice.

Increased absolute kidney weight and increased severity of chronic nephropathy was noted in both sexes of rats exposed chronically to ETBE through inhalation and in drinking water (JPEC 2010a,b). Changes in clinical chemistry suggestive of kidney toxicity (e.g. increased BUN, cholesterol, and protein urea) were also noted in both male and female rats. However, dose-related increased incidence of urothelial hyperplasia of the renal pelvis was observed in male rats in chronic oral and inhalation bioassays but was not found in female rats by either route of exposure. In summary, while male rats appear to be more sensitive to ETBE meditated kidney toxicity, indications of nephropathy were also observed in female rats. Therefore, this criterion has not been met.

Comparison of ETBE and tert-butanol α_{2u}-globulin data

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. A more recent retrospective analysis indicating that several steps in the sequence of pathological events are not required for tumor development has demonstrated this by evaluating several α_{2u}-globulin-inducing chemicals which 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 chemicals that induce the α_{2u}-globulin process such as d-limonene, decalin, propylene glycol mono-t-butyl ether, and Stoddard Solvent IICA (SS IICA). Although some of these chemicals induced dose-response effects for a few endpoints, all failed to induce a dose-response for at all 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 affected in a dose-related manner 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 IICA, 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 could be operative for inducing kidney tumors in male rats.

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

Tubule hyperplasia and renal tumors were both observed following 2-year exposure to *tert*-butanol but not to ETBE, despite similar internal concentrations of *tert*-butanol following ETBE exposure (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010b). The failure of ETBE to induce several histopathological lesions in the α_{2u}-globulin pathological sequence, including renal tubule hyperplasia and tumors, at similar internal *tert*-butanol concentrations as those that induced hyperplasia and tumorigenesis following exposure to *tert*-butanol suggests a lack of coherence across the two data sets.

c) Chronic Progressive Nephropathy (CPN)

Exacerbation of CPN has been proposed as another rat-specific mechanism of nephrotoxicity that is not relevant to humans (Hard et al., 2009). CPN is an age-related renal disease that occurs in rats of both sexes (NTP, 2015, 2014; Hard et al., 2013; Melnick et al., 2012; U.S. EPA, 1991a). CPN is more severe in males than in females and is particularly common in the Sprague-Dawley and Fischer 344 strains. Dietary and hormonal factors play a role in modifying CPN, though its etiology is unknown.

CPN has been suggested as a key event in the onset of renal tubule tumors, and a sequence of key events in the MOA is as follows: (1) metabolic activation, (2) chemically exacerbated CPN, (3) increased tubule cell proliferation, (4) tubule hyperplasia, and (5) adenomas (Hard et al., 2013). Arguments against this MOA also have been proposed (Melnick et al., 2012). ETBE exposure

increased CPN severity following 2-year inhalation and 13-week oral exposure but, unlike tert-butanol, did not affect tubule hyperplasia or increase renal tubule tumor incidence.

Additional markers associated with CPN include elevated proteinuria and albumin in the urine and increased BUN, creatinine, and cholesterol in the serum, of which proteinuria is the major urinary effect and a very sensitive measure of CPN (Hard et al., 2009). In the case of ETBE exposure, however, increased severity or incidence of proteinuria was not correlated with increased severity of CPN in male rats possibly due to high background severity of CPN. In female rats, background severity of CPN was much milder, thus increased proteinuria was observable only when CPN was increased as in the 2-year inhalation exposure study (Saito et al., 2013). Elevated BUN and creatinine typically are not observed until very late in CPN progression. This was true for ETBE, as most of these markers were elevated only after 2-year exposures.

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){Travlos, 2011, 1239901}.

CPN often is more severe in males than in females. While background severity of CPN in controls was higher in male rats, increased severity of CPN was reported in both male and female rats with ETBE exposure, and was statistically significant at the highest exposure groups of both sexes following chronic inhalation (see Table 1-2). Some of the observed renal lesions in male rats following exposure to ETBE are effects commonly associated with CPN. A strong, statistically significant, treatment-related relationship was observed between chronic ETBE exposure and increased incidence of urothelial hyperplasia in male (but not female) rats in both the inhalation and oral studies (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Urothelial hyperplasia is both increased by dose and weakly correlated with CPN, which is also dose-related (Table 1-5 and Table 1-6). Thus, disentangling the contributions of dose and nephropathy in the development of urothelial hyperplasia is not possible with the currently available information. Moreover, no evidence is available to support that urothelial hyperplasia is not related toETBE treatment, given the robust dose-response relationship in male rats treated with ETBE.

Finally, because *tert*-butanol is a major metabolite of ETBE and both chemicals induce similar noncancer kidney effects, *tert*-butanol could be the active toxic moiety responsible for these effects. The three noncancer kidney endpoints (kidney weights, urothelial hyperplasia, CPN) were evaluated on an internal dose basis using PBPK modeling to compare these data from ETBE and *tert*-butanol studies (Salazar et al., 2015). The results demonstrate that noncancer kidney effects, including kidney weight changes, urothelial hyperplasia, and exacerbated CPN, yielded consistent dose-response relationships across routes of exposure and across ETBE and *tert*-butanol studies using *tert*-butanol blood concentration as the dose metric. These results are consistent with the hypothesis that *tert*-butanol mediates the noncancer kidney effects following ETBE administration,

however contributing toxicity from the parent compound and other metabolites has not been ruled out.

Overall Conclusion on MOA for Kidney Effects

ETBE increases α_{2u}-globulin deposition and hyaline droplet accumulation in male rat kidneys, but only one of the five additional steps in the pathological sequence (linear mineralization) was consistently observed (see Table 1-8). These data are insufficient to conclude that ETBE induces α_{2u}-globulin nephropathy, however, the observation of α_{2u}-globulin accumulation in hyaline droplets with ETBE exposure adds uncertainty regarding the human relevance of the associated nephropathy in male rats CPN and the exacerbation of CPN could play a role in the observed nephropathy. Currently, the MOA for the observed ETBE-induced exacerbation of CPN is unknown, especially in female rats and in male and female mice which are not affected by α_{2u}-globulin nephropathy. Collectively, the evidence indicates other, unknown processes contribute to the observed nephrotoxicity following ETBE exposure, particularly in female rats.

Integration of Kidney Effects

Kidney effects (increases in severity of nephropathy, increased kidney weight, alterations in blood biomarkers, hyaline droplets, linear mineralization, and urothelial hyperplasia of the renal pelvis) were observed across multiple studies, predominantly in rats; chronic bioassays found no treatment-related increases in renal tumors. The available evidence indicates that multiple processes induce the noncancer kidney effects.

Some endpoints in male rats (hyaline droplets, linear mineralization) are components of the α_{2u}-globulin process. U.S. EPA (1991a) states that"[i]f a compound induces α_{2u}-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially occuring in humans." Therefore, in the case of ETBE exposure, endpoints directly associated with α_{2u}-globulin processes were not considered an indication of human health hazard for noncancer kidney toxicity. Because α2u-globulin nephropathy is strictly a male rat phenomenon, dose-related kidney effects in female rats and mice are not confounded by α2u-globulin nephropathy.

It has been observed that chemicals that bind to α_{2u}-globulin can lead to increased incidence and/or severity of CPN {Travlos, 2011, 1239901}{U.S. EPA, 1991, 635839}{Frazier, 2012, 2919046}. CPN is a common and well-established constellation of age-related lesions in the kidney of male and female rats, and there is no known counterpart to CPN in aging humans. However, CPN is not a specific diagnosis on its own but an aggregate term describing a spectrum of effects, employed to reduce the time and effort required to grade each component of the disease. The individual lesions associated with CPN (e.g. tubular degeneration, thickening of basement membranes, glomerular sclerosis, etc.) also occur in the human kidney {Lusco, 2016, 5926047}{Frazier, 2012, 2919046}{Zoja, 2015, 5926046}{Abrass, 2000, 5426141}. Although CPN has no known analog in the aging human kidney {Hard, 2009, 667590}{NIEHS, 2019, 5098230}, the

etiology is unknown {NIEHS, 2019, 5098230}{Hard, 2004, 782757}{Peter, 1986, 194755}. Given that there is no definitive pathogenesis for CPN, it cannot be ruled out that a chemical which exacerbates CPN in rats could also exacerbate existing disease processes in the human kidney {NIEHS, 2019, 5098230}. Therefore, increased incidence of kidney effects with ETBE exposure in the female rat (including increased kidney weight and increased severity of CPN) are considered relevant to humans.

Several noncancer endpoints were concluded to result from ETBE exposure including increased absolute kidney weight, histopathological changes, and increased blood biomarkers in female rats, with the effects in males tending to be stronger than in females (potentially due to confounding by α_{2u}-globulin processes). A PBPK model-based analysis yielded consistent dose-response relationships between kidney weight, urothelial hyperplasia, and chronic progressive nephropathy (CPN) using *tert*-butanol blood concentration as the dose metric, consistent with the hypothesis that *tert*-butanol mediates the noncancer kidney effects following ETBE administration (Salazar 2015). Based on dose-related increases in these noncancer endpoints in rats, kidney effects are a potential human hazard of ETBE exposure. The hazard and dose-response conclusions regarding these noncancer endpoints associated with ETBE exposure are discussed further in Section 1.3.1.

1.2.2. Liver Effects

Synthesis of Effects in Liver

This section reviews the studies that investigated whether exposure to ETBE can cause liver noncancer or cancer effects in humans or animals. The database for ETBE-induced liver effects includes nine studies conducted in animals, all but two of which were performed in rats. A description of the studies comprising the database is provided in Section 1.2.1. Briefly, exposures ranged from 13 weeks to 2 years and both inhalation and oral exposure routes are represented. Studies using 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 provide data informative of MOA or hazard identification. Studies are arranged in evidence tables first by effect and then in alphabetical order by author. The design, conduct, and reporting of each study were reviewed, and each study was considered adequate to provide information pertinent to this assessment.

Liver weight. Increased liver weight was observed with ETBE exposure in male and female rats treated for various durations orally or by inhalation. Several factors associated with the 2-year organ weight data could confound consideration for hazard identification. Proliferative lesions (altered hepatocellular foci) were observed in rat livers, especially males, in both 2-year oral and inhalation studies, which complicates interpretation of changes in organ weight. Furthermore, inhalation exposure significantly increased liver adenomas and carcinomas in male rats at the highest dose, corresponding to increased liver weights in those dose groups (Saito et al., 2013; JPEC, 2010b). Organ weight data obtained from studies of shorter duration, however, are less

complicated by these age-associated factors (e.g., tumors, mortality) and therefore could be appropriate for hazard identification.

Chronic and subchronic studies by both oral and inhalation routes reported consistent, statistically significant, dose-related increases in liver weights (see Figure 1-9, Figure 1-10, Table 1-9). Liver weight and body weight have been demonstrated to be proportional, and liver weight normalized to body weight was concluded to be optimal for data analysis (Bailey et al., 2004); thus, only relative liver weight is considered in the determination of hazard. Relative liver weights were consistently increased at similar exposure concentrations in four of five studies for males and three of four studies for females; however, statistically significant increases often occurred only at the highest tested concentration with increases in relative liver weight ranging from 17 to 27% in males and 8 to 18% in females. Relative liver weights in rats were increased at only the highest dose following oral exposures of 16 weeks or longer (Miyata et al., 2013; Fujii et al., 2010; JPEC, 2008c; Gaoua, 2004b). In utero exposure yielded similar effects on F1 liver weights, in terms of the magnitude of percent change, from adult exposure (Gaoua, 2004b). Inhalation exposure increased liver weight at the highest dose in female rats, but not in males, following 13-week exposure (JPEC, 2008b). Following a 28-day recovery period, male but not female liver weights were increased (JPEC, 2008b). Short-term studies observed similar effects on liver weight (JPEC, 2008a; White et al., 1995).

Liver histopathology. Centrilobular hypertrophy and acidophilic (eosinophilic) and basophilic focal lesions were the only dose-related types of pathological lesions observed in the liver. Centrilobular hypertrophy was inconsistently increased throughout the evidence base, but also was observed at the same concentrations that induced liver weight changes in rats of both sexes after 13-week inhalation and 26-week oral exposures (see Table 1-10; Figure 1-9, Figure 1-10). 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. In addition, 2-year oral and inhalation studies in rats reported increased liver weight in male and female rats.

Acidophilic (eosinophilic) and basophilic preneoplastic lesions were increased in male, but not female rats, at the highest tested dose following a 2-year inhalation exposure to ETBE (Saito et al., 2013; JPEC, 2010b). Following 2-year drinking water exposure to ETBE, an increasing, but not statistically significant, trend in basophilic preneoplastic lesions was observed in the liver of male rats, while incidence of these lesions decreased in female rats (Suzuki et al., 2012; JPEC, 2010a).

Serum liver enzymes. Serum liver enzymes were inconsistently affected across exposure routes (see Table 1-11; Figure 1-9, Figure 1-10). No enzyme levels were affected in studies of exposure durations less than 2 years (Miyata et al., 2013; JPEC, 2008b). Gamma-glutamyl transpeptidase (GGT) was significantly increased in male rats at one intermediate 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. With the exception of a dose-related increase in serum GGT in male rats and an increase in AST at the highest dose in females, no other dose-related changes in liver enzyme levels were observed that were directionally consistent with the liver weight and hypertrophy effects.

Liver tumors. Data on liver tumor induction by ETBE are presented in Table 1-12. Liver tumors were statistically significantly increased in male F344 rats at the high dose, but not in females, following 2-year inhalation exposure (Saito et al., 2013; JPEC, 2010b). The incidence of combined adenomas and carcinomas of 0/50, 2/50, 1/50 and 10/50 at 0, 2,090, 6,270, 20,900 mg/m^3, also resulted in a statistically significant, positive exposure-response trend (Peto's test $p < 0.001$). In this study, preneoplastic lesions, acidophilic (eosinophilic) and basophilic foci, were also increased in male rats following a similar exposure pattern.

At the highest exposure dose in male rats, the dose at which the majority of liver tumors were observed, a 25% reduction in body weight was seen, raising some question as to whether the liver tumors observed in the highest exposure group in male rats are solely the result of excessive toxicity rather than carcinogenicity of the tested agent. EPA's 2005 Cancer Guidelines discuss the determination of an "excessively high dose" as compared to an "adequately high dose" and describe the process as one of expert judgment which requires that "...adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent" {U.S. EPA, 2005, 86237}. In the case of the 2-year inhalation study, the study authors did not report any overt toxicity or altered toxicokinetics at the high dose {JPEC, 2010, 1517421}. In addition, the high-dose female rats had a similar reduction in body weight (22%) and no liver tumors (or increase in preneoplastic foci) were observed.

No significant increase in tumors was observed following two chronic oral bioassays (Suzuki et al., 2012; JPEC, 2010a; Maltoni et al., 1999). However, one bioassay {Maltoni, 1999, 87642} was confounded by extremely low survival in controls (25-28% at 2 years), potentially due to widespread respiratory infections (see discussion in Section 1.2.5). This extreme depression in survival likely impacts this study's power to detect potential carcinogenicity. The other available two year oral cancer bioassay was well designed, conducted, and reported and did not observed significant increases in liver tumors, however an increased, but not statistically significant, trend in basophilic preneoplastic lesions was observed in the liver of male rats, while incidence of these lesions decreased in female rats (Suzuki et al., 2012; JPEC, 2010a).

Two-stage "initiation, promotion" studies in male F344 and Wistar rats administered mutagens for 2–4 weeks reported statistically significant increases in liver adenomas, carcinomas, or total neoplasms after 19–23 weeks of ETBE exposure via oral gavage (Hagiwara et al., 2015; Hagiwara et al., 2011). Liver tumors were not observed in male F344 rats exposed to ETBE for 23

weeks (n=12) without prior mutagen exposure (Hagiwara et al., 2011), while liver tumorigenesis without prior mutagen exposure was not evaluated in Wistar rats (Hagiwara et al., 2015).

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

Reference and study design	Results			
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage P0, male (24/group): 0, 100, 300, 1,000 mg/kg-d daily for 16 wk beginning 10 wk prior to mating P0, female (24/group): 0, 100, 300, 1,000 mg/kg-d daily for 17 wk beginning 10 wk prior to mating to lactation day (LD) 21	Response relative to control			
	P0, Male		**P0, Female**	
	Dose (mg/kg-d)	Relative weight	Dose (mg/kg-d)	Relative weight
	0	-	0	-
	100	1%	100	-1%
	300	2%	300	3%
	1,000	21%*	1,000	9%*

Reference and study design	Results
Gaoua (2004b) rat, Sprague-Dawley oral – gavage P0, male (25/group): 0, 250, 500, 1,000 mg/kg-d daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1,000 mg/kg-d daily for a total of 18 wk beginning 10 wk before mating until PND 21 F1, male (25/group): 0, 250, 500, 1,000 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, 1,000 mg/kg-d P0 dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of F2 pups	Response relative to control **P0, Male**: Dose (mg/kg-d) / Relative weight: 0 / -; 250 / 3%; 500 / 6%; 1,000 / 24%* **P0, Female**: Dose (mg/kg-d) / Relative weight: 0 / -; 250 / 10%; 500 / 8%; 1,000 / 4% **F1, Male**: Dose (mg/kg-d) / Relative weight: 0 / -; 250 / 0%; 500 / 11%*; 1,000 / 25%* **F1, Female**: Dose (mg/kg-d) / Relative weight: 0 / -; 250 / 3%; 500 / 6%; 1,000 / 9%*
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral – gavage male (12/group): 0, 1,000 mg/kg-d daily for 23 wk	Response relative to control **Male**: Dose (mg/kg-d) / Relative weight: 0 / -; 1,000 / 27%*
JPEC (2008b) rat, CRL:CD(SD) inhalation – vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3) dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	Response relative to control **Male**: Dose (mg/m^3) / Relative weight: 0 / -; 627 / 5%; 2,090 / 5%; 6,270 / 5%; 20,900 / 10% **Female**: Dose (mg/m^3) / Relative weight: 0 / -; 627 / 4%; 2,090 / -1%; 6,270 / 6%; 20,900 / 18%*

Reference and study design	Results			
JPEC (2008b) rat, CRL:CD(SD) inhalation – vapor male (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b]; female (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk followed by a 28-d recovery period; generation method, analytical concentration, and method reported	Response relative to control			
	Male		**Female**	
	Dose (mg/m^3)	Relative weight	Dose (mg/m^3)	Relative weight
	0	-	0	-
	20,900	9%*	20,900	7%
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral – gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 26 wk	Response relative to control			
	Male		**Female**	
	Dose (mg/kg-d)	Relative weight	Dose (mg/kg-d)	Relative weight
	0	-	0	-
	5	5%	5	1%
	25	7%	25	1%
	100	9%	100	4%
	400	17%*	400	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.
Percent change compared to controls calculated as 100 × [(treated value – control value) ÷ control value].

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

Reference and study design	Results			
Gaoua (2004b) rat, Sprague-Dawley oral – gavage P0, male (25/group): 0, 250, 500, 1,000 mg/kg-d daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1,000 mg/kg-d daily for a total of 18 wk beginning 10 wk before mating until PND 21	**P0, Male**		**P0, Female**	
	Dose (mg/kg-d)	Incidence of centrilobular hypertrophy	Dose (mg/kg-d)	Incidence of centrilobular hypertrophy
	0	0/25	0	0/25
	250	0/25	250	0/25
	500	0/25	500	0/25
	1,000	3/25	1,000	0/25

Reference and study design	Results			
JPEC (2008b) rat, CRL:CD(SD) inhalation – vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3) dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	**Male**		**Female**	
	Dose (mg/m^3)	Incidence of centrilobular hypertrophy	Dose (mg/m^3)	Incidence of centrilobular hypertrophy
	0	0/10	0	0/10
	627	0/10	627	0/10
	2,090	0/10	2,090	0/10
	6,270	0/10	6,270	0/10
	20,900	4/10*	20,900	6/10*
JPEC (2008b) rat, CRL:CD(SD) inhalation – vapor male (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b]; female (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk followed by a 28-d recovery period; generation method, analytical concentration, and method reported	**Male**		**Female**	
	Dose (mg/m^3)	Incidence of centrilobular hypertrophy	Dose (mg/m^3)	Incidence of centrilobular hypertrophy
	0	0/6	0	0/6
	20,900	0/6	20,900	0/6
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation – vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	**Male**		**Female**	
	Dose (mg/m^3)	Incidence of centrilobular hypertrophy	Dose (mg/m^3)	Incidence of centrilobular hypertrophy
	0	0/11	0	0/10
	2,090	0/11	2,090	0/11
	7,320	0/11	7,320	0/11
	20,900	0/11	20,900	0/11
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation – vapor male (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	**Male**		**Female**	
	Dose (mg/m^3)	Incidence of centrilobular hypertrophy	Dose (mg/m^3)	Incidence of centrilobular hypertrophy
	0	0/15	0	0/13
	2,090	0/15	2,090	2/15
	7,320	2/15	7,320	1/15
	20,900	8/10*	20,900	9/14*

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Miyata et al. (2013); JPEC (2008c)
rat, CRL:CD(SD)
oral – gavage
male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d
daily for 26 wk</td><td>
<table>
<tr><th colspan="2">Male</th><th colspan="2">Female</th></tr>
<tr><th>Dose (mg/kg-d)</th><th>Incidence of centrilobular hypertrophy</th><th>Dose (mg/kg-d)</th><th>Incidence of centrilobular hypertrophy</th></tr>
<tr><td>0</td><td>0/15</td><td>0</td><td>0/15</td></tr>
<tr><td>5</td><td>0/15</td><td>5</td><td>0/15</td></tr>
<tr><td>25</td><td>0/15</td><td>25</td><td>0/15</td></tr>
<tr><td>100</td><td>0/15</td><td>100</td><td>0/15</td></tr>
<tr><td>400</td><td>6/15*</td><td>400</td><td>6/15*</td></tr>
</table>
</td></tr>
<tr><td>Saito et al. (2013); JPEC (2010b)
rat, Fischer 344
inhalation – vapor
male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]
dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method reported</td><td>
Male
<table>
<tr><th>Dose (mg/m^3)</th><th>Acidophilic foci in liver</th><th>Basophilic foci in liver</th><th>Bile duct hyperplasia</th><th>Centrilobular hypertrophy</th></tr>
<tr><td>0</td><td>31/50</td><td>18/50</td><td>48/50</td><td>0/50</td></tr>
<tr><td>2,090</td><td>28/50</td><td>10/50</td><td>44/50</td><td>0/50</td></tr>
<tr><td>6,270</td><td>36/49</td><td>13/49</td><td>46/49</td><td>0/49</td></tr>
<tr><td>20,900</td><td>39/50*</td><td>33/50*</td><td>41/50</td><td>0/50</td></tr>
</table>
Female
<table>
<tr><th>Dose (mg/m^3)</th><th>Acidophilic foci in liver</th><th>Basophilic foci in liver</th><th>Bile duct hyperplasia</th><th>Centrilobular hypertrophy</th></tr>
<tr><td>0</td><td>2/50</td><td>36/50</td><td>5/50</td><td>0/50</td></tr>
<tr><td>2,090</td><td>1/50</td><td>31/50</td><td>8/50</td><td>0/50</td></tr>
<tr><td>6,270</td><td>4/50</td><td>32/50</td><td>7/50</td><td>0/50</td></tr>
<tr><td>20,900</td><td>2/50</td><td>28/50</td><td>6/50</td><td>0/50</td></tr>
</table>
</td></tr>
</table>

Reference and study design	Results
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a] daily for 104 wk	**Male** Dose (mg/kg-d) / Acidophilic foci in liver / Basophilic foci in liver / Bile duct hyperplasia / Centrilobular hypertrophy 0 / 14/50 / 14/50 / 49/50 / 0/50 28 / 12/50 / 18/50 / 47/50 / 0/50 121 / 17/50 / 20/50 / 48/50 / 0/50 542 / 13/50 / 22/50 / 47/50 / 0/50 **Female** Dose (mg/kg-d) / Acidophilic foci in liver / Basophilic foci in liver / Bile duct hyperplasia / Centrilobular hypertrophy 0 / 2/50 / 36/50 / 1/50 / 0/50 46 / 2/50 / 25/50* / 4/50 / 0/50 171 / 1/50 / 31/50 / 4/50 / 0/50 560 / 0/50 / 30/50* / 3/50 / 0/50
Weng et al. (2012) 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)[b]; female (5/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber, 6 hr/d, 5 d/wk for 13 wk; generation methods not reported, but analytical methods (gas chromatograph) and concentration reported	**Male** / **Female** Dose (mg/m^3) / Incidence of centrilobular hypertrophy / Dose (mg/m^3) / Incidence of centrilobular hypertrophy 0 / 1/5 / 0 / 0/5 2,090 / 0/5 / 2,090 / 0/5 7,320 / 0/5 / 7,320 / 1/5 20,900 / 5/5* / 20,900 / 5/5*
Weng et al. (2012) mice, *Aldh2*-/- inhalation – vapor male (5/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (5/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber, 6 hr/d, 5 d/wk for 13 wk; generation methods were not reported, but analytical methods (gas chromatograph) and concentration reported	**Male** / **Female** Dose (mg/m^3) / Incidence of centrilobular hypertrophy / Dose (mg/m^3) / Incidence of centrilobular hypertrophy 0 / 0/5 / 0 / 0/5 2,090 / 3/5 / 2,090 / 0/5 7,320 / 2/5 / 7,320 / 0/5 20,900 / 5/5* / 20,900 / 4/5*

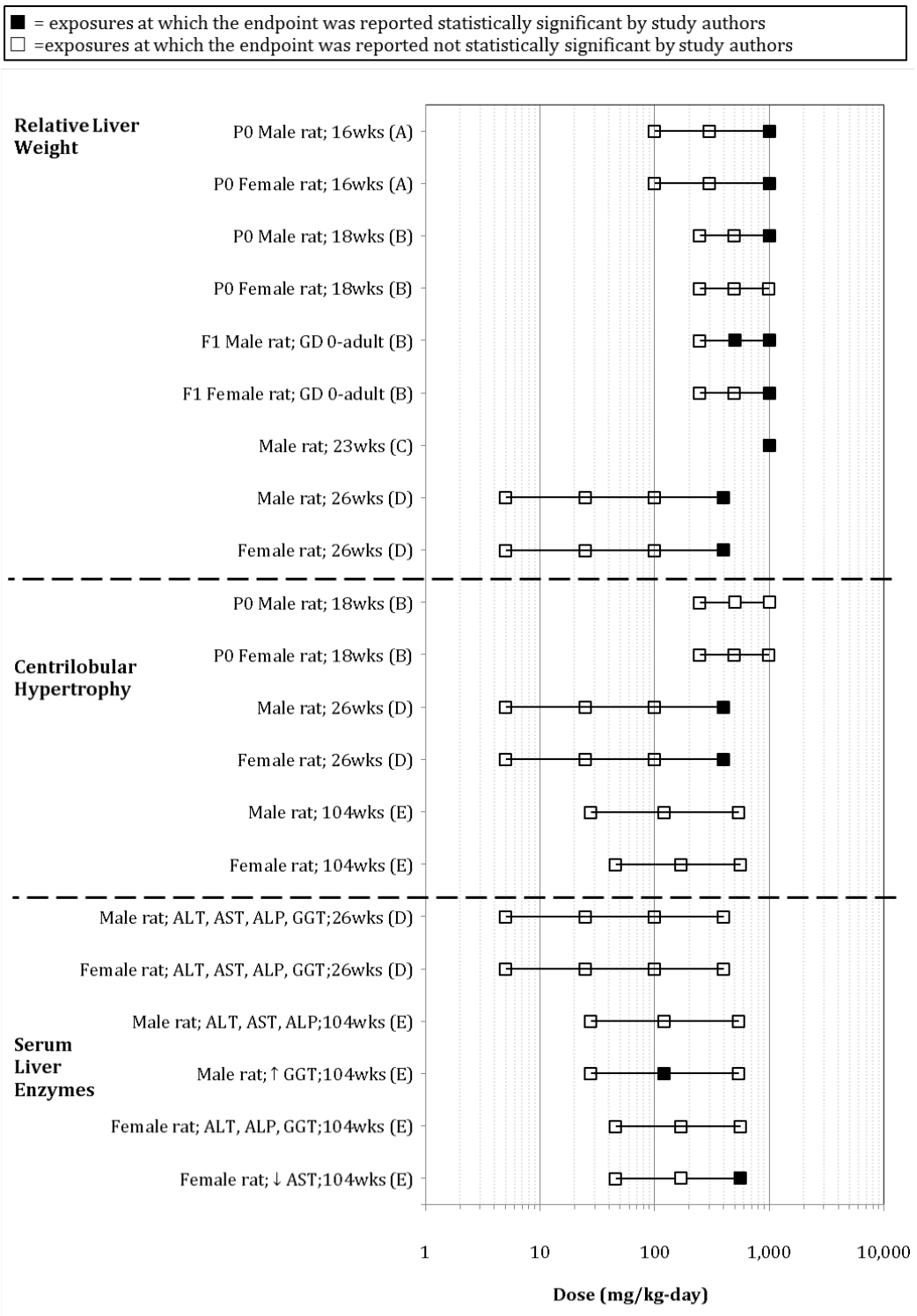
[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.

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

Reference and study design	Results
JPEC (2008b) rat, CRL:CD(SD) inhalation – vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3) dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	Response relative to control **Male** Dose (mg/m^3) / ALT / ALP / AST / GGT 0 / - / - / - / - 627 / 9% / 13% / 3% / 11% 2,090 / 0% / 12% / 1% / 0% 6,270 / 5% / -12% / -7% / 11% 20,900 / 12% / -9% / 4% / -100% **Female** Dose (mg/m^3) / ALT / ALP / AST / GGT 0 / - / - / - / - 627 / -1% / -3% / 2% / 25% 2,090 / 11% / -12% / -95% / 12% 6,270 / -5% / -7% / 12% / 25% 20,900 / 26% / 5% / 0% / 25%
Miyata et al. (2013); JPEC (2008c) rat, CRL:CD(SD) oral – gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 d	Response relative to control **Male** Dose (mg/kg-d) / ALT / ALP / AST / GGT 0 / - / - / - / - 5 / 10% / 2% / 16% / 25% 25 / 48% / 12% / 19% / 50% 100 / 13% / -7% / 20% / 25% 400 / 35% / 27% / 23% / 100% **Female** Dose (mg/kg-d) / ALT / ALP / AST / GGT 0 / - / - / - / - 5 / 11% / 6% / 10% / 40% 25 / 21% / -21% / 13% / 20% 100 / 46% / -18% / 19% / 0% 400 / 21% / -19% / 4% / -20%

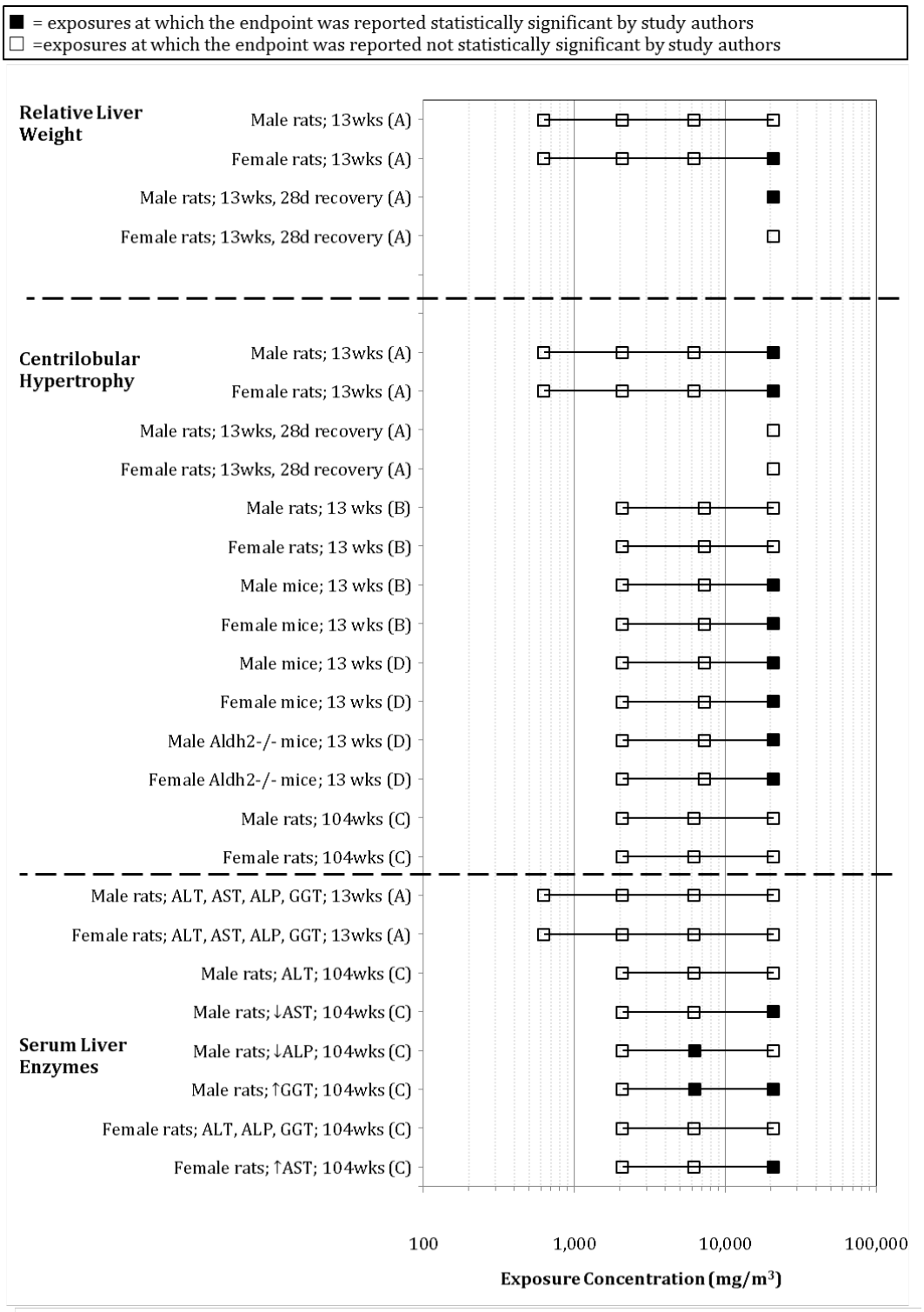
Reference and study design	Results
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation – vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method reported	Response relative to control **Male** Dose (mg/m^3) / ALT / ALP / AST / GGT 0 / - / - / - / - 2,090 / 53% / 0% / 29% / 33% 6,270 / -3% / -21%* / -16% / 50%* 20,900 / 24% / -5% / -2%* / 200%* **Female** Dose (mg/m^3) / ALT / ALP / AST / GGT 0 / - / - / - / - 2,090 / 2% / 12% / 22% / 50% 6,270 / -5% / -4% / 10% / 0% 20,900 / 4%* / 4% / 18%* / 150%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a]; daily for 104 wk	Response relative to control **Male** Dose (mg/kg-d) / ALT / ALP / AST / GGT 0 / - / - / - / - 28 / -17% / -5% / -21% / 0% 121 / 2% / 3% / -3% / 43%* 542 / -4% / 0% / -1% / 29% **Female** Dose (mg/kg-d) / ALT / ALP / AST / GGT 0 / - / - / - / - 46 / -10% / -16% / -19% / 0% 171 / -15% / 2% / -17% / 0% 560 / -26% / -15% / -46%* / 33%

[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].
Abbreviations: ALT = alanine aminotransferase, ALP = alkaline phosphatase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase.



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 1-9. Exposure-response array of noncancer 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 1-10. Exposure-response array of noncancer liver effects following inhalation exposure to ETBE.

Table 1-12. Evidence pertaining to liver tumor effects in animals exposed to ETBE

Reference and study design	Results
Hepatocellular Adenoma and Carcinoma	
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral – drinking water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a] daily for 104 wk	**Male** Dose (mg/kg-d) / Adenoma / Carcinoma / Adenoma or carcinoma 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 **Female** Dose (mg/kg-d) / Adenoma / Carcinoma / Adenoma or carcinoma 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
Maltoni et al. (1999) rat, Sprague-Dawley oral – gavage in olive oil male (60/group): 0, 250, 1,000 mg/kg-d; female (60/group): 0, 250, 1,000 mg/kg-d 4 d/wk for 104 wk; observed until natural death (depressed survival 25-28% seen in controls at 104 wks) NOTE: Tumor data not reanalyzed by Malarkey and Bucher (2011).	**Male** Dose (mg/kg-d) / Adenoma or carcinoma 0 / 0/60 250 / 0/60 1,000 / 0/60 **Female** Dose (mg/kg-d) / Adenoma or carcinoma 0 / 0/60 250 / 0/60 1,000 / 0/60

Reference and study design	Results			
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation – vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method reported	**Male** Dose (mg/m^3)	Adenoma	Carcinoma	Adenoma or carcinoma
	0	0/50	0/50	0/50
	2,090	2/50	0/50	2/50
	6,270	1/50	0/50	1/50
	20,900	9/50*	1/50	10/50*
	Female Dose (mg/m^3)	Adenoma	Carcinoma	Adenoma or carcinoma
	0	1/50	0/50	1/50
	2,090	0/50	0/50	0/50
	6,270	1/50	0/50	1/50
	20,900	1/50	0/50	1/50
Initiation-Promotion Studies				
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral – gavage in olive oil male (30/group): 0, 300, 1,000 mg/kg-d daily for 23 wk following a 4-wk tumor initiation by DMBDD[c] [+] no DMBDD initiation	**Male** Dose (mg/kg-d)	Adenoma	Carcinoma	
	0	0/30	1/30	
	300	1/30	0/30	
	1,000	6/30*	0/30	
	0[+]	0/12	0/12	
	1,000[+]	0/12	0/12	
Hagiwara et al. (2015) rat, Wistar oral – gavage in olive oil male (30/group): 0,100, 300, 500, 1,000 mg/kg-d daily for 19 wk following 2-wk tumor initiation by N-ethyl-N-hydroxyethylnitrosamine (EHEN)	**Male** Dose (mg/kg-d)	Adenoma	Carcinoma	Adenoma or carcinoma
	0	4/30	0/30	4/30
	100	5/30	2/30	7/30
	300	8/30	0/30	8/30
	500	8/30	3/30	10/30
	1,000	15/30*	5/30*	17/30*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
[c]Diethylnitrosamine (DEN), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), N-methyl-N-nitrosourea (MNU), 1,2-dimethylhydrazine dihydrochloride (DMH), and N-bis(2-hydroxypropyl)nitrosamine (DHPN).
*: 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.

Mode of Action Analysis - Liver Effects

Key characteristics of carcinogens

Mechanistic information was grouped into 10 "key characteristics" useful for summarizing and organizing the mechanistic data relevant to carcinogens (Smith et al., 2016). The evidence available for each characteristic is summarized in Table 1-13. Altogether, experimental evidence informing several of the key characteristics of carcinogens was identified in the available literature.. ETBE was found to have the potential for the formation of electrophilic metabolites, but it was concluded that there was inadequate evidence that ETBE induced any of the remaining key characteristics.

Table 1-13.Evidence of key characteristics of carcinogens for ETBE.

Characteristic	Evidence
1. Is electrophilic or can be metabolically activated to electrophiles	Metabolized to acetaldehyde in the liver.[1,3]
2. Is genotoxic	Inadequate evidence to draw a conclusion from 12 studies examining micronucleus, DNA strand breaks, chromosomal aberration, and gene mutation assays[2,3]
3. Alters DNA repair or causes genomic instability	No pertinent studies identified
4. Induces epigenetic alterations	No pertinent studies identified
5. Induces oxidative stress	Inadequate evidence to draw a conclusion from 3 studies examining 8-OHdG, 8-hOGG1 formation[3,4]
6. Induces chronic inflammation	No pertinent studies identified
7. Is immunosuppressive	No pertinent studies identified
8. Modulates receptor-mediated effects	Inadequate evidence to draw a conclusion from 2 studies examining PPAR, CAR, and PXR activation[5]
9. Causes immortalization	No pertinent studies identified
10. Alters cell proliferation, cell death, or nutrient supply	Inadequate evidence to draw a conclusion from 3 studies examining basophilic, acidophilic foci and cellular proliferation[5]

[1]See Supplemental Information section B.1.3.

[2]See Supplemental Information section B.2.2.

[3]See Acetaldehyde-mediated liver toxicity and genotoxicity in this section.

[4]See Oxidative stress in this section.

[5]See Receptor-mediated effects in this section.

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, while *tert*-butanol undergoes systemic circulation and ultimate excretion 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 IRIS assessment of *tert*-butanol) and acetaldehyde are relevant to evaluating the MOA for liver effects observed after ETBE exposure.

tert-Butanol induces thyroid tumors in mice and kidney tumors in male rats, but has not been observed to affect the incidence of rodent liver tumors following a 2-year oral exposure. Although some data suggest *tert*-butanol could be genotoxic, the overall evidence is inadequate to establish a conclusion. One study reported that *tert*-butanol might induce centrilobular hypertrophy in mice after 2 weeks (Blanck et al., 2010); however, no related liver pathology was observed in other repeat-exposure rodent studies including both subchronic and 2-year bioassays. Although Blanck et al. (2010) reported some limited induction of mouse liver enzymes following short-term *tert*-butanol exposure, no corresponding evidence exists in rats following any exposure duration. Therefore, a role for *tert*-butanol in liver carcinogenesis of ETBE appears unlikely.

In comparison, acetaldehyde associated with the consumption of alcoholic beverages is genotoxic and mutagenic (IARC, 1999a), and acetaldehyde produced in the liver as a result of ethanol metabolism has been suggested to be 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 relative liver weights in male and female rats and increased hepatocellular adenomas and carcinomas in males (Saito et al., 2013; JPEC, 2010b). In addition to the increased centrilobular hypertrophy, which is one possible indication of liver enzyme induction, chronic exposure induced focal proliferative lesions (including basophilic and acidophilic foci) that could be more directly related to tumorigenesis. The centrilobular hypertrophy was increased in rats of both sexes via both oral and inhalation exposure at subchronic time points; not at 2 years, although significantly increased liver weight was observed. Liver tumors were only observed in one sex (males) following one route of exposure (inhalation), however, indicating that subchronic hypertrophy may not be associated with later tumor development. This process was investigated in several studies to determine whether nuclear receptor activation is involved.

Centrilobular hypertrophy is induced through several possible mechanisms, many of which are via activation of 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, induction of gene expression driving 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, which has no established MOA, is hypothesized to progress from PXR activation to liver tumors in a similar manner as CAR. This progression would include PXR activation, cell proliferation, hypertrophy, CYP3A induction, and clonal expansion resulting in foci development. One study that orally exposed male rats to low and high concentrations of ETBE reported that several key sequences in the PPARα, PXR, and CAR pathways were affected (Kakehashi et al., 2013).

PPAR

Limited evidence suggests that ETBE could activate PPAR-mediated events (Kakehashi et al., 2013). For instance, mRNA expression was significantly elevated for PPARα and PPARγ after 1 week of exposure but not after 2 weeks. In addition, several 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. Additional effects in the PPAR pathway such as DNA damage (8-OHdG) and apoptosis (ssDNA) also were significantly increased after 2 weeks at the highest concentration of ETBE. Cell proliferation was increased after 3 days (Kakehashi et al., 2015), unchanged after 1 week, significantly decreased after 2 weeks (Kakehashi et al., 2013) and increased after 28 days (Kakehashi et al., 2015). The number of peroxisomes per hepatocyte was increased greater than fivefold after 2 weeks of treatments. Finally, the incidences of preneoplastic basophilic and acidophilic foci were significantly increased in males after 2 years of inhalation exposure to ETBE (Saito et al., 2013; JPEC, 2010b).

PPARα mediated genes were investigated in one study (Kakehashi et al., 2013). The high dose of ETBE (2,000 mg/kg-day) which induced the most consistent changes in PPARα, *Cyp4a*, *Cyp1a*, and *Cyp3a* in the oral gavage study (Kakehashi et al., 2013) yielded a higher internal metabolic rate in the liver (3.98 mg ETBE/hr) than from the 20,700 mg ETBE/m^3 inhalation dose (3.34 mg ETBE/hr) that increased liver tumors in the 2-year inhalation study (Saito et al., 2013; JPEC, 2010b). Only *Cyp2b* genes associated with PPARα expression were affected at the low gavage dose (300mg/kg-day), thus demonstrating poor dose-response relationships between PPAR-mediated genes and downstream effects. Finally, 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).However, several measures required for a full evaluation of the PPAR MOA were absent. Selective clonal expansion and gap junction intercellular communication were not examined in any study. No evidence is available in wild-type or PPARα-

null mice to demonstrate if PPARα gene expression changes in KO mice. Overall, these data are inadequate to conclude that ETBE induces liver tumors via a PPARα MOA.

CAR/PXR

Kakehashi et al. (2013) reported several CAR- and PXR-mediated events following ETBE exposure. After 2 weeks of exposure at the high dose of ETBE, CAR- and PXR-regulated xenobiotic metabolic enzymes were upregulated, including *Cyp2b1*, *Cyp2b2*, *Cyp3a1*, and *Cyp3a2* as determined by mRNA 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 CAR and PXR. However, with the exception of *Cyp2b*, these genes were only increased at the high dose, which yielded an internal rate of ETBE metabolism (3.98 mg/hr) that was greater than the metabolism rate (3.34 mg/hr) associated with liver tumors in the 2-year inhalation study (Saito et al., 2013; JPEC, 2010b). Histological evidence (preneoplastic foci) supporting increased liver cell proliferation is available following chronic, but not subchronic exposures (Saito et al., 2013; JPEC, 2010b). Several data gaps were not evaluated, such as a lack of clonal expansion and gap junction communication. These data provide evidence that CAR and PXR are activated at high concentrations in the liver following acute ETBE exposure; however, due to crosstalk of CAR and PXR on downstream effects such as cell proliferation, preneoplastic foci, and apoptosis. . Furthermore, the data do not provide enough information to determine dose-response relationships or temporal associations, which are helpful for establishing an MOA. Altogether, these data are inadequate to conclude that ETBE induces liver tumors via a CAR/PXR MOA.

In summary, several gaps in the receptor mediated effects data (for PPARα, CAR, and PXR) are noted such as evidence in only one species, lack of any studies in PPAR KO mice, lack of dose response concordance between receptor mediated gene changes and tumors, and lack of any receptor mediated data outside of the 1 and 2 week time points, which preclude establishing temporal associations. Overall, these data are inadequate to conclude that ETBE induces liver tumors via a PPARα or CAR/PXR MOA.

Acetaldehyde-mediated liver toxicity and genotoxicity

Another possible MOA for increased tumors could be due to genotoxicity and mutagenicity resulting from 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, although evidence is not sufficient to show 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), although no effect of acetaldehyde on

CAR or PXR activation has 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 among some subpopulations such as people of East Asian origin (IARC, 2012; Brennan et al., 2004). IARC (2012) found that *ALDH2* status was associated with increased esophageal cancer. Although IARC (2012) found inconclusive evidence for a contribution of *ALDH2* to liver cancer, Eriksson (2015) concluded that reduced aldehyde metabolism is associated with liver cancer by further analyzing the *ALDH2* compositions of the controls in the case-control studies.

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 male *Aldh2* knockout (KO) mice compared with wild type (WT), while females appeared to be less sensitive, similar to controls (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 increased 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 (8-hOGG1) in leukocytes were observed to be statistically significantly increased and dose responsive only in male *Aldh2* KO mice (Weng et al., 2013). Together, although these data suggest a potential role for acetaldehyde in the increased liver tumor response observed in male rats exposed to ETBE, the available data are inadequate to conclude that ETBE induces liver tumors via acetaldehyde-mediated mutagenicity.

Oxidative Stress

Studies with pertinent information on the evaluation of oxidative stress are limited to a few studies measuring oxidative DNA damage in leukoyctes and hepatocytes in mice (Weng et al., 2012) and one study in the liver of rats (Kakehashi et al., 2013). Hepatocytes in male mice had increased levels of 8-OHdG after 13 weeks of inhalation exposure to the concentration of ETBE that induced liver tumors following 2 years of inhalation exposure. No significant dose response was reported. Similarly, 8-OHdG was increased after 2 weeks of oral gavage in rats (Kakehashi et al., 2013) at a concentration two-fold greater than that inducing rat liver tumors in two-stage initiation-promotion assays (Hagiwara et al., 2015; Hagiwara et al., 2011). In addition, as discussed in the previous paragraph, oxidative DNA damage was also induced in *Aldh2* KO mice (Weng et al., 2013) and *Aldh2* heterozygous mice {Weng, 2019, 5343910}. Overall, these data are inadequate to conclude that ETBE induces liver tumors via oxidative stress.

Overall Conclusions on MOA for Liver Effects

Several reviews of the available mechanistic data suggest that the PPAR, PXR, and CAR pathways induce liver tumors in a manner not relevant to humans (Elcombe et al., 2014; Klaunig et al., 2003), although this conclusion has been questioned (Guyton et al., 2009). The database is inadequate to determine if nuclear receptor-mediated pathways (i.e., PPAR and CAR/PXR) contribute to the tumorigenesis observed in ETBE-treated male rats. Furthermore, centrilobular hypertrophy was observed at the same concentrations that induced liver weight changes in rats of both sexes after 13-week inhalation and 26-week oral exposure, yet liver tumors were observed only following oral exposure in male rats. This observation suggests that these effects are not associated with the observed rat liver tumorigenesis. Therefore, given the available data, ETBE-induced liver tumors in male rats are relevant to human hazard identification and are scientifically supported.

Evidence suggests that metabolism of ETBE to acetaldehyde could 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). The database, however, is inadequate to conclude that ETBE induces liver tumors via acetaldehyde-mediated mutagenic MOA.

Integration of Liver Effects

Liver effects were observed in oral and inhalation studies with exposure durations of 13 weeks to 2 years. Evidence for ETBE-induced noncancer liver effects is available from rat and mouse studies that include centrilobular hypertrophy, increased liver weight, and changes in serum liver enzyme levels. Based on dose-related increases in relative liver weight and increases in hepatocellular hypertrophy in male and female rats, and considering the poor temporal correlation of serum biomarkers and pathological lesions indicative of accumulating damage, evidence of liver effects associated with ETBE exposure is suggestive. The hazard and dose-response conclusions regarding these noncancer endpoints associated with ETBE exposure are further discussed in Section 1.3.1.

The carcinogenic effects observed include increased hepatocellular adenomas and carcinomas in males in a 2-year bioassay and ETBE-promoted liver tumorigenesis after 23 weeks following mutagen pretreatment. Although only one carcinoma was observed, rodent liver adenomas could progress to malignancy, eventually forming carcinomas (Liau et al., 2013; McConnell et al., 1986). 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 in transgenic mice suggest that lack of Aldh2 enhances ETBE-induced liver toxicity and genotoxicity, which is consistent with the observed genotoxicity being mediated by the ETBE metabolite acetaldehyde, although the database is inadequate to conclude that ETBE induces liver tumors via acetaldehyde-mediated mutagenic MOA. The hazard and dose-

response conclusions regarding the liver tumors associated with ETBE exposure are further discussed as part of the overall weight of evidence for carcinogenicity in Section 1.3.2.

1.2.3. Reproductive Effects

Synthesis of Effects Related to Male Reproduction

The database examining male reproductive effects following ETBE exposure contains no human data but is comprised of animal data from rats and mice. Effects on male reproduction, including fertility, male reproductive organ weights, histopathology, sperm parameters, and hormone levels were evaluated in a one-generation oral study (Fujii et al., 2010), a two-generation oral study (Gaoua, 2004b), 13- and 9-week inhalation studies (Weng et al., 2014), and a 14-day oral study (de Peyster et al., 2009). Additional data on male reproductive organ weights and histopathology were obtained from two 2-year carcinogenicity studies [oral: Suzuki et al. (2012); JPEC (2010a); inhalation: Saito et al. (2013); JPEC (2010b)], a medium term carcinogenicity study (23-week oral exposure) (Hagiwara et al., 2011; JPEC, 2008d), a 180-day oral study (Miyata et al., 2013; JPEC, 2008c), a 90-day inhalation study (JPEC, 2008b), and a 13-week inhalation study (Medinsky et al., 1999). These studies were conducted in Sprague-Dawley rats, Fischer 344 rats, CD-1 mice, and C57BL/6 mice, and the design, conduct, and reporting of each study were of sufficient quality to inform human health hazard assessment. Selected endpoints from these studies are summarized in

Table 1-14.

The one- and two-generation reproductive toxicity studies found no effects on copulation, fertility, or sperm parameters in adult male Sprague-Dawley rats exposed to ETBE by oral gavage at concentrations up to 1,000 mg/kg-day for 10 weeks prior to mating (Fujii et al., 2010; Gaoua, 2004b), nor in F1 male offspring exposed during gestation, lactation, and post-weaning in the diet (Gaoua, 2004b). No dose-related changes in testicular histopathology were observed in F0 or F1 males (Gaoua, 2004b). Furthermore, no dose-related histopathological changes or significant changes in absolute male reproductive organ weight were observed in the 2-year carcinogenicity studies in Fischer 344 rats at oral doses up to 542 mg/kg-day (Suzuki et al., 2012; JPEC, 2010a) or at inhalation exposure concentrations up to 20,900 mg/m^3 (Saito et al., 2013; JPEC, 2010b); in the medium term carcinogenicity study in Fischer 344 rats (Hagiwara et al., 2011; JPEC, 2008d); in the 180-day oral study in Sprague-Dawley rats at doses up to 400 mg/kg-day (Miyata et al., 2013; JPEC, 2008c); in the 90-day inhalation study in Sprague-Dawley rats at doses up to 20,900 mg/m^3 (JPEC, 2008b); or in the 14-day oral study in Fischer 344 rats at doses up to 1,800 mg/kg-day (de Peyster et al., 2009). In some cases, dose-related increases in relative organ weights were observed, including significant increases in relative testis weight (Fujii et al., 2010; JPEC, 2010b; Gaoua,

2004b) and relative prostate weight (Gaoua, 2004b) at the highest doses tested, which may have been attributable to reduced body weight gain in these groups.

In contrast, testicular degeneration was observed in two 13-week ETBE inhalation studies in which rats and mice were exposed to concentrations ranging from 2,090–20,900 mg/m^3. In Fischer 344 rats, a statistically significant increase in the percentage of seminiferous tubules with spermatocyte degeneration was observed; however, there were no significant microscopic findings in CD-1 mice under these same exposure conditions and no changes in male reproductive organ weights in rats or mice (Medinsky et al., 1999). In C57BL/6 wild type and *Aldh2* KO mice, there was a dose-related increase in the incidence of atrophy of seminiferous tubules (described by the authors as "slight" or "extremely slight" atrophy), with a greater incidence of atrophy occurring in *Aldh2* KO mice compared to wild type (Weng et al., 2014). ETBE-exposed mice also had significant decreases in sperm head numbers and sperm mobility (expressed as the percentage of motile sperm, percentage of static sperm, and percentage of sperm with rapid movement) and a significant increase in sperm DNA damage (expressed as strand breaks and oxidative DNA damage), with effects on sperm parameters reaching statistical significance at lower exposure concentrations in *Aldh2* KO mice (2,090 mg/m^3) compared to wild type (7,320–20,090 mg/m^3). Significantly decreased epididymis weight was observed in *Aldh2* KO mice but not wild type mice.

Weng et al. (2014) also conducted a 9-week inhalation study using lower ETBE exposure concentrations (209–2,090 mg/m^3) and three mouse genotypes (wild type, *Aldh2* KO, and *Aldh2* heterozygous; n=5/group). Wild type mice had little to no change in male reproductive organ weights or sperm parameters at any of the tested concentrations, whereas significant effects were observed on sperm count, sperm mobility, and sperm DNA damage in *Aldh2* KO and heterozygous mice at exposure concentrations as low as 836 mg/m^3 ETBE. *Aldh2* heterozygous mice had significantly decreased relative testis and epididymis weight in the 20,090 mg/m^3 exposure group. However, for unknown reasons, several reproductive effects were noted to be more pronounced in the heterozygous mice as compared to the *Aldh2* KO mice. Taken together, the results of Weng et al. (2014) indicate that populations with inactive *Aldh2* variants may be more susceptible to male reproductive toxicity following exposure to ETBE. However, these effects are considered to be preliminary due to the small sample size (n=5) in one species, in one study, and the unconvincing magnitude of many of the statistically significant effects (including the observation that the heterozygotes exhibited more robust changes than the knockouts).

Although testicular lesions were not found in the 14-day oral study in Fischer 344 rats (de Peyster et al., 2009), plasma estradiol levels in these animals were increased by up to 106% compared to controls. Plasma testosterone in the 1,800 mg/kg-day dose group was decreased by 34% compared to controls, but the difference was not statistically significant and was not observed in any other ETBE dose group. The authors conducted a separate in vitro experiment to evaluate testosterone production in isolated Sprague-Dawley rat Leydig cells and found reduced

testosterone production in ETBE-treated cells compared to controls (data not shown in evidence table).

Table 1-14. Evidence pertaining to male reproductive effects in animals exposed to ETBE

Reference and Study Design	Results
Male Fertility	
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	**F0 Generation-Parent** Dose (mg/kg-d) / Copulation index (%) / Absolute change from control (%) / Fertility index (%) / Absolute change from control (%) 0 / 100 / - / 87.5 / - 100 / 91.7 / -8.3 / 100 / 12.5 300 / 95.8 / -4.2 / 95.7 / 8.2 1,000 / 100 / 0 / 91.7 / 4.2
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups F0 Generation-Parent	**F0 Generation-Parent** Dose (mg/kg-d) / Male mating index[a] (%) / Absolute change from control (%) / Male fertility index[b] (%) / Absolute change from control (%) 0 / 100 / - / 92 / - 250 / 100 / 0 / 84 / -8 500 / 100 / 0 / 88 / -4 1,000 / 100 / 0 / 100 / 8 **F1 Generation-Offspring** Dose (mg/kg-d) / Male mating index[a] (%) / Absolute change from control (%) / Male fertility index[b] (%) / Absolute change from control (%) 0 / 96 / - / 92 / - 250 / 96 / 0 / 92 / 0 500 / 100 / 4 / 88 / -4 1,000 / 96 / 0 / 96 / 4

Reference and Study Design	Results		
Testicular Histopathology			
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation – vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk	Dose (mg/m^3)	Incidence of spermatocyte degeneration	Incidence of sloughed epithelium
	0	11/11	7/11
	2,090	11/11	3/11
	7,320	11/11	3/11
	20,900	10/11	7/11
	Dose (mg/m^3)	Mean seminiferous tubules with spermatocyte degeneration (%)	Absolute change from control (%)
	0	2.1	-
	2,090	2.4	0
	7,320	7.8*	6
	20,900	12.7*	11
	Dose (mg/m^3)	Mean seminiferous tubules with lumenal debris (%)	Absolute change from control (%)
	0	2.1	-
	2,090	0.7	-1
	7,320	2.8	1
	20,900	1	-1

<table>
<tr><th>Reference and Study Design</th><th>Results</th></tr>
<tr><td>Weng et al. (2014)
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]
dynamic whole body inhalation; 6 h/d, 5 d/wk for 13 wk; methods described in Weng et al. (2012)</td><td>Wild Type Mice; 13-week Exposure
<table><tr><th>Dose (mg/m^3)</th><th>Incidence of "extremely slight" atrophy</th><th>Incidence of "slight" atrophy</th><th>Total incidence of atrophy of seminiferous tubules</th></tr><tr><td>0</td><td>1/5</td><td>0/5</td><td>1/5</td></tr><tr><td>2,090</td><td>0/5</td><td>0/5</td><td>0/5</td></tr><tr><td>7,320</td><td>2/5</td><td>0/5</td><td>2/5</td></tr><tr><td>20,900</td><td>3/5</td><td>0/5</td><td>3/5</td></tr></table>Knockout Mice (Aldh2-/-); 13-week Exposure
<table><tr><th>Dose (mg/ m^3)</th><th>Incidence of "extremely slight" atrophy</th><th>Incidence of "slight" atrophy</th><th>Total incidence of atrophy of seminiferous tubules</th></tr><tr><td>0</td><td>2/5</td><td>0/5</td><td>2/5</td></tr><tr><td>2,090</td><td>2/5</td><td>3/5</td><td>5/5</td></tr><tr><td>7,320</td><td>4/5</td><td>1/5</td><td>5/5</td></tr><tr><td>20,900</td><td>3/5</td><td>2/5</td><td>5/5</td></tr></table></td></tr>
<tr><td colspan="2">Sperm Parameters</td></tr>
<tr><td>Gaoua (2004b)
rat, Sprague-Dawley
oral – gavage
F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d
dosed daily for 18 wks from 10 wks premating until weaning of F1 pups
F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d
dosed daily from PND 22 until weaning of F2 pups</td><td>F0 Males
<table><tr><th>Dose (mg/kg-d)</th><th>Mean epididymal spermatozoa count (n) ± SD</th><th>% change from control</th><th>Mean epididymal sperm motility (%) ± SD</th><th>Absolute change from control (%)</th></tr><tr><td>0</td><td>923 ± 200</td><td>-</td><td>99.7 ± 1.5</td><td>-</td></tr><tr><td>250</td><td>938 ± 205</td><td>2</td><td>100 ± 0</td><td>0</td></tr><tr><td>500</td><td>935 ± 159</td><td>1</td><td>98.6 ± 4</td><td>-1</td></tr><tr><td>1,000</td><td>918 ± 194</td><td>-1</td><td>97.6 ± 6.6</td><td>-2</td></tr></table><table><tr><th>Dose (mg/kg-d)</th><th>Mean epididymal sperm with normal morphology (%) ± SD</th><th>Absolute change from control (%)</th><th>Mean testicular sperm heads (10^6/gram of testis) ± SD</th><th>% change from control</th></tr><tr><td>0</td><td>93 ± 19</td><td>-</td><td>114.8 ± 18.7</td><td>-</td></tr><tr><td>250</td><td>93 ±19</td><td>0</td><td>109 ± 13.1</td><td>-5</td></tr><tr><td>500</td><td>97 ± 2</td><td>4</td><td>108.1 ± 18.6</td><td>-6</td></tr><tr><td>1,000</td><td>96 ± 2</td><td>3</td><td>109.8 ± 16.5</td><td>-4</td></tr></table></td></tr>
</table>

Reference and Study Design	Results
Gaoua (2004b) (continued)	

Dose (mg/kg-d)	Mean daily testicular sperm production (10^6/gram of testis)	% change from control	N (epididymal sperm count)	N (other sperm parameters)
0	18.8 ± 3.1	-	25	25
250	17.9 ± 2.2	-5	25	25
500	17.7 ± 3.1	-6	25	25
1,000	18 ± 2.7	-4	24	25

F1 Males

Dose (mg/kg-d)	Mean epididymal spermatozoa count (n) ± SD	% change from control	Mean epididymal sperm motility (%) ± SD	Absolute change from control (%)
0	725 ± 150	-	84.6 ± 34.1	-
250	673 ± 197	-7	87.1 ± 31.6	3
500	701 ± 97	-3	93.3 ± 22	9
1,000	688 ± 177	-5	88.3 ± 29.4	4

Dose (mg/kg-d)	Mean epididymal sperm with normal morphology (%) ± SD	Absolute change from control (%)	Mean testicular sperm heads (10^6/gram of testis) ± SD	% change from control
0	84 ± 30	-	100.6 ± 36.7	-
250	86 ± 28	2	97.8 ± 32.3	-3
500	86 ± 27	2	105.3 ± 27.2	5
1,000	88 ± 24	4	99.8 ± 38.9	-1

Dose (mg/kg-d)	Mean daily testicular sperm production (10^6/gram of testis)	% change from control	N (epididymal sperm count)	N (other sperm parameters)
0	16.5 ± 6	-	22	24
250	16 ± 5.3	-3	24	25
500	17.3 ± 4.5	5	23	24
1,000	16.4 ± 6.4	-1	24	25

Reference and Study Design	Results
Weng et al. (2014) 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] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 13 wk; methods described in Weng et al. (2012)	**Wild Type Mice; 13-week Exposure** Dose (mg/m^3) / Mean sperm head numbers (testis) (x 10^6/g) ± SD / % change from control / Motile sperm (epididymal) ± SE / Absolute change from control (%) 0 / 166.62 ± 21.9 / - / 67.34 ± 3.45 / - 2,090 / 167.74 ± 28.02 / 1 / 69.64 ± 3.45 / 2 7,320 / 167.78 ± 25.52 / 1 / 62.73 ± 1.73 / -5 20,900 / 150.94 ± 23.07 / -9 / 58.13 ± 2.30 / -9 Dose (mg/m^3) / % Static sperm (epididymal) / Absolute change from control (%) / % Sperm with rapid movement (epididymal) / Absolute change from control (%) 0 / 32.57 ± 3.00 / - / 55.00 ± 3.75 / - 2,090 / 30.86 ± 3.86 / -2 / 56.25 ± 3.13 / 1 7,320 / 37.29 ± 1.71 / 5 / 49.38 ± 3.13 / -6 20,900 / 42.43 ± 2.57 / 10 / 46.25* ± 2.50 / -9 Dose (mg/m^3) / Epididymal sperm DNA breaks (tail intensity in comet assay) / % change from control / Epididymal sperm DNA damage (measurement of 8-OH-dG in comet assay) / % change from control 0 / 4.91 ± 0.34 / - / 3.46 ± 0.45 / - 2,090 / 5.91 ± 0.35 / 20 / 4.23 ± 0.22 / 23 7,320 / 7.60* ± 0.69 / 55 / 5.16* ± 0.46 / 49 20,900 / 7.91* ± 0.52 / 61 / 6.55 ± 1.13 / 89 **Knockout Mice (*Aldh2*-/-); 13-week Exposure** Dose (mg/m^3) / Mean sperm head numbers (testis) (x 10^6/g) ± SD / % change from control / Motile sperm (epididymal) ± SE / Absolute change from control (%) 0 / 169.15 ± 28.33 / - / 75.07 ± 2.88 / - 2,090 / 127.08 ± 17.32 / -25 / 61.23 ± 5.03 / -14 7,320 / 124.6* ± 11.96 / -26 / 61.05* ± 5.75 / -16 20,900 / 124.72* ± 18.72 / -26 / 57.27* ± 5.77 / -20

Reference and Study Design	Results
Weng et al. (2014) (continued)	

Dose (mg/m^3)	% Static sperm (epididymal)	Absolute change from control (%)	% Sperm with rapid movement (epididymal)	Absolute change from control (%)
0	25.46 ± 2.56	-	66.74 ± 2.17	-
2,090	40.34 ± 5.14	15	51.54 ± 2.84	-15
7,320	41.51* ± 5.57	16	47.74* ± 5.66	-19
20,900	45.27* ± 5.58	20	45.03* ± 3.97	-22

Dose (mg/m^3)	Epididymal sperm DNA breaks (tail intensity in comet assay)	% change from control	Epididymal sperm DNA damage (measurement of 8-OH-dG in comet assay)	% change from control
0	4.90 ± 0.52	-	3.64 ± 0.61	-
2,090	7.71 ± 0.69	58	5.45 ± 0.15	50
7,320	10.44* ± 0.78	113	7.65* ± 0.61	110
20,900	9.46* ± 0.69	93	7.95* ± 1.52	119

Weng et al. (2014)
mice, *C57BL/6*
inhalation – vapor
male (NR): 0, 50, 200, 500 ppm (0, 209, 836, 2,090 mg/m^3)[a]
dynamic whole body inhalation; 6 hr/d, 5 d/wk for 9 wk; methods described in Weng et al. (2012)

Wild Type Mice; 9-Week Exposure

Dose (mg/m^3)	Mean sperm head numbers (testis) (x 10^6/g) ± SD	% change from control	Motile sperm (epididymal) ± SE	Absolute change from control (%)
0	199.62 ± 27.22	-	85.82 ± 4.26	-
209	173.35 ± 23.35	-13	78.72 ± 1.42	-7
836	170.47 ± 25.37	-15	82.27 ± 2.13	-4
2,090	173.13 ± 16.28	-13	80.14 ± 1.42	-6

Dose (mg/m^3)	% Static sperm (epididymal)	Absolute change from control (%)	% Sperm with rapid movement (epididymal)	Absolute change from control (%)
0	13.02 ± 3.38	-	71.11 ± 2.78	-
209	21.74 ± 2.96	9	65.56 ± 2.22	-6
836	17.78 ± 2.11	5	67.22 ± 2.22	-4
2,090	16.36 ± 1.68	3	67.22 ± 2.78	-4

Reference and Study Design	Results
Weng et al. (2014) (continued)	

Dose (mg/m^3)	Epididymal sperm DNA breaks (tail intensity in comet assay)	% change from control	Epididymal sperm DNA damage (measurement of 8-OH-dG in comet assay)	% change from control
0	4.10 ± 0.26	-	3.88 ± 0.30	-
209	4.04 ± 0.10	-2	3.73 ± 0.15	-4
836	4.40 ± 0.26	7	4.25 ± 0.30	10
2,090	4.59 ± 0.26	12	4.48 ± 0.37	15

Knockout Mice (*Aldh2*-/-); 9-week Exposure

Dose (mg/m^3)	Mean sperm head numbers (testis) (x 10^6/g) ± SD	% change from control	Motile sperm (epididymal) ± SE	Absolute change from control (%)
0	216.19 ± 12.46	-	84.17 ± 2.88	-
209	198.21 ± 20.54	-8	83.45 ± 2.88	-1
836	180.71* ± 23.5	-16	77.70 ± 2.88	-6
2,090	165.8* ± 43.52	-23	69.06 ± 6.47	-15

Dose (mg/m^3)	% Static sperm (epididymal)	Absolute change from control (%)	% Sperm with rapid movement (epididymal)	Absolute change from control (%)
0	14.57 ± 1.71	-	69.79 ± 2.84	-
209	16.29 ± 4.29	2	68.65 ± 3.97	-1
836	21.43 ± 3.00	7	63.55 ± 2.27	-6
2,090	30.00* ± 6.00	15	52.20* ± 5.11	-18

Dose (mg/m^3)	Epididymal sperm DNA breaks (tail intensity in comet assay)	% change from control	Epididymal sperm DNA damage (measurement of 8-OH-dG in comet assay)	% change from control
0	4.65 ± 0.17	-	3.66 ± 0.30	-
209	4.67 ± 0.09	0	3.96 ± 0.30	8
836	5.71* ± 0.34	23	4.48 ± 0.30	22
2,090	7.01* ± 0.26	51	4.85* ± 0.22	33

Reference and Study Design	Results
Weng et al. (2014) (continued)	**Haplotype Mice (*Aldh2* heterozygous); 9-week Exposure**

Dose (mg/m^3)	Mean sperm head numbers (testis) (x 10^6/g) ± SD	% change from control	Motile sperm (epididymal) ± SE	Absolute change from control (%)
0	202.76 ± 14.59	-	85.61 ± 2.16	-
209	202.26 ± 26.31	0	85.61 ± 2.16	0
836	109.53* ± 21.56	-46	73.38* ± 3.60	-12
2,090	96.31* ± 33.4	-53	76.98* ± 3.60	-9

Dose (mg/m^3)	% Static sperm (epididymal)	Absolute change from control (%)	% Sperm with rapid movement (epididymal)	Absolute change from control (%)
0	15.00 ± 1.71	-	70.14 ± 2.24	-
209	15.00 ± 2.14	0	68.59 ± 2.24	-2
836	27.43* ± 3.86	12	49.42* ± 6.24	-21
2,090	24.00* ± 3.00	9	58.08* ± 1.69	-12

Dose (mg/m^3)	Epididymal sperm DNA breaks (tail intensity in comet assay)	% change from control	Epididymal sperm DNA damage (measurement of 8-OH-dG in comet assay)	% change from control
0	3.51 ± 0.25	-	4.04 ± 0.22	-
209	3.70 ± 0.34	5	4.45 ± 0.14	10
836	5.32* ± 0.43	52	4.86 ± 0.43	20
2,090	5.86* ± 0.42	67	5.34* ± 0.50	32

Organ Weights	
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	**F0 Parents-Absolute Organ Weights**

Dose (mg/kg-d)	Mean testis weight (g) ± SD	% change from control	Mean epididymis weight (mg) ± SD	% change from control
0	3.47 ± 0.31	-	1371 ± 136	-
100	3.48 ± 0.28	0	1360 ± 83	-1
300	3.57 ± 0.24	3	1381 ± 73	1
1,000	3.57 ± 0.31	3	1349 ± 95	-2

Reference and Study Design	Results
Fujii et al. (2010); JPEC (2008e) (continued)	

Dose (mg/kg-d)	Mean prostate weight (mg) ± SD	% change from control	Mean seminal vesicle weight (g) ± SD	% change from control
0	787 ± 180	-	2.16 ± 0.23	-
100	778 ± 158	-1	2.1 ± 0.32	-3
300	752 ± 172	-4	2.19 ± 0.24	1
1,000	816 ± 136	4	2.19 ± 0.23	1

F0 Parents-Relative Organ Weights

Dose (mg/kg-d)	Mean testis: body weight ratio (%) ± SD	Absolute change from control (%)	Mean epididymis: body weight ratio (%) ± SD	Absolute change from control (%)
0	0.554 ± 0.065	-	219 ± 30	-
100	0.572 ± 0.062	0.02	223 ± 18	4
300	0.589 ± 0.076	0.03	228 ± 25	9
1,000	0.61* ± 0.074	0.06	230 ± 24	11

Dose (mg/kg-d)	Mean prostate: body weight ratio (%) ± SD	Absolute change from control (%)	Mean seminal vesicle: body weight ratio (%) ± SD	Absolute change from control (%)
0	125 ± 28	-	0.345 ± 0.054	-
100	128 ± 30	3	0.343 ± 0.051	0.00
300	124 ± 30	-1	0.361 ± 0.052	0.02
1,000	139 ± 23	14	0.373 ± 0.042	0.03

Gaoua (2004b)
rat, Sprague-Dawley
oral – gavage
F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d
dosed daily for 18 wks from 10 wks premating until weaning of F1 pups
F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d
dosed daily from PND 22 until weaning of F2 pups

F0 Parents-Absolute Organ Weights

Dose (mg/kg-d)	Mean testis weight (left) (g) ± SD	% change from control	Mean testis weight (right) (g) ± SD	% change from control
0	1.78 ± 0.116	-	1.76 ± 0.105	-
250	1.73 ± 0.181	-3	1.76 ± 0.179	0
500	1.78 ± 0.142	0	1.76 ± 0.13	0
1,000	1.75 ± 0.237	-2	1.79 ± 0.126	2

Reference and Study Design	Results
Gaoua (2004b) (continued)	

Dose (mg/kg-d)	Mean epididymis weight (left) (g) ± SD	% change from control	Mean epididymis weight (right) (g) ± SD	% change from control
0	0.77008 ± 0.054	-	0.78148 ± 0.053	-
250	0.77092 ± 0.077	0	0.78698 ± 0.092	1
500	0.77784 ± 0.067	1	0.77492 ± 0.062	-1
1,000	0.80988 ± 0.189	5	0.77528 ± 0.056	-1

Dose (mg/kg-d)	Mean prostate weight ± SD	% change from control	Mean seminal vesicle weight ± SD	% change from control	N
0	1.41 ± 0.272	-	2.06 ± 0.309	-	25
250	1.63 ± 0.32	16	2.26 ± 0.595	10	25
500	1.37 ± 0.285	-3	2.19 ± 0.439	6	25
1,000	1.62 ± 0.396	15	2.28 ± 0.574	11	25

F0 Parents-Relative Organ Weights

Dose (mg/kg-d)	Mean testis weight: body weight ratio (left) (g) ± SD	Absolute change from control (%)	Mean testis weight: body weight ratio (right) (g) ± SD	Absolute change from control (%)
0	0.297488 ± 0.029	-	0.29488 ± 0.029	-
250	0.29005 ± 0.025	-0.01	0.29427 ± 0.025	0.00
500	0.307 ± 0.033	0.01	0.30321 ± 0.033	0.01
1,000	0.31052 ± 0.049	0.01	0.31497* ± 0.029	0.02

Dose (mg/kg-d)	Mean epididymis weight (left): body weight ratio (g) ± SD	Absolute change from control (%)	Mean epididymis: body weight ratio (right) (%) ± SD	Absolute change from control (%)
0	0.12886 ± 0.014	-	0.13072 ± 0.013	-
250	0.12947 ± 0.013	0.00	0.13245 ± 1.014	0.00
500	0.13434 ± 0.016	0.01	0.13383 ± 0.015	0.00
1,000	0.14209 ± 0.027	0.01	0.1367 ± 0.012	0.01

Reference and Study Design	Results
Gaoua (2004b) (continued)	

Dose (mg/kg-d)	Mean prostate weight: body weight ratio ± SD	Absolute change from control (%)	Mean seminal vesicle: body weight ratio ± SD	Absolute change from control (%)	N
0	0.23582 ± 0.054	-	0.34605 ± 0.066	-	25
250	0.27279 ± 0.053	0.04	0.37895 ± 0.098	0.03	25
500	0.23656 ± 0.054	0.00	0.37615 ± 0.073	0.03	25
100	0.28593* ± 0.069	0.05	0.40207 ± 0.1	0.06	25

F1 Offspring-Absolute Organ Weights

Dose (mg/kg-d)	Mean testis weight (left) (g) ± SD	% change from control	Mean testis weight (right) (g) ± SD	% change from control
0	1.79 ± 0.11	-	1.84 ± 0.137	-
250	1.77 ± 0.39	-1	1.75 ± 0.337	-5
500	1.84 ± 0.21	3	1.86 ± 0.226	1
1,000	1.84 ± 0.171	3	1.82 ± 0.255	-1

Dose (mg/kg-d)	Mean epididymis weight (left) (g) ± SD	% change from control	Mean epididymis weight (right) (g) ± SD	% change from control
0	0.71683 ± 0.11	-	0.75575 ± 0.041	-
250	0.69636 ± 0.123	-3	0.70512 ± 0.148	-7
500	0.71904 ± 0.123	0	0.75008 ± 0.113	-1
1,000	0.6898 ± 0.12	-4	0.71244 ± 0.127	-6

Dose (mg/kg-d)	Mean prostate weight ± SD	% change from control	Mean seminal vesicle weight ± SD	% change from control	N
0	1.470 ± 0.311	-	1.71 ± 0.295	-	24
250	1.48 ± 0.249	1	1.94 ± 0.567	13	25
500	1.38 ± 0.23	-6	1.86 ± 0.422	9	24
1,000	1.41 ± 0.279	-4	1.92 ± 0.436	12	25

Reference and Study Design	Results
Gaoua (2004b) (continued)	**F1 Offspring-Relative Organ Weights**

Dose (mg/kg-d)	Mean testis weight: body weight ratio (left) (g) ± SD	Absolute change from control (%)	Mean testis weight: body weight ratio (right) (g) ± SD	Absolute change from control (%)
0	0.30842 ± 0.065	-	0.31441 ± 0.036	-
250	0.30222 ± 0.067	-0.01	0.29746 ± 0.059	-0.02
500	0.30679 ± 0.037	0.00	0.31004 ± 0.04	0.00
1,000	0.31198 ± 0.042	0.00	0.30958 ± 0.05	0.00

Dose (mg/kg-d)	Mean epididymis weight (left): body weight ratio (g) ± SD	Absolute change from control (%)	Mean epididymis weight (right) (g) ± SD	Absolute change from control (%)
0	0.12299 ± 0.023	-	0.12915 ± 0.012	-
250	0.11863 ± 0.021	0.00	0.12002 ± 0.025	-0.01
500	0.1198 ± 0.021	0.00	0.12492 ± 0.018	0.00
1,000	0.11693 ± 0.021	-0.01	0.12065 ± 0.022	-0.01

Dose (mg/kg-d)	Mean prostate weight: body weight ratio ± SD	Absolute change from control (%)	Mean seminal vesicle: body weight ratio ± SD	Absolute change from control (%)	N
0	0.25136 ± 0.057	-	0.29278± 0.055	-	24
250	0.25239 ± 0.043	0.00	0.33038 0.085	0.04	25
500	0.23059 ± 0.043	-0.02	0.3165 ± 0.113	0.02	24
1,000	0.2374 ± 0.04	-0.01	0.32424 ± 0.073	0.03	25

Weng et al. (2014)
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]
dynamic whole body inhalation; 6 hr/d, 5 d/wk for 13 wk; methods described in Weng et al. (2012)

Wild Type Mice; 13-Week Exposure

Dose (mg/m^3)	Mean testis: body weight ratio (%) ± SD	Absolute change from control (%)	Mean epididymis: body weight ratio (%) ± SD	Absolute change from control (%)
0	0.7 ± 0.06	-	0.24 ± 0.02	-
2,090	0.74 ± 0.04	0.04	0.26 ± 0.02	0.02
7,320	0.67 ± 0.09	-0.03	0.25 ± 0.01	0.01
20,900	0.7 ± 0.02	0.00	0.24 ± 0.02	0.00

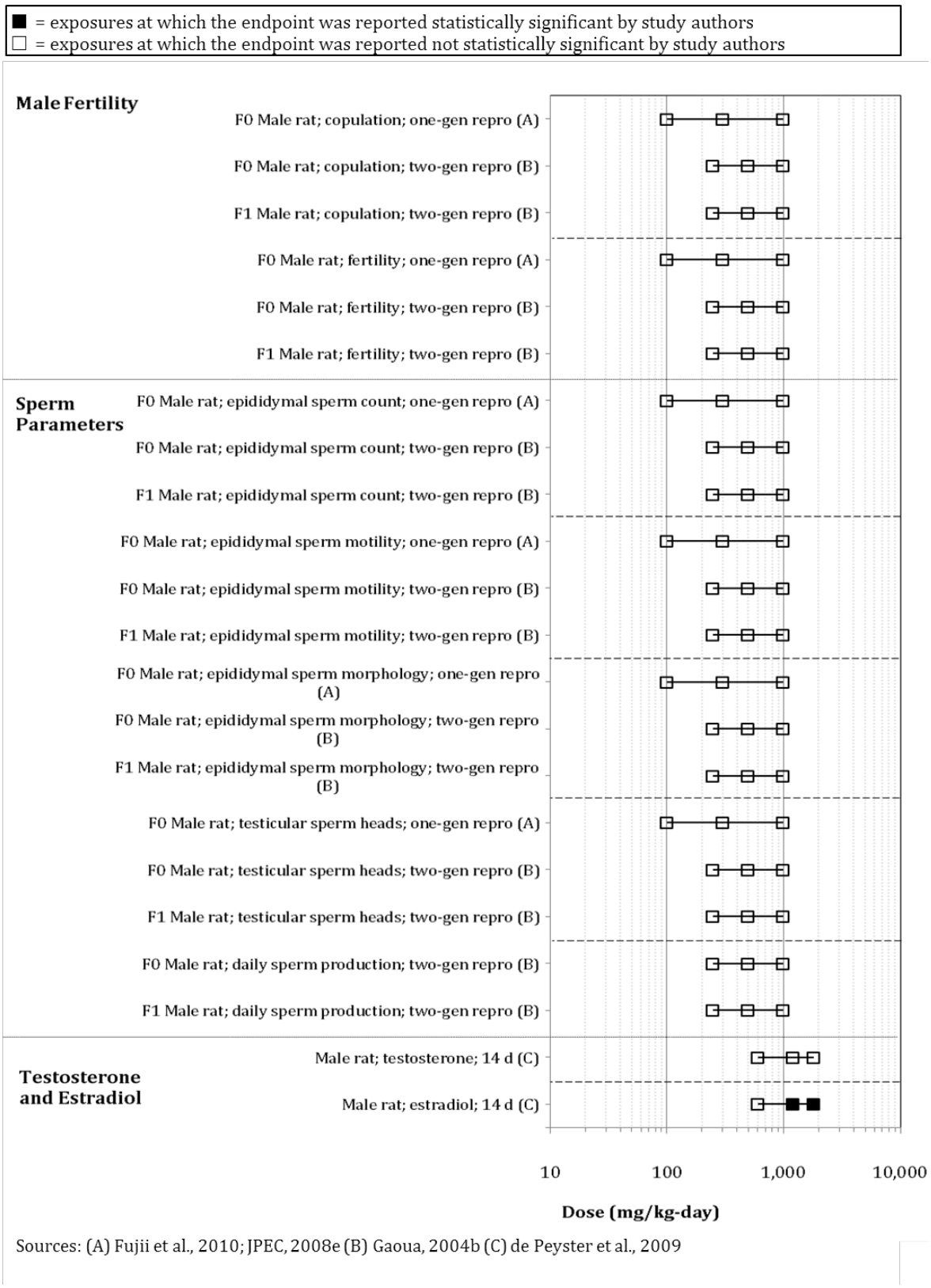
Reference and Study Design	Results
Weng et al. (2014) (continued)	**Knockout Mice (*Aldh2*-/-); 13-week Exposure** Dose (mg/m^3) | Mean testis: body weight ratio (%) ± SD | Absolute change from control (%) | Mean epididymis: body weight ratio (%) ± SD | Absolute change from control (%) 0 | 0.76 ± 0.04 | - | 0.26 ± 0.01 | - 2,090 | 0.71 ± 0.11 | -0.05 | 0.24 ± 0.02 | -0.02 7,320 | 0.72 ± 0.05 | -0.04 | 0.24* ± 0.02 | -0.02 20,900 | 0.71 ± 0.07 | -0.05 | 0.23* ± 0.02 | -0.03
Weng et al. (2014) mice, *C57BL/6* inhalation – vapor male (NR): 0, 50, 200, 500 ppm (209, 836, 2,090 mg/m^3)[a] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 9 wk; methods described in Weng et al. (2012)	**Wild Type Mice; 9-Week Exposure** Dose (mg/m^3) | Mean testis: body weight ratio (%) ± SD | Absolute change from control (%) | Mean epididymis: body weight ratio (%)± SD | Absolute change from control (%) 0 | 0.8 ± 0.12 | - | 0.26 ± 0.03 | - 209 | 0.77 ± 0.09 | -0.03 | 0.25 ± 0.03 | -0.01 836 | 0.77 ± 0.09 | -0.03 | 0.25 ± 0.02 | -0.01 2,090 | 0.78 ± 0.08 | -0.02 | 0.25 ± 0.02 | -0.01 **Knockout Mice (*Aldh2*-/-); 9-week Exposure** Dose (mg/m^3) | Mean testis: body weight ratio (%) ± SD | Absolute change from control (%) | Mean epididymis: body weight ratio (%)± SD | Absolute change from control (%) 0 | 0.8 ± 0.06 | - | 0.27 ± 0.02 | - 209 | 0.76 ± 0.05 | -0.04 | 0.26 ± 0.02 | -0.01 836 | 0.79 ± 0.07 | -0.01 | 0.27 ± 0.01 | 0.00 2,090 | 0.74 ± 0.01 | -0.06 | 0.25 ± 0.03 | -0.02 **Haplotype Mice (*Aldh2* heterozygous); 9-week Exposure** Dose (mg/m^3) | Mean testis: body weight ratio (%) ± SD | Absolute change from control (%) | Mean epididymis: body weight ratio (%)± SD | Absolute change from control (%) 0 | 0.82 ± 0.07 | - | 0.26 ± 0.02 | - 209 | 0.8 ± 0.06 | -0.02 | 0.26 ± 0.01 | 0.00 836 | 0.81 ± 0.09 | -0.01 | 0.26 ± 0.02 | 0.00 2,090 | 0.73 ± 0.03 | -0.09 | 0.24 ± 0.01 | -0.02

Reference and Study Design	Results
de Peyster et al. (2009) rat, Fischer 344 oral – gavage P0, male (12/group): 0, 600, 1,200, 1,800 mg/kg-d daily for 14 days	**Absolute Organ Weights** Dose (mg/kg-d) | Mean testis weight (g) ± SD | % change from control | Mean epididymis weight (mg) ± SD | % change from control 0 | 2.55 ± 0.09 | - | 0.696 ± 0.016 | - 600 | 2.53 ± 0.05 | -1 | 0.693 ± 0.027 | 0 1,200 | 2.49 ± 0.07 | -2 | 0.701 ± 0.026 | 1 1,800 | 2.47 ± 0.1 | -3 | 0.663 ± 0.029 | -5 Dose (mg/kg-d) | Mean prostate weight (g) ± SD | % change from control | Mean seminal vesicle weight (g) ± SD | % change from control 0 | 0.238 ± 0.018 | - | 0.781 ± 0.022 | - 600 | 0.309 ± 0.034 | 30 | 0.733 ± 0.024 | -6 1,200 | 0.252 ± 0.018 | 6 | 0.749 ± 0.037 | -4 1,800 | 0.269 ± 0.036 | 13 | 0.701 ± 0.041 | -10 Dose (mg/kg-d) | Mean weight of combined accessory sex organs (g) ± SD | % change from control 0 | 1.712 ± 0.041 | - 600 | 1.735 ± 0.057 | 1 1,200 | 1.702 ± 0.063 | -1 1,800 | 1.633 ± 0.059 | -5 **Relative Organ Weights** Dose (mg/kg-d) | Mean testis: body weight ratio (%) ± SD | Absolute change from control (%) | Mean epididymis: body weight ratio (%) ± SD | Absolute change from control (%) 0 | 0.997 ± 0.036 | - | 0.272 ± 0.007 | - 600 | 1.014 ± 0.027 | 0.02 | 0.275 ± 0.009 | 0.00 1,200 | 1.097 ± 0.03 | 0.10 | 0.308 ± 0.009 | 0.04 1,800 | 1.097 ± 0.045 | 0.10 | 0.294 ± 0.014 | 0.02 Dose (mg/kg-d) | Mean prostate: body weight ratio (%) ± SD | Absolute change from control (%) | Mean seminal vesicle: body wt. ratio (%) ± SD | Absolute change from control (%) 0 | 0.092 ± 0.007 | - | 0.304 ± 0.008 | - 600 | 0.124 ± 0.015 | 0.03 | 0.292 ± 0.012 | -0.01 1,200 | 0.111 ± 0.076 | 0.02 | 0.328 ± 0.012 | 0.02 1,800 | 0.123 ± 0.021 | 0.03 | 0.31 ± 0.017 | 0.01

Reference and Study Design	Results
de Peyster et al. (2009) (continued)	Dose (mg/kg-d) / Mean combined accessory sex organs:body weight ratio (%) ± SD / Absolute change from control (%) 0 / 0.668 ± 0.018 / - 600 / 0.691 ± 0.026 / 0.02 1,200 / 0.746 ± 0.019 / 0.08 1,800 / 0.727 ± 0.035 / 0.06
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation – vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk	Organ weights of Fisher 344 rats and CD-1 mice were not altered by exposure to ETBE.

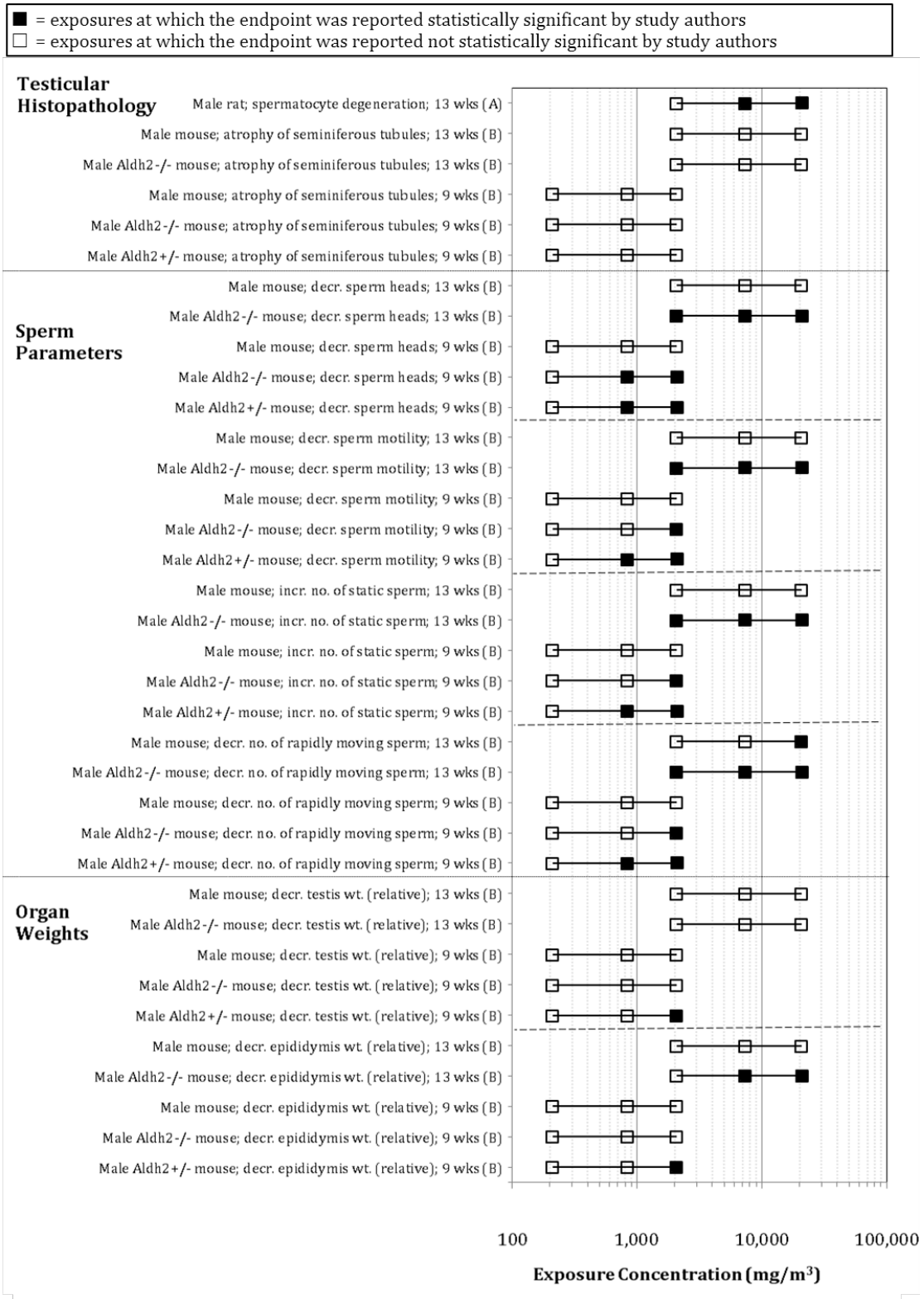
Reference and Study Design	Results			
Testosterone and Estradiol				
de Peyster et al. (2009) rat, Fischer 344 oral – gavage P0, male (12/group): 0, 600, 1,200, 1,800 mg/kg-d daily for 14 days	Dose (mg/kg-d)	N	Mean plasma testosterone (ng/ml) ± SE	% change from control
	0	12	2.07 ± 42	-
	600	12	3.1 ± 0.78	50
	1,200	11	2.61 ± 0.55	26
	1,800	10	1.36 ± 0.39	-34
	Dose (mg/kg-d)	N	Mean plasma estradiol (pg/ml)	% change from control
	0	12	1.085 ± 0.1	-
	600	12	1.395 ± 0.403	29
	1,200	11	2.238* ± 0.377	106
	1,800	9	2.224* ± 0.611	105

[a]4.18 mg/m^3 = 1 ppm.
[b]Conversion performed by study authors.
[c]Male mating index (%) = (No. males able to mate with at least one female / Total males) x 100.
[d]Male fertility index (%) = (No. males with at least one pregnant partner / Males that mated at least once) x 100
*: 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.
% change from control = [(treated group value –control value)/control value] x 100.
Absolute change from control (%) = control value (%) – treated group value (%).



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

Figure 1-11. Exposure-response array of male reproductive effects following oral exposure to ETBE.



Sources: (A) Medinsky et al., 1999; Bond et al., 1996b (B) Weng et al., 2014

Figure 1-12. Exposure-response array of male reproductive effects following inhalation exposure to ETBE.

Mechanistic Evidence

No mechanistic evidence for male reproductive effects was identified by the literature search.

Integration of Male Reproductive Effects

The male reproductive endpoints examined in this database were not consistently affected across studies or across doses in wildtype animals. The 13-week and 9-week inhalation studies conducted in rats and mice (Weng et al., 2014; Medinsky et al., 1999) provide suggestive evidence of ETBE-induced testicular degeneration and effects on sperm count, sperm mobility, and sperm DNA damage. In contrast, no male reproductive toxicity was observed in any of the other studies examined in this database, including one- and two-generation reproductive toxicity studies, 2-year carcinogenicity studies, and sub-chronic studies. For example, the 2-year inhalation carcinogenicity study (Saito et al., 2013; JPEC, 2010b) used the same rat strain, same route of exposure, and similar range of exposure concentrations as Medinsky et al. (1999) and did not observe any dose-related effects on testicular histopathology. Weng et al. (2014), however, found that *Aldh2* KO and heterozygous mice had consistently reduced numbers of sperm heads and sperm motility as well as reductions in male reproductive organ weights, suggesting that populations with ALDH2 polymorphisms could be more susceptible to effects from ETBE exposure (discussed in Section 1.3.3). The 14-day study by de Peyster et al. (2009) observed increased estradiol and decreased testosterone in ETBE-exposed rats, which is a potential mechanism for testicular degeneration; however, no effects on testicular histopathology or organ weight were observed in this study. Collectively, although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify male reproductive effects as a potential human hazard of ETBE, and male reproductive effects are not carried forward as a hazard. While the ALDH2 knock out data suggest a potential sensitive subpopulation for male reproductive effects, these findings are considered preliminary.

Synthesis of Effects Related to Female Reproduction

The available evidence for ETBE-induced effects on the female reproductive system includes no human data. The evidence was obtained primarily from a one-generation reproductive toxicity study (Fujii et al., 2010; JPEC, 2008e), a two-generation reproductive toxicity study (Gaoua, 2004b), and three developmental toxicity studies (Aso et al., 2014; Asano et al., 2011; JPEC, 2008h, i; Gaoua, 2004a). In addition, some evidence was obtained from two 90-day toxicity studies (JPEC, 2008b; Medinsky et al., 1999; Bond et al., 1996a), one subchronic (180-day) study (Miyata et al., 2013; JPEC, 2008c), two 2-year carcinogenicity studies (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b), and a short-term study evaluating ETBE-induced oocyte effects (Berger and Horner, 2003). These studies evaluated the effects of ETBE exposure on maternal body weight change (Aso et al., 2014; Asano et al., 2011; Fujii et al., 2010; JPEC, 2008e, h, i; Gaoua, 2004a, b), fertility, mating, and pregnancy parameters (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b; Berger and Horner,

2003), fecundity (Aso et al., 2014; Asano et al., 2011; Fujii et al., 2010; JPEC, 2008e, h, i; Gaoua, 2004a, b), estrous cyclicity (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b), and organ weights (Aso et al., 2014; Miyata et al., 2013; Saito et al., 2013; Suzuki et al., 2012; Asano et al., 2011; Fujii et al., 2010; JPEC, 2010a, b, 2008b, c, e, h, i; Gaoua, 2004b; Medinsky et al., 1999; Bond et al., 1996a). ETBE-induced effects were examined in pregnant rats and rabbits and non-pregnant female rats after oral or whole body inhalation exposures, and the design, conduct, and reporting of each study were of sufficient quality to inform human health hazard assessment. Selected female reproductive toxicity endpoints from these studies are summarized in Table 1-15.

The one- and two-generation reproductive toxicity studies and developmental studies evaluated maternal toxicity and several endpoints related to fertility, pregnancy, and pregnancy outcomes in rats and rabbits up to 1,000 mg/kg-day ETBE. Maternal toxicity, as shown by decreased maternal body weight and corrected (for the gravid uterus) body weight, was observed following gestational exposure to 1,000 mg/kg-day ETBE from GD 5–19; however, this effect was not observed in another developmental exposure study in which ETBE was administered at the same dose and exposure duration (Aso et al., 2014; JPEC, 2008h). Further, administration of ETBE during the pre-mating through lactation periods in parental and F1 generations (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b) did not affect maternal body weight parameters in rats. Maternal body weight during the entire pregnancy (GD 0–28) and corrected body weight change were decreased in rabbits administered 1,000 mg/kg-day ETBE (Asano et al., 2011; JPEC, 2008i); however, the lack of change in body weight during the treatment period (GD 6–27), the lack of a dose-related response, and the inherent variability in body weight parameters during pregnancy in rabbits (U.S. EPA, 1991b) limit the interpretation of this effect. ETBE did not affect indices of mating or fertility, and pre-coital times and gestation lengths were similarly unaffected in rats in the parental (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b) and the F1 generation (Gaoua, 2004b). In addition, the number of corpora lutea in pregnant rats and rabbits (Aso et al., 2014; Asano et al., 2011; JPEC, 2008h, i), the average estrous cycle length, and the percent of females with normal estrous cycles (Fujii et al., 2010; JPEC, 2008e) were not significantly affected by ETBE when compared to control values. Further supporting these findings, oocyte quality and fertilizability was shown to be unaffected by ETBE (Berger and Horner, 2003). Litter size was evaluated by Fujii et al. (2010), JPEC (2008e), Gaoua (2004b), Aso et al. (2014), JPEC (2008h), and Asano et al. (2011), JPEC (2008i), and no significant, dose-related effects were observed in rats or rabbits following ETBE exposure.

Reproductive organ weights were also reported after oral and inhalation exposures to ETBE. Gravid uterine weights were not affected following ETBE exposure during gestation in rabbits (Asano et al., 2011; JPEC, 2008i) nor were ovary and uterine weights affected after exposure during pre-mating through lactation periods in rats (Fujii et al., 2010; JPEC, 2008e). Consistent with these findings, ovary and uterine weights in non-pregnant females were not affected by ETBE after 90-day inhalation (JPEC, 2008b; Medinsky et al., 1999; Bond et al., 1996a), 180-day oral (Miyata et al., 2013; JPEC, 2008c), and 2-year oral (Suzuki et al., 2012; JPEC, 2010a) exposure assessments. In

a 2-year inhalation study in rats (Saito et al., 2013; JPEC, 2010b), however, a significant increase in relative (but not absolute) ovary weight was observed at exposures of 1,500 and 5,000 ppm ETBE. It is possible the finding of increased relative ovary weight was influenced by concurrent decreases in bodyweight (9-22%) at these exposures.

Table 1-15. Evidence pertaining to female reproductive effects in animals exposed to ETBE

Reference and study design	Results				
Maternal Body Weight					
Gaoua (2004a) rat, Sprague-Dawley oral – gavage P0, female (24/group): 0, 250, 500, 1,000 mg/kg-d dams exposed from GD 5 to GD 19	Dose (mg/kg-d)	Body wt change ± SD, GD 5-20 (g)	% change from control	Net body wt change ± SD (g)	% change from control
	0	132 ± 22	-	61.8 ± 13	-
	250	132 ± 12	-2	59.4 ± 8.1	-4
	500	134 ± 19	-1	60 ± 11.3	-3
	1,000	120* ± 15	-11	51.5* ± 10.3	-17
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d)	F0: Body wt change ± SD (g)	% change from control	F1: Body wt change ± SD (g)	% change from control
	0	132 ± 15	-	146 ± 21	-
	250	134 ± 14	2	145 ± 15	-1
	500	136 ± 25	3	141 ± 21	-3
	1,000	136 ± 12	3	137 ± 12	-6
Aso et al. (2014); JPEC (2008h) rat, Sprague-Dawley oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 5 to GD 19 C-section GD 20	Dose (mg/kg-d)	Body wt ± SD, GD 5 (g)	Body wt ± SD, GD 20 (g)	Body wt change ± SD, GD 5-20 (g)	% change from control
	0	280.9 ± 16.7	394.4 ± 26.9	113.5	-
	100	273.4 ± 10.8	380.3 ± 23.9	106.9	-6
	300	280 ± 13.4	389.8 ± 25.9	109.8	-3
	1,000	277.7 ± 15.9	382.4 ± 27.1	104.7	-8
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage	Dose (mg/kg-d)	F0: Body wt change ± SD, GD 5-20 (g)	% change from control		

Reference and study design	Results		
F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	0	124.9 ± 22	-
	100	119.6 ± 20.3	-4
	300	135.2 ± 21.5	8
	1,000	140.2* ± 19.1	12

Asano et al. (2011); JPEC (2008i) rabbit, New Zealand White oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 6 to GD 27 C-section GD 28	Dose (mg/kg-d)	Body wt change ± SD, GD 6-28 (kg)	% change from control	Body wt change ± SD, GD 0-28 (kg)	Adjusted body wt change ± SD (kg)	% change from control
	0	0.26 ± 0.12	-	0.40 ± 0.12	0.02 ± 0.14	-
	100	0.23 ± 0.12	-12	0.35 ± 0.12	-0.06 ± 0.12	-400
	300	0.28 ± 0.08	8	0.40 ± 0.08	0 ± 0.1	-100
	1,000	0.12 ± 0.19	-54	0.25* ± 0.21	-0.07 ± 0.19	-450

Fertility, Mating, and Pregnancy

Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	Dose (mg/kg-d)	Copulation index[c] (%)	Fertility index[d] (%)
	0	100	87.5
	100	95.8	100
	300	100	95.8
	1,000	100	91.7

Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d)	Pregnant/mated females, F0	Fertility index, F0 (%)	Pregnant/mated females, F1	Fertility index, F1 (%)
	0	23/25	92	22/25	88
	250	21/25	84	22/24	92
	500	22/25	88	22/25	88
	1,000	25/25	100	22/23	96

Aso et al. (2014); JPEC (2008h) rat, Sprague-Dawley	Dose (mg/kg-d)	Mean no. corpora lutea ± SD	% change from control

Reference and study design	Results		
oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 5 to GD 19 C-section GD 20	0	15.5 ± 1.54	-
	100	14.1 ± 1.48	-9
	300	14.4 ± 1.85	-7
	1,000	14.6 ± 2.44	-6

Reference and study design	Results				
Litter Size					
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	Dose (mg/kg-d) 0 100 300 1,000	Mean no. pups delivered ± SD (kg) 11.8 ± 3.2 10.4 ± 3.4 12.1 ± 2.3 13.0 ± 1.9	% change from control - -12 3 10		
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d) 0 250 500 1,000	Litter size at birth, F0 14.3 14.1 14.9 14.2	% change from control, F0 - -1 4 -1	Pregnant/mated females, F1 13.7 13.7 13.7 14	% change from control, F1 - 0 0 2
Aso et al. (2014); JPEC (2008h) rat, Sprague-Dawley oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 5 to GD 19 C-section GD 20	Dose (mg/kg-d) 0 100 300 1,000	Mean no. live fetuses ± SD (kg) 13.6 ± 1.5 12.0 ± 2.65 12.6 ± 2.58 12.3 ± 2.8	% change from control - -12 -7 -10		
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand White oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 6 to GD 27 C-section GD 28	Dose (mg/kg-d) 0 100 300 1,000	Mean no. live fetuses ± SD (kg) 7.8 ± 3.1 7.9 ± 3.2 8.4 ± 2.0 6.9 ± 3.2	% change from control - 1 8 -12		

Reference and study design	Results
Gestation Length	
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	Dose (mg/kg-d) / Mean gestation length ± SD (days) / % change from control 0 / 22.2 ± 0.4 / - 100 / 22.1 ± 0.4 / 0 300 / 22.2 ± 0.4 / 0 1,000 / 22.6 ± 0.5 / 2
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d) / Gestation length (days), F0 / % change from control, F0 / Gestation length (days), F1 / % change from control, F1 0 / 21.7 / - / 21.5 / - 250 / 21.5 / -1 / 21.6 / 0 500 / 21.5 / -1 / 21.6 / 0 1,000 / 21.8 / 0 / 21.6 / 0
Estrous Cyclicity	
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	Dose (mg/kg-d) / % Females w/normal estrous cycles, F0 / Mean estrous cycle length ± SD (days) / % change from control 0 / 91.7 / 4.03 ± 0.09 / - 100 / 97.1 / 4.1 ± 0.29 / 2 300 / 97.1 / 4.06 ± 0.17 / 1 1,000 / 95.8 / 4.29 ± 0.61 / 6
Organ Weights	
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	**Absolute Weight** Dose (mg/kg-d) / Mean ovary wt ± SD (mg) / % change from control / Mean uterus wt ± SD (mg) / % change from control 0 / 98.8 ± 14.9 / - / 468 ± 68 / - 100 / 92.5 ± 16.6 / -6 / 513 ± 151 / 10 300 / 95.3 ± 11.1 / -4 / 523 ± 157 / 12 1,000 / 100.9 ± 16.9 / 2 / 516 ± 136 / 10

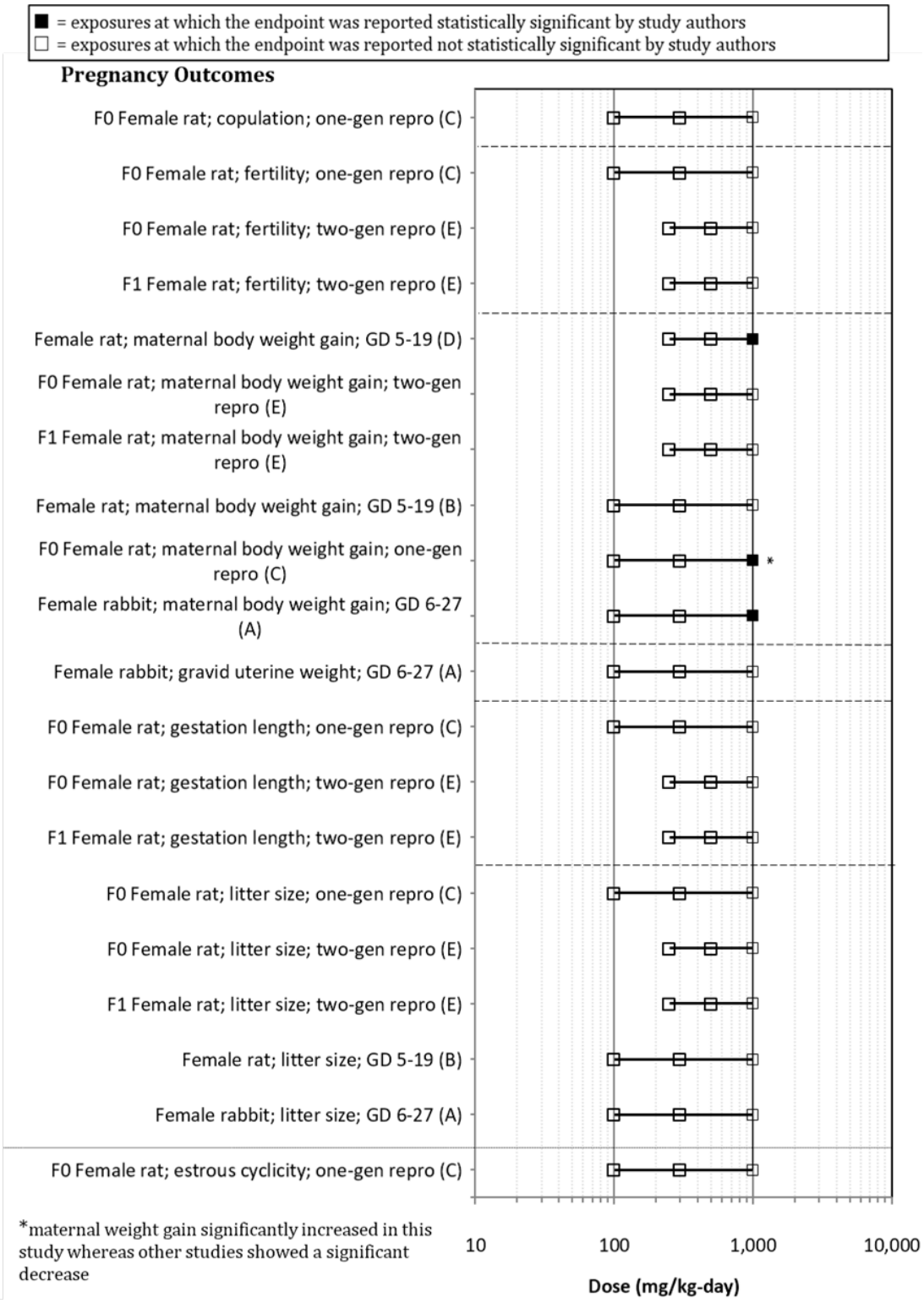
Reference and study design	Results
Fujii et al. (2010); JPEC (2008e) (continued)	**Relative Weight** Dose (mg/kg-d) / Mean ovary wt ± SD (mg) / % change from control / Mean uterus wt ± SD (mg) / % change from control 0 / 30.7 ± 4.7 / - / 146 ± 27 / - 100 / 28.6 ± 6 / -7 / 158 ±49 / 8 300 / 29.3 ± 3.6 / -5 / 162 ± 53 / 11 1,000 / 29.9 ± 4.9 / -3 / 154 ± 46 / 5
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (19-25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (19–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d) / Mean ovary Wt. ± SD (g) / % change from control / Mean uterus Wt. ± SD (g) / % change from control **Absolute Weight, F0** 0 / 0.168 ± 0.025 / - / 0.54 ± 0.096 / - 250 / 0.167 ± 0.027 / -1 / 0.587 ± 0.231 / 9 500 / 0.167 ± 0.022 / -1 / 0.483 ± 0.102 / -11 1,000 / 0.164 ± 0.023 / -2 / 0.576 ± 0.218 / 7 **Absolute Weight, F1** 0 / 0.164 ± 0.027 / - / 0.557 ± 0.13 / - 250 / 0.172 ± 0.028 / 5 / 0.577 ± 0.161 / 4 500 / 0.168 ± 0.031 / 2 / 0.538 ± 0.141 / -3 1,000 / 0.163 ± 0.049 / -1 / 0.547 ± 0.122 / -2
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation – vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk	Dose (mg/m^3) / Mean ovary wt ± SD (g) / % change from control 0 / 0.085 ± 0.022 / - 2,090 / 0.095 ± 0.016 / 12 7,320 / 0.088 ± 0.12 / 4 20,900 / 0.090 ± 0.19 / 6

Reference and study design	Results		
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand White oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 6 to GD 27 C-section GD 28	Dose (mg/kg-d)	Gravid uterus wt ± SD (g)	% change from control
	0	383 ± 98	-
	100	398 ± 128	4
	300	403 ± 91	5
	1,000	323 ± 128	-16
Miyata et al. (2013); JPEC (2008c) rat, Sprague-Dawley oral – gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 days	Dose (mg/kg-d)	Mean absolute ovary wt ± SD (mg)	% change from control
	0	70.0 ± 18.7	-
	5	71.0 ± 21.7	1
	25	73.8 ± 16.6	5
	100	67.7 ± 17.7	-3
	400	76.6 ± 18.2	9
	Dose (mg/kg-d)	Mean relative ovary wt ± SD (mg/100g)	% change from control
	0	20.4 ± 5.4	-
	5	21.4 ± 5	5
	25	21.8 ± 4.8	7
	100	20.0 ± 4.9	-2
	400	22.8 ± 5.5	12

Reference and study design	Results					
JPEC (2008b) rat, Sprague-Dawley inhalation – vapor male (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a]; female (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration, and method reported	Dose (mg/m^3)	N	Mean ovary wt ± SD (mg)	% change from control	Mean uterus wt ± SD (g)	% change from control
	Absolute Weight, Day 92					
	0	10	91.47 ± 10.26	-	0.709 ± 0.222	-
	627	10	87.36 ± 15.83	0	0.819 ± 0.38	16
	2,090	10	84.92 ± 16.91	0	0.654 ± 0.159	-8
	6,270	10	78.39 ± 9.83	0	0.712 ± 0.198	0
	20,900	10	91.94 ± 21.84	0	0.702 ± 0.205	-1
	Absolute Weight, Day 120					
	0	6	82.82 ± 17.89	-	0.965 ± 0.332	-
	627	-	-	-	-	-
	2,090	-	-	-	-	-
	6,270	-	-	-	-	-
	20,900	6	90.38 ± 15.88	9	0.818 ± 0.286	-15
	Relative Weight, Day 92					
	0	10	27.19 ± 3.8	-	0.21 ± 0.066	-
	627	10	27.58 ± 4.35	1	0.269 ± 0.151	28
	2,090	10	27.03 ± 4.55	0	0.211 ± 0.055	0
	6,270	10	25 ± 2.67	-6	0.228 ± 0.061	9
	20,900	10	30.39 ± 6.46	9	0.231 ± 0.071	10
	Relative Weight, Day 120					
	0	6	25.02 ± 4.03	-	0.298 ± 0.107	-
	627	-	-	-	-	-
	2,090	-	-	-	-	-
	6,270	-	-	-	-	-
	20,900	6	26.72 ± 4.79	7	0.24 ± 0.089	-19

Reference and study design	Results		
JPEC (2010a) rat, Fischer 344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[b]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[b] daily for 104 wk	Dose (mg/kg-d)	Mean ovary wt ± SD (g)	% change from control
	0	0.194 ± 0.238	-
	46	0.18 ± 0.146	-7.21649
	171	0.153 ± 0.035	-21.134
	560	0.147 ± 0.023	-24.2268

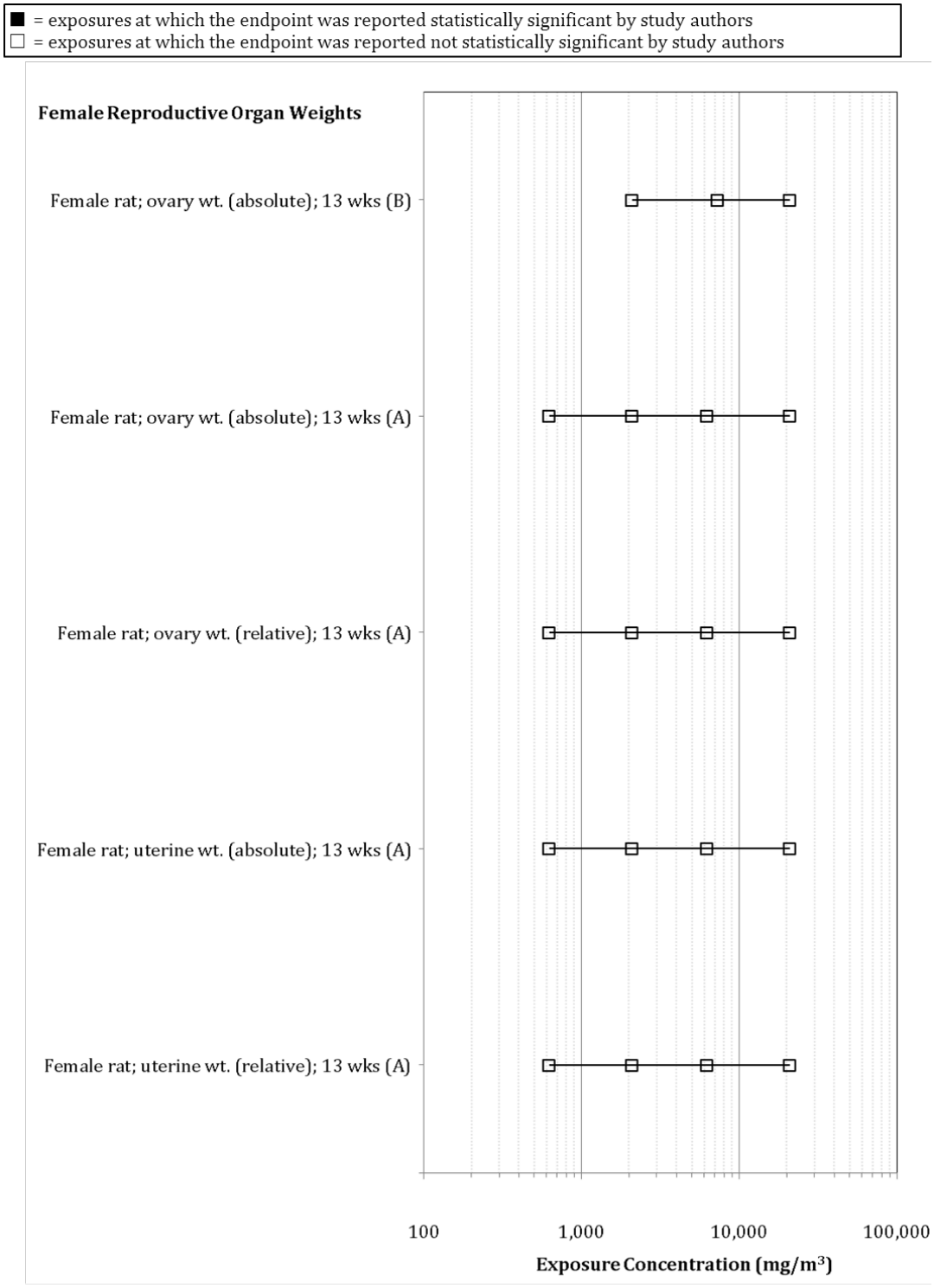
[a]4.18 mg/m^3 = 1 ppm.
[b]Conversion performed by study authors.
[c]Copulation index (%) = (no. of rats with successful copulation/no. of rats paired) x 100.
[d]Fertility index (%) = (no. females pregnant or no. of males sired/no. of rats with successful copulation) x 100.
*: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
% change from control = (control value – treated group value)/control value] x 100.
Absolute change from control (%) = control value (%) – treated group value (%).



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

Figure 1-13. Exposure-response array of female reproductive effects following oral exposure to ETBE.

1



Source: (A) JPEC 2008b (B) Medinsky et al., 1999; Bond et al., 1996b

2

3 **Figure 1-14. Exposure-response array of female reproductive effects following**
4 **inhalation exposure to ETBE.**

Mechanistic Evidence

No mechanistic evidence for female reproductive effects was identified by the literature search.

Integration of Female Reproductive Effects

The available evidence to assess female reproductive effects consists of one- and two-generation reproductive toxicity studies, developmental toxicity studies, and 90-day through 2-year oral and inhalation exposure studies that adequately evaluate the relevant female reproductive endpoints. These studies show that ETBE does not adversely affect maternal body weight gain, fertility, mating, pregnancy parameters, or reproductive organ weights in all but one study up to 1,000 mg/kg-day (oral exposure) or 5,000 ppm (whole body inhalation exposure) in the female rat or rabbit. Relative, but not absolute ovary weights were significantly increased following ETBE inhalation exposure in one 2-year study but not observed in other 2-year, 180-/90-day, reproductive, or developmental studies. Collectively, although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify female reproductive effects as a potential human hazard of ETBE, and female reproductive effects are not carried forward as a hazard.

1.2.4. Developmental Effects

Synthesis of Effects Related to Development

The database examining developmental effects following ETBE exposure includes no human data; it is composed of data from toxicology studies conducted in Sprague-Dawley rats or New Zealand White rabbits in which ETBE was administered via oral gavage. These consisted of three prenatal developmental toxicity studies [two in rats: (Aso et al., 2014; JPEC, 2008h) and (Gaoua, 2004a) and one in rabbits: (Asano et al., 2011; JPEC, 2008i)], a one-generation reproductive toxicity study in rats (Fujii et al., 2010; JPEC, 2008e), and a two-generation reproductive toxicity study in rats (Gaoua, 2004a). The design, conduct, and reporting of all five studies were of sufficient quality to inform human health hazard assessment. The highest dose level tested in each study was 1,000 mg/kg-d, the recommended limit dose for prenatal developmental toxicology studies (OECD, 2001; U.S. EPA, 1998c).

Developmental endpoints evaluated after ETBE exposure include prenatal and postnatal survival, growth, and morphological development. In addition, limited assessments of postnatal neurological functional development were conducted. Selected developmental toxicity data are summarized in

Table 1-16.

Evidence of effects of ETBE treatment on pre- or postnatal survival was minimal. In the developmental toxicity study in rats by (Aso et al., 2014; JPEC, 2008h), increased preimplantation loss was observed in the treated groups. The percent preimplantation loss in the 1,000 mg/kg-day dams was 81.8% greater than control, while it was increased 37.9% at 100 mg/kg-day and 21.2% at 300 mg/kg-day. Statistical significance was not reported. Increased preimplantation loss was not observed in the other available developmental toxicity studies in rats or rabbits [(Gaoua, 2004a) and (Asano et al., 2011; JPEC, 2008i), respectively]. Postnatal survival was not affected by ETBE treatment in either the first or second generation of the reproductive toxicity study by Gaoua (2004b). Viability indices throughout the lactation period were similar between control and treated groups during both generations of this study. In the one-generation reproductive toxicity study (Fujii et al., 2010; JPEC, 2008e), there was evidence of a non-significant decrease (10.5% as compared to control) in the PND 4 viability index at 1,000 mg/kg-day. Examination of the individual animal data indicated that total litter loss in three litters had resulted in the majority of pup deaths that occurred from PND 0–4. For two of these litters, severe maternal toxicity had led to moribund sacrifice of the dams in early lactation; this is the only evidence in the available ETBE data where adverse outcomes in the offspring were definitively associated with maternal toxicity. The third dam with total litter loss had no evidence of treatment-related toxicity.

Neither prenatal nor postnatal growth were affected by ETBE treatment. Mean fetal weights were comparable between control and ETBE-treated groups in the prenatal developmental toxicity studies in rats and rabbits (Aso et al., 2014; Asano et al., 2011; JPEC, 2008h, i; Gaoua, 2004a). Similarly, pup weights from PND 0–21 were not affected by treatment in the reproductive toxicity studies (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b). Additionally, (Fujii et al., 2010; JPEC, 2008e) no effects were observed in the rate of completion of development landmarks in male and female F1 offspring, specifically pinna detachment on PND 3, incisor eruption on PND 11, and eye opening on PND 15. Organ weights (brain, spleen, and thymus) were evaluated in PND 21 pups in the one- and two-generation reproduction studies (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b); no significant differences were observed between control and treated groups (not shown in evidence table). At the termination of adult animals in the reproductive toxicity studies, a number of organ weights were measured. Sections 1.2.1 and 1.2.2 discuss increased mean kidney and liver weights, respectively, observed in the adult F1 offspring of the two-generation reproduction study (Gaoua, 2004b). The findings in the F1 adults were similar to those in the P adults, indicating an absence of life stage-related susceptibility for these outcomes.

No evidence existed of treatment-related effects on postnatal morphological assessments that consisted of PND 1 anogenital distance measurements in F1 and F2 pups (Gaoua, 2004b) and the age of F1 sexual maturation (preputial separation in males and vaginal opening in females) (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b).

In the prenatal developmental toxicity studies with ETBE (Aso et al., 2014; Asano et al., 2011; JPEC, 2008h, i; Gaoua, 2004a), the evidence of treatment-related alterations in fetal development at 1,000 mg/kg-day were sporadic, and there was no consistent pattern of effect.

In Aso et al. (2014), a >3-fold increase in the number and percent of rat fetuses with skeletal variations was noted at 1,000 mg/kg-day compared to control. Examination of the individual litter data revealed that this increase was primarily attributable to a statistically significant >6-fold increase in the number of fetuses (and >3-fold increase in the number of litters) with rudimentary lumbar rib at that dose. The study authors dismissed the relevance of this finding, reporting that it is within a historical control range (1.1–21.2%) for the strain of rat used in the study and because the effect has sometimes been viewed as transient [e.g., (Chernoff et al., 1991)]. Nevertheless, the incidence of this finding is significantly increased as compared to the concurrent control, which is considered more relevant and preferable to historical control and the finding might have been the result of an alteration of vertebral development; therefore, it is considered potentially treatment-related.

In Gaoua (2004a), a statistically significant 37% increase in the number of fetuses with unossified 4th metacarpal as compared to control was observed at 1,000 mg/kg-day. Further evaluation of the fetuses, which were double-stained with alcian blue, revealed that a cartilage precursor was present, suggesting that the finding represented a treatment-related delay in development rather than a malformation.

An increase in the number of rabbit fetuses and litters with visceral malformations at 1,000 mg/kg-day was noted in Asano et al. (2011) and JPEC (2008i). This was specifically attributed to observations of fetuses with absent right atrioventricular valve of the heart. The incidences of this finding did not achieve statistical significance. Also in Asano et al. (2011) and JPEC (2008i), a 66% increase in the number of rabbit fetuses with skeletal variations at 1,000 mg/kg-day as compared to control was found to be primarily attributed to incidences of unossified talus (in 12 fetuses, 6 litters).

Limited evaluation of postnatal functional neurological development in F1 male and female offspring in reproductive toxicity studies were conducted by Fujii et al. (2010), JPEC (2008e), and Gaoua (2004b). No treatment-related effects were found in assessments of reflex ontogeny, which included surface righting reflex on PND 5 (Fujii et al., 2010; JPEC, 2008e; Gaoua, 2004b), negative geotaxis on PND 8 (Fujii et al., 2010; JPEC, 2008e), cliff avoidance on PND 11 (Gaoua, 2004b), and air righting reflex on PND 17 (Gaoua, 2004b) or PND 18 (Fujii et al., 2010; JPEC, 2008e). Gaoua (2004b) also conducted tests in F1 males and females of acoustic startle response [postnatal week (PNW) 4], pupil constriction (PNW 4), and motor activity (PNW 7 and 8). The motor activity testing was performed using an automated device that measured the number of movements within the front or back of the cage, back and forth movements, and vertical movements. Two 10-minute trials were conducted 1 week apart. No treatment-related effects were found.

Table 1-16. Evidence pertaining to developmental effects in animals following exposure to ETBE

Reference and study design	Results
Prenatal Survival	
Aso et al. (2014); JPEC (2008h) rat, Sprague-Dawley oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 5 to GD 19 C-section GD 20	Dose (mg/kg-d) / No. Litters / No. preimplan-tation loss / % change from control / % Preimplan-tation loss[a] / % change from control 0 / 21 / 22 / - / 6.6 / - 100 / 22 / 25 / 13.6 / 9.1 / 37.9 300 / 20 / 25 / 13.6 / 8.0 / 21.2 1,000 / 22 / 39 / 77.3 / 12.0 / 81.8 Dose (mg/kg-d) / No. resorptions / % Postimplantation loss[b] 0 / 18 / 5.8 100 / 22 / 7.2 300 / 12 / 4.2 1,000 / 13 / 5
Gaoua (2004a) rat, Sprague-Dawley oral – gavage female (24/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from GD 5 to GD 19 C-section GD 20	Dose (mg/kg-d) / No. Litters / No. preimplantation loss / % Preimplantation loss[a] 0 / 21 / 48 / 17.8 250 / 19 / 36 / 14.9 500 / 20 / 38 / 14.3 1,000 / 22 / 47 / 16.8 Dose (mg/kg-d) / No. Postimplantation loss / % Postimplantation loss[b] 0 / 14 / 5.2 250 / 16 / 6.6 500 / 19 / 7.2 1,000 / 21 / 7.5
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand White oral – gavage	Dose (mg/kg-d) / No. litters / % Preimplantation loss[a] / % Postimplantation loss[b] 0 / 22 / 19.6 / 11.0

Reference and study design	Results			
female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 6 to GD 27 C-section GD 28	100	22	15.3	11.3
	300	20	10.7	7.0
	1,000	23	22.9	8.7

Reference and study design	Results
Postnatal Survival	
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	Dose (mg/kg-d) / Viability index PND 0 ± SD / Viability index PND 4 ± SD / % change from control (PND 4) / Total litter loss (PND 0-4)[c] 0 / 98.9 ± 3.7 / 97.4 ± 4.7 / - / 0 100 / 97.9 ± 5.6 / 96.7 ± 8.1 / -0.7 / 0 300 / 99.5 ± 2.6 / 99.6 ± 1.9 / 2.3 / 0 1,000 / 93.6 ± 15.5 / 87.2 ± 29.8 / -10.5 / 3 Dose (mg/kg-d) / Viability Index - PND 21 ± SD 0 / 97 ± 11.1 100 / 95.8 ± 11.4 300 / 95.7 ± 11.1 1,000 / 92.5 ± 23.1
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d) / Viability index PND 0 / Viability index PND 4 / Total litter loss (PND 0-4) / Viability index PND 21 *F1* 0 / 100 / 97.6 / 0 / 94.6 250 / 100 / 92.9 / 1 / 91.7 500 / 100 / 82.3 / 0 / 96.1 1,000 / 100 / 97.7 / 1 / 99.5 *F2* 0 / 100 / 97.6 / 0 / 97.6 250 / 100 / 94.8 / 0 / 98.8 500 / 100 / 97.0 / 3 / 100 1,000 / 100 / 92.9 / 0 / 99.3
Prenatal Growth	
Aso et al. (2014); JPEC (2008h) rat, Sprague-Dawley oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 5 to GD 19 C-section GD 20	Dose (mg/kg-d) / No. litters / Mean fetal weight ± SD male (g) / Mean fetal weight ± SD female (g) 0 / 21 / 4.1 ± 0.3 / 3.89 ± 0.25 100 / 22 / 4.14 ± 0.33 / 3.92 ± 0.23 300 / 20 / 4.23 ± 0.22 / 4.01 ± 0.22 1,000 / 22 / 4.14 ± 0.34 / 3.91 ± 0.39

Reference and study design	Results			
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d)	No. litters	Mean fetal weight ± SD male (g)	Mean fetal weight ± SD female (g)
	0	21	3.92 ± 0.58	3.77 ± 0.5
	250	19	4.03 ± 0.32	3.82 ± 0.33
	500	20	3.94 ± 0.35	3.75 ± 0.32
	1,000	22	3.91 ± 0.33	3.66 ± 0.39
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand White oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 6 to GD 27 C-section GD 28	Dose (mg/kg-d)	No. litters	Mean fetal weight ± SD male (g)	Mean fetal weight ± SD female (g)
	0	22	33.5 ± 4.1	31.5 ± 3.7
	100	22	33.4 ± 6.2	31.5 ± 4.8
	300	20	33.9 ± 2.5	32.0 ± 3.6
	1,000	23	32.3 ± 6.5	30.1 ± 6.0
Postnatal Growth				
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	Dose (mg/kg-d)	No. litters	Mean ± SD PND 0 (g)	Mean ± SD PND 4 precull (g)	Mean ± SD PND 21 (g)
	F1-Male Pup Weight				
	0	21	6.9 ± 0.7	11.0 ± 2.0	61.3 ± 6.3
	100	22	6.9 ± 0.8	11.0 ± 1.8	61.0 ± 7.0
	300	23	6.9 ± 0.6	10.8 ± 1.4	61.6 ± 4.6
	1,000	22	7.0 ± 0.7	10.4 ± 1.7	61.6 ± 6.4
	F1-Female Pup Weight				
	0	21	6.5 ± 0.7	10.4 ± 1.8	59.3 ± 6.4
	100	22	6.5 ± 0.6	10.4 ± 1.6	58.5 ± 6.4
	300	23	6.5 ± 0.6	10.2 ± 1.4	58.5 ± 6.4
	1,000	22	6.6 ± 0.6	10.0 ± 1.8	59.7 ± 5.2

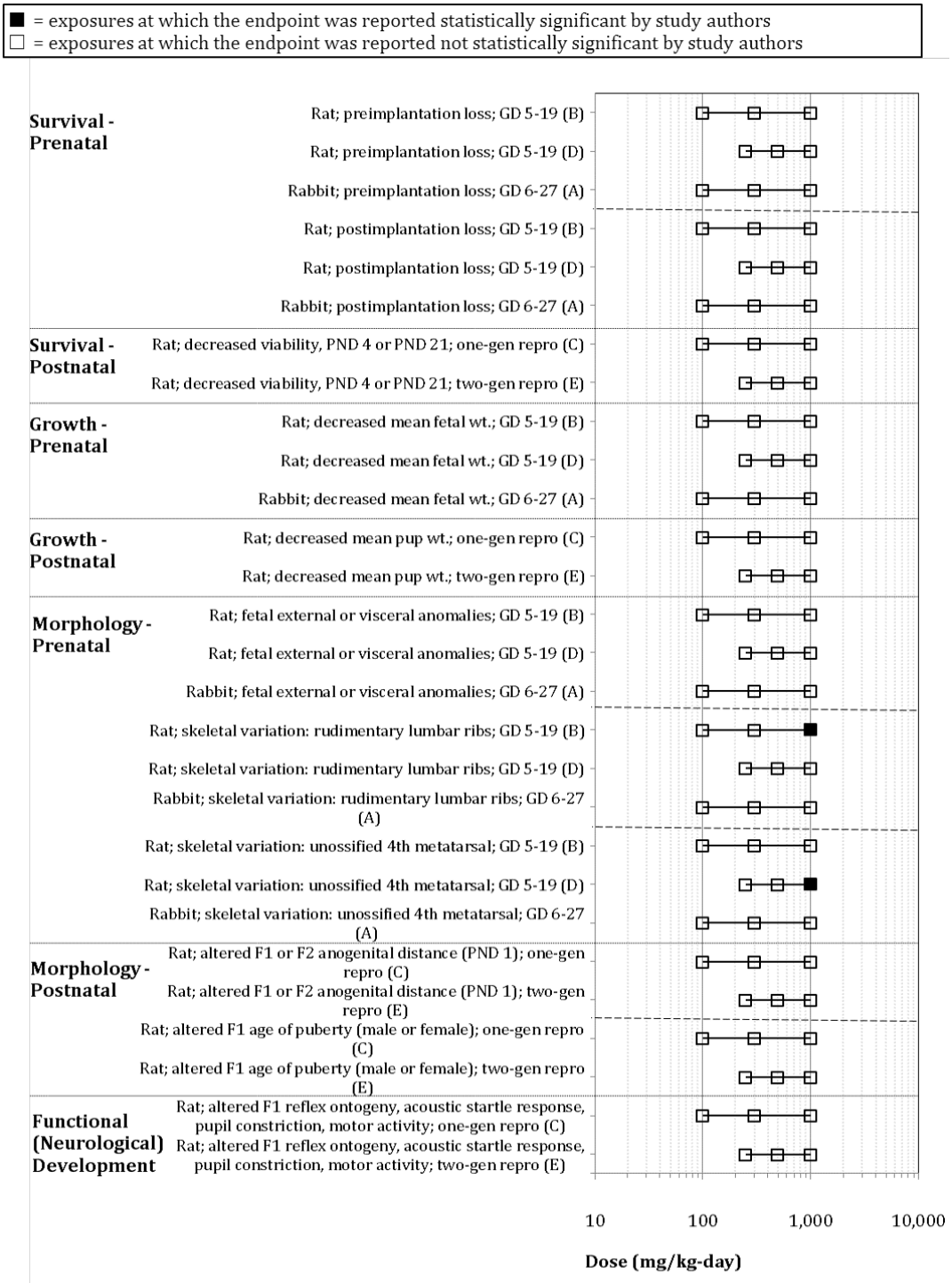
Reference and study design	Results			
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d)	Mean ± SD PND 1 (g)	Mean ± SD PND 4 precull (g)	Mean ± SD PND 21 (g)
		F1-Male Pup Weight		
	0	6.8 ± 0.7	9.1 ± 1.4	50.1 ± 4.9
	250	6.7 ± 0.6	9.0 ± 1.6	51.7 ± 4.1
	500	6.5 ± 0.7	8.7 ± 1.3	50.5 ± 6.7
	1,000	7.0 ± 0.7	9.3 ± 1.2	52.4 ± 4.5
		F1-Female Pup Weight		
	0	6.4 ± 0.6	8.6 ± 1.4	48.1 ± 6.1
	250	6.4 ± 0.6	8.5 ± 1.6	49.5 ± 4.3
	500	6.0 ± 0.6	8.1 ± 1.2	48.2 ± 5.9
	1,000	6.5 ± 0.6	8.9 ± 1.2	50.6 ± 4.4
		F2-Male Pup Weight		
	0	6.9 ± 0.6	9.5 ± 1.5	51.5 ± 7.2
	250	6.7 ± 0.6	9.3 ± 1.0	52.1 ± 4.4
	500	6.4 ± 0.5	9.2 ± 1.0	50.3 ± 5.8
	1,000	6.3 ± 0.6	9.2 ± 1.4	51.2 ± 3.6
		F2-Female Pup Weight		
	0	6.5 ± 0.6	8.9 ± 1.3	49.6 ± 6.2
	250	6.3 ± 0.6	8.8 ± 1.0	49.9 ± 3.6
	500	6.4 ± 0.5	8.9 ± 0.9	49.0 ± 5.5
	1,000	6.3 ± 0.6	8.7 ± 1.4	49.1 ± 3.7
Prenatal Morphology				
Aso et al. (2014); JPEC (2008h) rat, Sprague-Dawley oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 5 to GD 19 C-section GD 20	Dose (mg/kg-d)	No. fetuses (litters)[d]	No. fetuses examined for visceral anomalies	No. fetuses with visceral malformations	No. fetuses with visceral variations
	0	285(21)	146	3(3)	6(6)
	100	263(22)	137	2(2)	8(7)
	300	251(20)	132	2(2)	4(4)
	1,000	270(22)	139	0	8(7)

Reference and study design	Results
Aso et al. (2014); JPEC (2008h) (continued)	Dose (mg/kg-d) / No. fetuses examined for skeletal anomalies / No. fetuses with skeletal malformations / No. fetuses with skeletal variations / % fetuses (litters) with skeletal variations 0 / 139 / 0 / 9(8) / 6.5(38.1) 100 / 126 / 0 / 3(3) / 2.4(13.6) 300 / 119 / 0 / 3(3) / 2.5(15.0) 1,000 / 131 / 0 / 29(13) / 22.1(59.1) Dose (mg/kg-d) / No. fetuses (litters) with rudimentary lumbar rib / % fetuses (litters) with rudimentary lumbar rib 0 / 4(4) / 2.9(19.0) 100 / 0 / 0(0) 300 / 2(2) / 1.7(10.0) 1,000 / 25*(11) / 19.1*(50.0)
Gaoua (2004a) rat, Sprague-Dawley oral – gavage female (24/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from GD 5 to GD 19 C-section GD 20	Dose (mg/kg-d) / No. fetuses (litters)[d] / No. fetuses with external malformations / No. fetuses examined for visceral anomalies / No. fetuses with visceral malformations 0 / 255(21) / 0 / 120 / 0 250 / 226(19) / 1(1) / 109 / 0 500 / 246(20) / 0 / 116 / 0 1,000 / 258(22) / 0 / 122 / 1(1) Dose (mg/kg-d) / No. fetuses with visceral variations / No. fetuses examined for skeletal anomalies / No. fetuses with skeletal malformations / No. fetuses with skeletal variations 0 / 1(1) / 135 / 1(1) / 125(21) 250 / 2(2) / 117 / 2(2) / 101(19) 500 / 1(1) / 130 / 1(1) / 116(20) 1,000 / 3(3) / 136 / 2(2) / 112(22) No. fetuses with unossified 4th metacarpal / % change from control / % fetuses with unossified 4th metacarpal 0 / 27(9) / - / 20.0(42.9) 250 / 21(10) / -22.2 / 17.9(52.6) 500 / 24(9) / -11.1 / 18.5(45.0) 1,000 / 43*(12) / 37.2 / 31.6(54.5)

Reference and study design	Results
Asano et al. (2011); JPEC (2008i) rabbit, New Zealand White oral – gavage female (24/group): 0, 100, 300, 1,000 mg/kg-d dams dosed daily from GD 6 to GD 27 C-section GD 28	Dose (mg/kg-d) / No. fetuses (litters)[d] / No. fetuses with external malformations / No. fetuses with visceral malformations / No. fetuses with skeletal malformations 0 / 171(22) / 0 / 1(1) / 5(4) 100 / 174(22)[e] / 1(1) / 1(1) / 4(4) 300 / 167(20) / 0 / 1(1) / 3(2) 1,000 / 159(23)[e] / 1(1) / 3(2) / 8(5) Dose (mg/kg-d) / No. fetuses with skeletal variations / Absent right atrioventricular valve / % change from control 0 / 9(7) / 0 / - 100 / 11(9) / 0 / 0(0) 300 / 6(6) / 1(1) / 0.6(5.0) 1,000 / 15(8) / 3(2) / 1.9(8.7)
Postnatal Morphology	
Fujii et al. (2010); JPEC (2008e) rat, Sprague-Dawley oral – gavage F0, male and female (24/sex/group): 0, 100, 300, 1,000 mg/kg-d; dosed daily for 17 wks, from 10 wks premating to lactation day 21	Dose (mg/kg-d) / No. litters / Male preputial separation - age (days) mean ± SD / Female vaginal opening - age (days) mean ± SD *F1* 0 / 21 / 41.0 ± 1.7 / 31.2 ± 1.4 100 / 22 / 41.4 ± 1.1 / 30.9 ± 1.7 300 / 23 / 40.6 ± 1.5 / 30.5 ± 2.2 1,000 / 19 / 41.2 ± 1.6 / 30.3 ± 2.1
Gaoua (2004b) rat, Sprague-Dawley oral – gavage F0, male and female (25/sex/group): 0, 250, 500, 1,000 mg/kg-d dosed daily for 18 wks from 10 wks premating until weaning of F1 pups F1, male and female (24–25/group): 0, 250, 500, 1,000 mg/kg-d dosed daily from PND 22 until weaning of F2 pups	Dose (mg/kg-d) / No. litters / Anogenital distance[f] - males (PND 1) mean ± SD / Anogenital distance[f] - females (PND 1) mean ± SD *F1* 0 / 21 / 2.48 ± 0.18 / 1.53 ± 0.18 250 / 22 / 2.45 ± 0.17 / 1.5 ± 0.14 500 / 23 / 2.4 ± 0.21 / 1.45 ± 0.14 1,000 / 20 / 2.43 ± 0.15 / 1.44 ± 0.2 *F2* 0 / 21 / 2.41 ± 0.18 / 1.51 ± 0.18 250 / 22 / 2.42 ± 0.25 / 1.47 ± 0.19 500 / 23 / 2.42 ± 0.23 / 1.51 ± 0.17 1,000 / 20 / 2.45 ± 0.21 / 1.57 ± 0.22

Reference and study design	Results			
Gaoua (2004b) (continued)	Dose (mg/kg-d)	No. litters	Male preputial separation - age (days) - mean ± SD	Female vaginal opening - age (days) - mean ± SD
		F1		
	0	25	35 ± 2	34 ± 3
	250	25	34 ± 2	34 ± 3
	500	25	35 ± 2	35 ± 2
	1,000	25	35 ± 2	33 ± 2

[a]Percent preimplantation loss = (no. preimplantation embryonic loss/no. corpora lutea) x100.
[b]Percent postimplantation loss = (no. resorptions and dead fetuses/no. implantations) x100.
[c]Two 1,000 mg/kg-d dams were killed in a moribund condition on PND 2 and 4, thus compromising the survival of their litters. In a third litter, all pups died between PND 1-4 although there was no evidence of maternal toxicity throughout the study.
[d]The parenthetical number following fetal incidence indicates the associated litter incidence for all findings.
[e]No. of fetuses examined for visceral and skeletal anomalies at 100 and 1,000 mg/kg-d were 173 and 158, respectively, because fetuses with external malformations were excluded.
[f]AGD/cube root of body weight.
*: 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.
% change from control = (control value – treated group value)/control value] x 100.
Absolute change from control (%) = control value (%) – treated group value (%).



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

Figure 1-15. Exposure-response array of developmental effects following oral exposure to ETBE.

Mechanistic Evidence

No mechanistic evidence for developmental effects was identified by the literature search

Integration of Developmental Effects

The evidence to assess developmental toxicity for ETBE consists of two prenatal developmental toxicity studies in rats and one in rabbits, a one-generation reproductive toxicity study in rats, and a two-generation reproductive toxicity study in rats. These studies included assessments of pre- and postnatal survival, growth, morphology, and functional neurological development following oral (gavage) administration during sensitive periods of development. Slight evidence of effects of ETBE treatment on prenatal or postnatal survival consisted of preimplantation loss in a developmental toxicity study in rats and decreased PND 0–4 pup viability that was associated with severe maternal toxicity. Pre- and postnatal growth (body weights and developmental landmarks), anogenital distance, sexual maturation, and evaluation of neurological function (including reflex ontogeny and assessments of acoustic startle response, pupil constriction, and motor activity in offspring) were not affected by treatment. Evidence of incidental structural (visceral and skeletal) fetal anomalies following in utero exposures to ETBE were observed at the highest dose tested (1,000 mg/kg-day). The findings were limited to increased incidences of rudimentary lumbar rib (Aso et al., 2014; JPEC, 2008h) and unossified 4th metatarsal (Gaoua, 2004b) in two rat studies and unossified talus and absent right atrioventricular valve in a rabbit study (Asano et al., 2011; JPEC, 2008i). The fetal, but not litter, incidences of skeletal findings in rats (rudimentary lumbar rib and unossified 4th metatarsal) were statistically significant at the highest dose tested (1,000 mg/kg-day). These skeletal observations were not confirmed in other species. No inhalation prenatal developmental or reproductive toxicity studies were conducted, thus potential effects of inhalation exposure on pre- and postnatal development have not been characterized. Overall, the available evidence is considered inadequate to draw conclusions regarding the development toxicity of ETBE, and developmental effects are not carried forward as a hazard.

1.2.5. 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. The evidence pertaining to tumorigenicity in the kidney and liver was previously discussed in Sections 1.2.1 and 1.2.2, respectively. The database for ETBE carcinogenicity consists of only animal data: three 2-year studies (two oral, one inhalation), and two "initiation, promotion" cancer bioassays 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 1-17, Table 1-18; Figure 1-16, Figure 1-17). Interpretation of the study results reported by Maltoni et al. (1999) is complicated by

the nonstandard histopathological diagnoses used and the greater than expected mortality in treated groups and controls compared with other laboratories. Survival was reduced at the highest exposure in males and females after 72 weeks (data not shown) and after 104 weeks, survival in the 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). The survival data in this study was potentially attributable to chronic respiratory infections in the controls and treated groups (Malarkey and Bucher, 2011), ultimately limiting the ability of this study to predict potential carcinogenicity.

In response to the concerns regarding high mortality and the utilization of nonstandard histopathological diagnoses, a pathology working group sponsored by EPA and the National Toxicology Program (NTP) reviewed the histopathological data (Malarkey and Bucher, 2011). In addition to recalculating tumor incidences, the working group found that the respiratory infections in the study animals confound interpretation of leukemia and lymphoma. Thus, the Malarkey and Bucher (2011) data were used when considering carcinogenicity in place of the published Maltoni et al. (1999) study, and leukemia and lymphoma incidences from this study were not considered.

Following 2-year exposure to ETBE, the incidence of leiomyomas was increased in the uterus of Sprague-Dawley 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. These neoplasms arise from nervous tissue and are not specific to uterine tissue. Leiomyomas and a carcinoma were observed in uterine/vaginal tissue, but no significant trend was observed (Malarkey and Bucher, 2011).

Several initiation-promotion studies have been conducted with ETBE {Hagiwara, 2011, 1248019;Hagiwara, 2015, 3046107;Hagiwara, 2013, 2321105}. While chronic cancer bioassays are considered key data for the evaluation of carcinogenicity, other types of studies, such as initiation-promotion studies, are considered supplemental lines of information which can aid in the interpretation of more standard toxicological evidence (e.g., rodent chronic bioassays), especially regarding potential modes of action {U.S. EPA, 2005, 86237}. A statistically significant and dose-dependent increase in incidence of neoplastic lesions was observed in the thyroid of F344 male rats following subchronic exposure to ETBE after a 4-week tumor initiation exposure to DMBDD (Hagiwara et al., 2011); incidences of colon and urinary bladder neoplasms also were statistically significantly increased (Hagiwara et al., 2013). Forestomach papilloma or hyperplasia incidence was elevated statistically significantly, while no cases were reported in control animals receiving 4 weeks of mutagenic treatment. This finding is consistent with the rarity of forestomach squamous cell papillomas in untreated animals (historical control rate = 0.08% in untreated male F344/N rats after 2 years; (NTP, 2011); comparability with JPEC controls unknown). While increased tumors were observed with ETBE following administration of tumor initiators, it is important to recognize the limitations of these experimental protocols. Such limitations include experimental manipulation of the carcinogenic process, a generally less than chronic exposure duration, and smaller groups of animals. Male F344 rats (n=12), exposed to ETBE via gavage for 23 weeks (in the absence of

DMBDD treatment) did not significantly induce tumor development in any organs evaluated (Hagiwara et al., 2011). Increased tumorigenesis in these tissues was not reported following 2 years of exposure to ETBE alone via drinking water or inhalation in male or female F344 rats (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010b).

Mechanistic Evidence

The available mechanistic evidence was previously discussed in the context of kidney and liver tumors (Sections 1.1.1 and 1.1.2). Aside from (predominantly negative) genotoxicity testing results, generally relevant to tumorigenesis in any tissue location (discussed in the Supplemental Information), no further mechanistic evidence was identified relevant to uterine, thyroid, colon, forestomach, or urinary bladder carcinogenesis.

Integration of Carcinogenicity Evidence

The evidence for carcinogenic effects other than liver or kidney is solely from rat studies. ETBE exposure following mutagen administration increased the incidence of thyroid adenomas or carcinomas, colon adenomas or carcinomas, forestomach papillomas, and urinary bladder carcinomas in male rats. Confidence in the data demonstrating an increase in the incidence of schwannomas is reduced due to the lack of a dose-response in Sprague-Dawley rats and lack of a similar effect reported in F344 rats from two other well-conducted 2-year studies, or in F344 or Wistar rats from the two-stage subchronic cancer bioassays. The hazard and dose-response conclusions regarding these carcinomas and adenomas associated with ETBE exposure are further discussed as part of the overall weight of evidence for carcinogenicity in Section 1.3.2.

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

Reference and study design	Results					
Thyroid adenomas/adenocarcinomas						
JPEC (2010a); Suzuki et al. (2012) rat, Fischer 344 oral – water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a] daily for 104 wk	**Male**			**Female**		
	Dose (mg/kg-d)	Thyroid follicular adenocarcinoma	Thyroid follicular adenoma	Dose (mg/kg-d)	Thyroid follicular adenocarcinoma	Thyroid follicular adenoma
	0	0/50	1/50	0	0/50	0/50
	28	1/50	0/50	46	1/50	0/50
	121	0/50	0/50	171	0/50	0/50
	542	0/50	0/50	560	0/50	0/50
JPEC (2010b);Saito et al. (2013) rat, Fischer 344 inhalation – vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2090, 6270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method reported	**Male**			**Female**		
	Dose (mg/m^3)	Thyroid follicular adenocarcinoma	Thyroid follicular adenoma	Dose (mg/m^3)	Thyroid follicular adenocarcinoma	Thyroid follicular adenoma
	0	0/50	1/50	0	1/50	0/50
	2,090	0/50	0/50	2,090	1/50	0/50
	6,270	0/50	1/50	6,270	1/50	0/50
	20,900	0/50	2/50	20,900	0/50	0/50
Maltoni et al. (1999) rat, Sprague-Dawley oral – gavage male (60/group): 0, 250, 1,000 mg/kg-d; female (60/group): 0, 250, 1,000 mg/kg-d 4 d/wk for 104 wk; observed until natural death NOTE: Tumor data not reanalyzed by Malarkey and Bucher (2011).	**Male**		**Female**			
	Dose (mg/kg-d)	Thyroid adenocarcinoma		Dose (mg/kg-d)	Thyroid adenocarcinoma	
	0	0/60		0	0/60	
	250	0/60		250	0/60	
	1,000	0/60		1,000	1/60	

Reference and study design	Results
Endometrial/Uterine carcinogenic effects	
JPEC (2010a);Suzuki et al. (2012) rat, Fischer 344 oral – water female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)[a] daily for 104 wk	**Female** Dose (mg/kg-d) / Endometrial stromal sarcoma / Uterine adenocarcinoma / Uterine fibroma 0 / 6/50 / 1/50 / 1/50 46 / 9/50 / 0/50 / 0/50 171 / 3/50 / 2/50 / 0/50 560 / 7/50 / 2/50 / 0/50
JPEC (2010b);Saito et al. (2013) rat, Fischer 344 inhalation – vapor female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method reported	**Female** Dose (mg/m^3) / Endometrial stromal sarcoma / Uterine adenocarcinoma 0 / 2/50 / 2/50 2,090 / 2/50 / 3/50 6,270 / 3/50 / 1/50 20,900 / 2/50 / 4/50
Malarkey and Bucher (2011); Maltoni et al. (1999) rat, Sprague-Dawley oral – gavage female (60/group): 0, 250, 1,000 mg/kg-d reanalysis of data from Maltoni et al. (1999) for which animals were dosed 4 d/wk for 104 wk	**Female** Dose (mg/kg-d) / Carcinoma of the uterus/ vagina / Uterine leiomyoma / Uterine leiomyosarcoma / Schwannoma of the uterus/vagina / Uterine carcinoma 0 / 0/60 / 0/60 / 1/60 / 0/60 / 0/60 250 / 1/60 / 0/60 / 0/60 / 7/60 / 1/60 1,000 / 0/60 / 3/60 / 0/60 / 2/60 / 0/60

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
*Statistically significant ($p < 0.05$) based on analysis of data conducted by study authors.

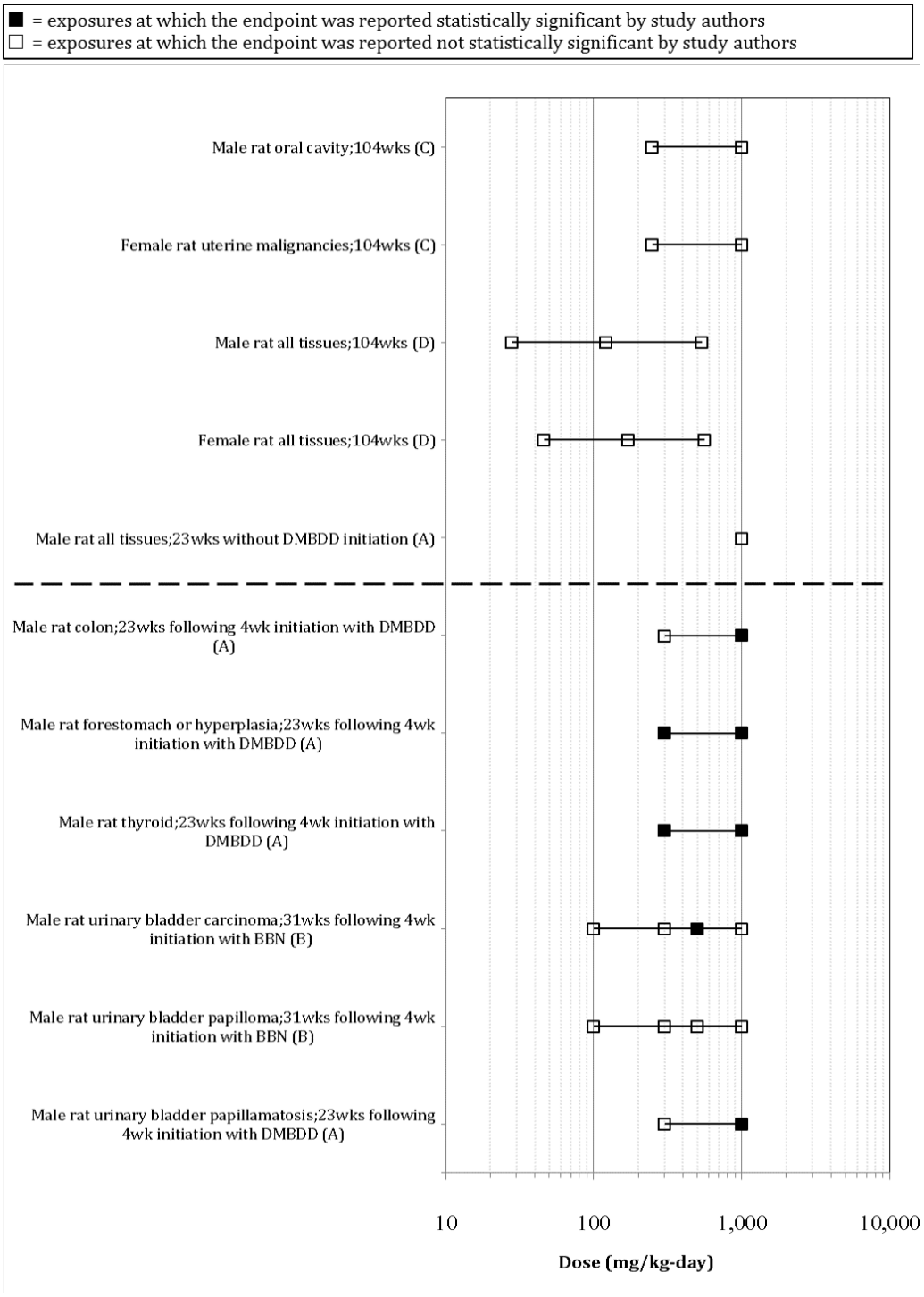
Table 1-18. Supplemental Evidence pertaining to ETBE promotion of mutagen-initiated tumors in animals

Reference and Dosing Protocol	Results by Endpoint		
Colon Adenoma or Carcinoma			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral – gavage male (30/group): 0, 300, 1,000 mg/kg-d		Dose (mg/kg-d)	Response (incidence)
	Male	0	25/30
		300	21/30

Reference and Dosing Protocol	Results by Endpoint		
daily for 23 wk following a 4-wk tumor initiation by DMBDD[a] [+]no DMBDD initiation		1,000 0[+] 1,000[+]	28/30* 0/12 0/12
Forestomach Papillomas or Hyperplasia			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral – gavage male (30/group): 0, 300, 1,000 mg/kg-d daily for 23 wk following a 4-wk tumor initiation by DMBDD[a] [+]no DMBDD initiation	**Male**	Dose (mg/kg-d) 0 300 1,000 0[+] 1,000[+]	Response (incidence) 0/30 6/30* 6/30* 0/12 0/12
Thyroid Gland Adenoma or Carcinoma			
Hagiwara et al. (2011); JPEC (2008d) rat, Fischer 344 oral – gavage male (30/group): 0, 300, 1,000 mg/kg-d daily for 23 wk following a 4-wk tumor initiation by DMBDD[a] [+]no DMBDD initiation	**Male**	Dose (mg/kg-d) 0 300 1,000 0[+] 1,000[+]	Response (incidence) 8/30 17/30* 20/30* 0/12 0/12
Urinary Bladder Carcinoma			
Hagiwara et al. (2013) rat, F344/DuCrlCrlj oral – water male (30/group): 0, 100, 300, 500, 1,000 mg/kg-d daily for 31 wk beginning 1 wk after a 4-wk exposure to BBN	**Male**	Dose (mg/kg-d) 0 100 300 500 1,000	Response (incidence) 5/30 7/30 6/30 14/30* 9/26

Reference and Dosing Protocol	Results by Endpoint		
Urinary Bladder Papilloma			
Hagiwara et al. (2013) rat, F344/DuCrlCrlj oral – water male (30/group): 0, 100, 300, 500, 1,000 mg/kg-d daily for 31 wk beginning 1 wk after a 4-wk exposure to N-butyl-N-(4-hydroxybutyl) (BBN)		Dose (mg/kg-d)	Response (incidence)
	Male	0	21/30
		100	13/30
		300	17/30
		500	17/30
		1,000	21/26
Urinary Bladder Papilloma or Carcinoma			
Hagiwara et al. (2013) rat, F344/DuCrlCrlj oral – water male (30/group): 0, 100, 300, 500, 1,000 mg/kg-d daily for 31 wk beginning 1 wk after a 4-wk exposure to N-butyl-N-(4-hydroxybutyl) (BBN)		Dose (mg/kg-d)	Response (incidence)
	Male	0	24/30
		100	18/30
		300	20/30
		500	25/30
		1,000	21/26
Urinary Bladder Papillamotosis			
Hagiwara et al. (2011); JPEC (2008d) rat, F344 oral – gavage male (12/group): 0, 1,000 mg/kg-d daily for 23 wk following a 4-wk tumor initiation by DMBDD[a] [+]no DMBDD initiation		Dose (mg/kg-d)	Response (incidence)
	Male	0	0/30
		300	0/30
		1,000	10/30*
		0[+]	0/12
		1,000[+]	2/12

[a]Diethylnitrosamine (DEN), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), N-methyl-N-nitrosourea (MNU), 1,2-dimethylhydrazine dihydrochloride (DMH), and N-bis(2-hydroxypropyl)nitrosamine (DHPN).



Sources: (A) Hagiwara et al., 2011; JPEC 2008d (B) Hagiwara et al., 2013 (C) Malarkey and Bucher, 2011 (reanalysis of Maltoni et al., 1999) Maltoni et al., 1999; (D) Suzuki et al., 2012; JPEC, 2010a

Figure 1-16. Exposure-response array of carcinogenic effects following oral exposure to ETBE.

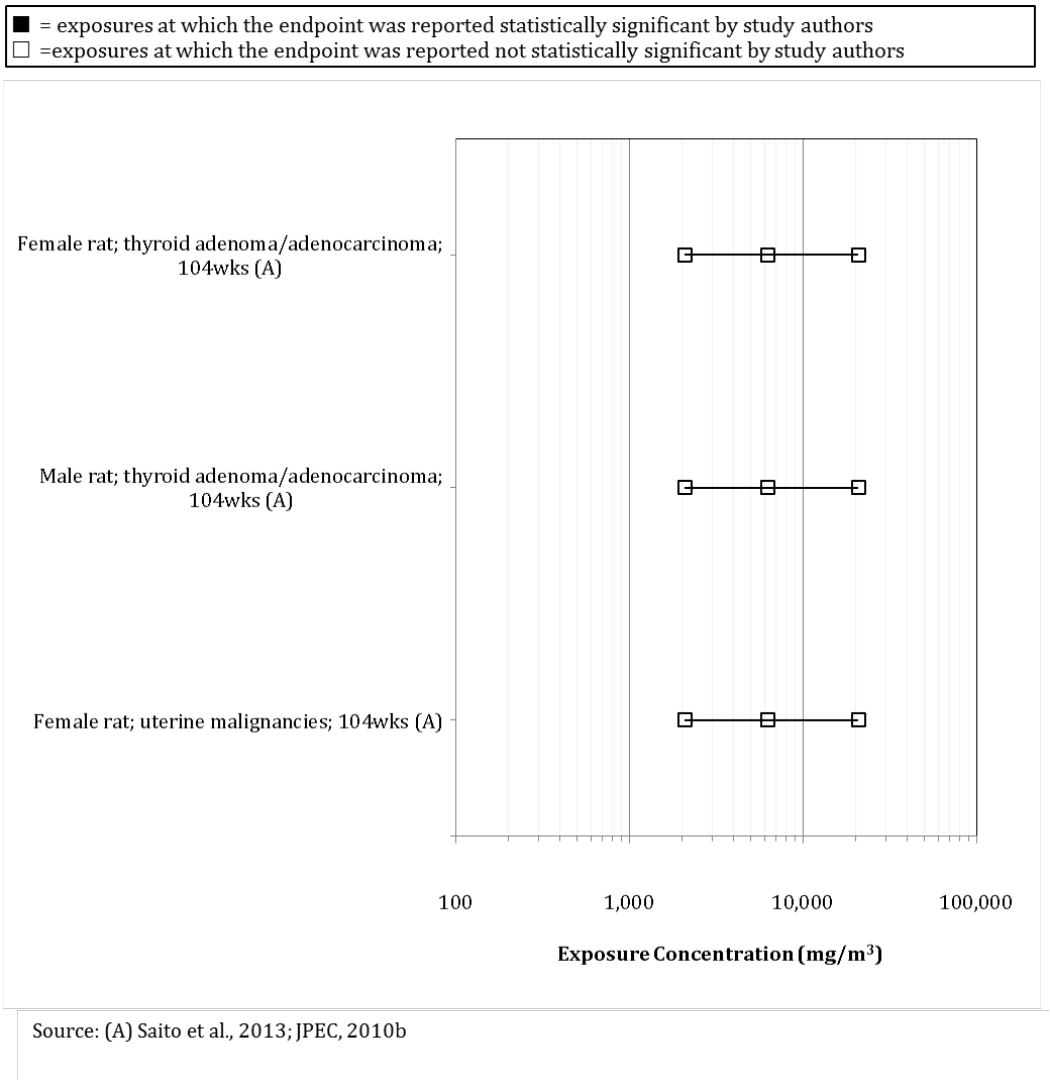


Figure 1-17. Exposure-response array of carcinogenic effects following inhalation exposure to ETBE.

1.2.6. Other Toxicological Effects

The evidence base for other effects includes 11 rodent studies, some of which reported decreased body weight, increased adrenal weights, altered spleen weights, and increased mortality. The available subchronic or chronic studies used inhalation, oral gavage, or drinking water routes of exposure for 90 days or more. Shorter-duration, multiple-exposure studies that examined immunological endpoints also were included. The design, conduct, and reporting of each study were reviewed, and each study was considered adequate.

At this time, the available evidence is considered inadequate to draw conclusions regarding these other toxic effects following ETBE exposure. For more information, see Appendix B.3.

1.3. INTEGRATION AND EVALUATION

1.3.1. Effects Other Than Cancer

Kidney effects were identified as a potential human hazard of ETBE exposure based on several affected endpoints in male and female rats, including kidney weight increases, urothelial hyperplasia (in male rats only), and—to a lesser extent—exacerbated severity of CPN, and increases in serum markers of kidney function such as cholesterol, BUN, and creatinine. These effects are similar to the kidney effects observed with the ETBE metabolite *tert*-butanol (e.g., CPN and transitional epithelial hyperplasia) and a related compound, MTBE (e.g., CPN and mineralization) (ATSDR, 1996). Changes in kidney parameters were consistently observed but the magnitude of change was generally moderate, while males had greater severity of effects compared to females. While the ETBE metabolite *tert*-butanol meets the criteria for α_{2u}-globulin nephropathy (https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=262086), ETBE binds to α_{2u}-globulin and meets some but not all the criteria in the EPA and IARC α_{2u}-globulin frameworks {U.S. EPA, 1991, 635839}{Capen, 1999, 699905}, see Section 1.2.1. {U.S. EPA, 1991, 635839@@author-year} notes that "[i]f a compound induces α_{2u}-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially occuring in humans" (Section XVIII, p. 89). However, as α_{2u}-globulin nephropathy is strictly a male rat phenomenon, dose-related kidney effects in female rats are not confounded by α_{2u}-globulin nephropathy.

It has been observed that chemicals that bind to α_{2u}-globulin also exacerbate the incidence and/or severity of background chronic progressive nephropathy (CPN) in male rats {Travlos, 2011, 1239901}{U.S. EPA, 1991, 635839}{Frazier, 2012, 2919046}. CPN has no known analog in the aging human kidney {Hard, 2009, 667590}{NIEHS, 2019, 5098230} and the etiology is unknown {Hard, 2004, 782757}{NIEHS, 2019, 5098230}{Peter, 1986, 194755}{Frazier, 2012, 2919046}. However, many of the same lesions observed in CPN (e.g. thickening of tubule basement membranes, tubule atrophy, tubule dilation, and glomerular sclerosis) are also observed in chronic kidney disease in humans {Lusco, 2016, 5926047}{Frazier, 2012, 2919046}{Zoja, 2015, 5926046}{Abrass, 2000, 5426141}. Because the mode of action is unknown, it cannot be ruled out that a chemical which

exacerbates CPN in rats could also exacerbate existing disease processes in the human kidney {NIEHS, 2019, 5098230}. Therefore, increased incidence of kidney effects with ETBE exposure, particularly in the female rat (including increased kidney weight and increased severity of CPN) are considered relevant to humans and were carried forward for dose-response analysis. Kidney effects in male rats were also modeled and presented for comparison.

Evidence is suggestive that liver effects are associated with ETBE exposure. Increased liver weight in male and female rats was consistently observed across studies. Centrilobular hypertrophy was observed at the same concentrations that induced liver weight changes in rats of both sexes after 13-week inhalation and 26-week oral exposures. No other histopathological findings were observed, and only one serum marker of liver toxicity (GGT) was elevated, although other markers (AST, ALT, and ALP) were not. The magnitude of change for these noncancer liver effects was considered modest 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 evidence that nuclear receptor-mediated pathways contribute to the tumorigenesis observed in ETBE-treated males is inadequate, thus these data remain relevant for human noncancer hazard identification. Due to the lack of data supporting the adversity of the liver weight increases with ETBE exposure (e.g. alterations in histology or clinical chemistry), liver effects were not considered further for dose-response analysis and the derivation of reference values.

At this time, there is insufficient information to draw conclusions regarding male reproductive effects, female reproductive effects, developmental effects, or other toxic effects as human hazards of ETBE exposure.

1.3.2. Carcinogenicity

Summary of Evidence

In F344 rats, administration of ETBE via inhalation increased hepatocellular adenomas in males in an exposure-dependent manner, as indicated by a significant positive trend ($p < 0.001$ with Peto's test). Hepatocellular tumors were not increased in female rats (Saito et al., 2013). A significantly increased incidence of hepatocellular adenomas or carcinomas (only one carcinoma observed) was observed at the highest dose tested in males, and three hepatocellular adenomas were observed at the two lower concentrations. Significant increases in preneoplastic foci (basophilic and eosinophilic foci) were also observed in male rats(Saito et al., 2013). Following 2 year gavage or drinking water exposure, liver tumors were not increased in Sprague-Dawley or F344 rats of either sex (Suzuki et al., 2012; Maltoni et al., 1999), although an apparent, but non-statistically significant increase in preneoplastic foci (basophilic) was observed in F344 male rats (Suzuki et al., 2012). Regarding the 2 year oral gavage study by {Maltoni, 1999, 87642@@author-year}, depressed survival (25-28% of male and female control rats survived to week 104) may have confounded the ability to detect carcinogenicity.

{Saito, 2013, 2321101@@author-year} compared the doses achieved in the 2 year drinking water bioassay compared with the 2 year inhalation bioassay {Suzuki, 2012, 1433129}{JPEC, 2010, 1517477} and calculated the highest intake in male rats to be 542 mg/kg-day as compared to the highest inhalation intake estimate of 3015 mg/kg-day (after adjusting for 6 hour/day, 5 day a week exposure, minute volume of 561 ml/min, and 100% ETBE absorption). However, the ranges of the internal dose metrics of ETBE expressed as either metabolized ETBE or metabolized t-butanol (one of the two primary breakdown products of ETBE) as computed by toxicokinetic modeling were similar for the oral and inhalation exposures of ETBE and the oral exposure of t-butanol in these three studies (see the range of the internal dose variables in Figure B-3 of Supplemental Information). Nonetheless, the incidence of liver tumors was not consistently correlated with any internal dose measure and the lack of a consistent dose-response relationship using any of these internal metrics suggest that differences in liver tumor responses between oral and inhalation exposures is not likely due to pharmacokinetic factors alone. Statistically significant increases in liver tumor incidence, were observed in the livers of male F344 and Wistar rats in supplemental initiation-promotion studies, after 19-23 weeks of ETBE exposure via oral gavage, only following an initial 2-4 week mutagen exposure (Hagiwara et al., 2015; Hagiwara et al., 2011); along with increases in, colon, thyroid, forestomach, and urinary bladder tumorigenesis in male F344 rats (Hagiwara et al., 2013; Hagiwara et al., 2011). No studies have evaluated chronic ETBE exposure in mice via any route.

The EPA Cancer Guidelines (U.S. EPA, 2005a) emphasize that knowledge of the biochemical and biological changes preceding tumor development could inform whether a cancer hazard exists and might help in understanding events relevant to potential mode of carcinogenic action. However, as discussed in Section 1.2.2, an MOA for liver carcinogenesis could not be established, and in the absence of information to indicate otherwise (U.S. EPA, 2005b), the liver tumors induced by ETBE following inhalation exposure are considered relevant to human hazard identification.

The EPA Cancer Guidelines (2005) indicate that information on metabolites can help inform the weight of evidence for carcinogenicity. ETBE is primarily metabolized into acetaldehyde and *tert*-butanol. Regarding the ETBE metabolite *tert*-butanol, drinking water exposure in F344 rats did not cause an increase in liver tumors, but resulted in renal tubule tumors, mostly adenomas, in males; drinking water exposure also increased the incidence of thyroid follicular cell adenomas in female B6C3F_1 mice and adenomas or carcinomas in males {NTP, 1995, 91022}. Regarding the ETBE metabolite acetaldehyde, inhalation exposures to acetaldehyde were concluded to cause carcinomas of the nasal mucosa in rats and carcinomas of the larynx in hamsters (IARC, 1999b). IARC classifies acetaldehyde as possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde. In addition, acetaldehyde associated with the consumption of alcoholic beverages is considered by IARC to be carcinogenic to humans (Group 1) (IARC, 1999a) and was concluded to be the key metabolite in cancer of the esophagus and aerodigestive tract (IARC, 2010). Acetaldehyde produced in the liver as a result of

ethanol metabolism has been suggested to be a contributor to liver toxicity and cancer (Setshedi et al., 2010).

Integration of evidence

The descriptor *suggestive evidence of carcinogenic potential* is appropriate when the evidence raises "a concern for potential carcinogenic effects in humans" but is not sufficient for a stronger conclusion and covers a spectrum of evidence associated with varying levels of concern for carcinogenicity. Such evidence can range from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. The results for ETBE raise a concern for cancer, however, the effects were limited to tumors in one tissue (liver), primarily at the high dose, by one route of exposure (inhalation) in male rats (females were negative and no ETBE bioassays are available in mice).

Regarding the animal database for carcinogenicity, EPA considers chronic bioassays as key evidence (i.e., the three chronic cancer bioassays, one inhalation and two oral), and other types of studies, including initiation promotion studies, as supplemental lines of information which can aid in the interpretation of more standard toxicological evidence, especially regarding potential modes of action {U.S. EPA, 2005, 86237}. Across three initiation-promotion studies, orally administered ETBE enhanced tumorigenesis in multiple tissues in male rats pre-exposed to mutagens, including kidney, liver, forestomach, thyroid, colon, and urinary bladder. Furthermore, no MOA has been identified for ETBE which could explain the potentiation of mutagen-induced carcinogenesis in the forestomach, thyroid, colon, and urinary bladder. This suggests that the available database is limited with regard to informing molecular mechanisms of ETBE carcinogenesis. The available evidence suggests that populations exposed to mutagenic agents prior to, or concomitant with, oral ETBE exposure might be more susceptible to chemically induced carcinogenesis than predicted by the results of ETBE 2-year rodent oral bioassays alone.

The carcinogenicity of ETBE appears to be route dependent, therefore multiple cancer descriptors are used in accordance with the Cancer Guidelines {U.S. EPA, 2005, 86237}. The evidence of carcinogenic potential for ETBE is determined to be *suggestive* for exposure via the *inhalation* route and *inadequate* for exposure via the *oral* route. These weight of evidence descriptors are based primarily on a positive carcinogenic response following inhalation exposure in the liver in a single animal study, along with significant increases in pre-neoplastic liver lesions and mechanistic data (i.e. the metabolism of ETBE to the genotoxic compound acetaldehyde in the liver) for the inhalation route and no increased liver tumors detected in two chronic oral studies in Sprague-Dawley or F344 rats for the oral route {Maltoni, 1999, 87642}{Suzuki, 2012, 1433129}{JPEC, 2010, 1517477}.

Biological considerations for dose-response analysis

This section addresses the cancer hazards to bring forward to Section 2 for dose-response analysis. The observed liver tumors in male rats following inhalation exposure are deemed

relevant for estimating human cancer hazard. The Saito et al. (2013) inhalation study observed a positive exposure-response trend in the incidence of hepatocellular tumors in male rats (although the majority of tumors were observed at the highest dose). This study was considered suitable for dose-response analysis, as it is part of a well-designed study that evaluated multiple dose levels (JPEC, 2010b). 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. Although decreased body weight gain and survival was noted in the high dose males and females, the study authors did not detect changes to the animals' general condition (e.g. abnormal behavior or clinical signs) associated with ETBE. Similar decreases in body weight were observed in male (75% of control) and female animals (78% of control), although significantly increased liver tumors were only observed in male rats. Some concern exists that decreased survival in ETBE treated dose groups (primarily from CPN in the male rats) could bias the cancer findings toward the null. This study was deemed appropriate for dose-response as there is no clear indication that overt toxicity or altered toxicokinetics {as discussed in \U.S. EPA, 2005, 86237} are responsible for the significantly increased incidence of liver tumors in male rats.

The results from MOA analysis can potentially inform dose-response analysis and extrapolation approaches (U.S. EPA, 2005a), however, for ETBE, no clear MOA was identified. As discussed above, the evidence was inadequate to determine the role of nuclear receptor activation in liver carcinogenesis, due in part to a lack of coherence between nuclear receptor activation and proliferation or apoptosis, key events in these pathways. Evidence also was inadequate to conclude that ETBE induces liver tumors via acetaldehyde-mediated mutagenic MOA, due in part to a paucity of evidence specifically evaluating intermediate key events following ETBE exposure in rats. No other systemic cancer MOAs were identified. In the absence of MOA information to indicate otherwise, dose-response analysis should use linear extrapolation (U.S. EPA, 2005a).

1.3.3. Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

Genetic polymorphisms of *ALDH2*, the enzyme that oxidizes acetaldehyde to acetic acid, might affect potential ETBE liver toxicity. The virtually inactive form, ALDH2*2, is responsible for alcohol intolerance and is found in about one-half of East Asian populations (Brennan, 2002). This variant is associated with slow metabolism of acetaldehyde and, hence, extended exposure to a genotoxic compound. Other studies also have linked *ALDH2* polymorphisms to hepatocellular cancers in humans (Eriksson, 2015). 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* KO mice or cells, genotoxicity was significantly increased compared with wild-type controls following ETBE exposure to similar doses where both cancer and noncancer effects were observed following chronic rodent exposure bioassays {Weng, 2011, 1062385;Weng, 2012, 1248016;Weng, 2013, 2279880;Weng, 2014, 2321096;Weng, 2019, 5343910}. Studies in

Aldh2 KO mice observed elevated blood concentrations of acetaldehyde following ETBE exposure compared with wild-type mice (Weng et al., 2013), increased alterations to sperm and male reproductive tissue (Weng et al., 2014), and increased incidence of centrilobular hypertrophy (Weng et al., 2013; Weng et al., 2012). Similar effects on genotoxicity and liver histopathology have also been observed in *Aldh2* heterozygous animals {Weng, 2019, 5343910}. 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 MOA information exists to account for the sex discrepancies in genotoxic effects. Finally, IARC (1999a) and IARC (2012) identified acetaldehyde produced as a result of ethanol metabolism as contributing to human carcinogenesis in the upper aerodigestive tract and esophagus following ethanol ingestion, with effects amplified by slower acetaldehyde metabolism. Altogether, these data present plausible evidence that diminished ALDH2 activity yields health effect outcomes that are more severe than those organisms with fully functional ALDH2. It is also plausible that individuals with non-coding region variants in ALDH2 (which could potentially affect gene expression), as well as individuals with other variants in alcohol metabolism may also be disproportionately impacted by ETBE exposure. 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 at least one variant (2A6*4) clearly has no catalytic activity (Fukami et al., 2004); however, the effect of this variability on ETBE toxicity is unknown. In addition, the data on ETBE-induced mutagenicity are inconclusive.

Regarding lifestages particularly susceptible to ETBE exposure, while certain lifestages (e.g. development) are generally thought to have heightened vulnerability to most chemical exposures, no specific data was identified to support heightened periods of susceptibility to ETBE exposure in the available database of developmental and reproductive studies. It should be noted that the majority of developmental and reproductive studies were performed by the oral route, so differential susceptibility from inhalation exposure cannot be ruled out, however, in general, the effects observed from ETBE exposure (except for liver tumors) appeared to be consistent across routes of exposure (e.g. kidney effects, liver hypertrophy).

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 (UF values) generally applied to reflect limitations of the data used.

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

Studies were evaluated using general study quality characteristics as discussed in Section 1.1.1; see also U.S. EPA (2002) to help inform the selection of studies from which to derive toxicity values.

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. No human occupational or epidemiological studies of oral exposure to ETBE, however, are available.

Animal studies were evaluated to determine which studies provided (1) the most relevant routes and durations of exposure, (2) multiple exposure levels that informed the shape of the dose-response curve, and (3) sufficient sample size to detect effects at low exposure levels (U.S. EPA, 2002). The database for ETBE includes several chronic and subchronic studies, mostly in rats, showing effects in the kidney that are suitable for use in deriving oral reference values. In general, lifetime exposures are preferred over subchronic exposures.

Kidney Toxicity

Kidney effects were identified as a potential human hazard of ETBE-induced toxicity based on findings in male and female rats (summarized in Section 1.3.1). Kidney toxicity was observed across several chronic and subchronic studies following oral and inhalation exposure, based on findings of organ weight changes, histopathology (urothelial hyperplasia in males), and altered serum biomarkers (cholesterol, creatinine, BUN) in rats. The strongest and most consistent findings across oral exposure routes and durations were for absolute kidney weight changes and urothelial hyperplasia in male rats; thus, only these endpoints were analyzed for dose-response. Kidney effects observed after chronic exposure, such as urothelial hyperplasia, could affect the ability of the kidney to filter waste, and changes in kidney weight could serve as a general indication of renal toxicity. 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). Chronic studies of oral exposure reported urothelial hyperplasia to be increased with treatment in male rats (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b).

Hagiwara et al. (2011), with only one dose group, was not considered further given its concordance with several other rat studies that had multiple groups. Additionally, as discussed in Section 1.1.1, 2-year organ weight data in male rats was not considered suitable due to the prevalence of age-associated confounders (e.g., CPN-related early deaths in male rats). Therefore, the urothelial hyperplasia data (observed in male rats only) and absolute kidney weight (female rats, only) were the only endpoints from the 2-year studies [JPEC, 2010a; with selected data published as Suzuki et al. (2012)], and absolute kidney weight was the only endpoint from the 13- to 26-week studies that were considered for dose-response analysis. These data and the absolute kidney weights from the remaining studies, JPEC [2008c; selected data published as Miyata et al. (2013)], Gaoua (2004b), Fujii et al. (2010), are discussed further below.

In the 2-year drinking water study (Suzuki et al., 2012; JPEC, 2010a), male and female F344 rats (50/sex/dose group) were exposed to doses of 0, 28, 121, or 542 mg/kg-day. Increased incidence of urothelial hyperplasia was observed only in males and significantly increased at 121 and 542 mg/kg-day.

In the JPEC (2008c) 26-week gavage study, male and female Crl:CD(SD) rats (15/sex/dose group) were exposed to daily doses of 0, 5, 25, 100, or 400 mg/kg-day. Absolute kidney weight was significantly increased in males and females treated with 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.

In the Gaoua (2004b) two-generation reproductive toxicity study, Sprague-Dawley rats (25/sex/dose group) were exposed via gavage to doses of 0, 250, 500, or 1,000 mg/kg-day; treatment commenced 10 weeks before mating and continued throughout the 2-week mating period, gestation, and the end of lactation (PND 21) for 18 weeks. Absolute kidney weights were significantly increased in all dose groups in P0 males, but not in P0 females, 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, thus P0 female kidney weights were not modeled. Absolute kidney weights were also increased in F1 animals, however, F1 animals appeared to be less impacted than P0 animals.

In the Fujii et al. (2010) one-generation reproductive toxicity study, male and female Crl:CD(SD) rats (24/sex/dose group) were exposed via gavage to doses of 0, 100, 300, or 1,000 mg/kg-day beginning 10 weeks prior to F0 mating and continuing throughout the reproductive period (mating, gestation, 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 1,000 mg/kg-day.

2.1.2. Methods of Analysis

No biologically based dose-response models are available for ETBE. In this situation, a range of dose-response models was evaluated to determine how best to model the dose-response relationship empirically in the range of the observed data. The models in EPA's Benchmark Dose Software (BMDS) were applied. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), the BMD and the BMDL are estimated using a benchmark response (BMR) to represent a minimal, biologically significant level of change. In the absence of information regarding what level of change is considered biologically significant, a BMR of 10% change from the control mean (relative deviation; RD) for kidney weight and a BMR of 10% extra risk on incidences of urothelial hyperplasia data were used to estimate the BMD and BMDL and to facilitate a consistent basis of comparison across endpoints, studies, and assessments. When modeling was feasible, the estimated BMDLs were used as points of departure (PODs); the PODs are summarized in Table 2-1. Details, including the modeling output and graphical results for the model selected for each endpoint are presented in Appendix C of the Supplemental Information to this Toxicological Review.

Human equivalent doses (HEDs) for oral exposures were derived from the PODs according to the hierarchy of approaches outlined 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). The preferred approach is physiologically based pharmacokinetic (PBPK) modeling. Other approaches include using chemical-specific information in the absence of a complete PBPK model. As discussed in Appendix B of the Supplemental Information, several rat 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 chemical-specific models or data to inform the derivation of human equivalent oral exposures, body-weight scaling to the ¾ power ($BW^{3/4}$) is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans to derive an oral RfD. $BW^{3/4}$ scaling was not used 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 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 using 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 DAF for rats is 0.24. Applying the DAF to the POD identified for effects in adult rats yields a POD_{HED} as follows (see Table 2-1):

$$POD_{HED} = \text{Duration-adjusted laboratory animal dose (mg/kg-day)} \times DAF$$

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 points of departure following oral exposure for up to 2 years

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; 2-year Suzuki et al. (2012); JPEC (2010a)	Male F344 rats	Quantal-Linear	10% ER	79.3	60.5	60.5	14.5
Increased absolute kidney weight; 2-year Suzuki et al. (2012); JPEC (2010a)	Male F344 rats	NOAEL[d,e] 121 mg/kg-d 5% ↑ in kidney weight				121	29.0
Increased absolute kidney weight; 2-year Suzuki et al. (2012); JPEC (2010a)	Female F344 rats	Exponential (M4)	10% RD	204	120	120	28.8
Increased absolute kidney weight; 26-week JPEC (2008c); Miyata et al. (2013)	Male Sprague-Dawley rats	Linear	10% RD	176	115	115	27.6
Increased absolute kidney weight; 26-week JPEC (2008c); Miyata et al. (2013)	Female Sprague-Dawley rats	Exponential (M4)	10% RD	224	57	57	13.7
Increased absolute kidney weight (P0 generation); 18-week Gaoua (2004b)	Male Sprague-Dawley rats	Hill	10% RD	244	94	94	22.6
Increased absolute kidney weight (P0 generation); 16-week Fujii et al. (2010)	Male Sprague-Dawley rats	Hill	10% RD	435	139	139	33.4

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); 17-week Fujii et al. (2010)	Female Sprague-Dawley rats	Polynomial 2°	10% RD	1,094	905	905	217

[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. This adjustment, however, was not required for the studies evaluated.
[c]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011).
ER = extra risk, RD = relative deviation.
[d]NOAEL was used due to lack of suitable model fit (see Appendix C).
[e] 18% increase in kidney weight at LOAEL

2.1.3. Derivation of Candidate Values

Consistent with EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), five possible areas of uncertainty and variability were considered when determining the application of UF values to the PODs presented in Table 2-1. 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 (U.S. EPA, 2002).

An interspecies uncertainty factor, UF_A, of 3 ($10^{0.5}$ = 3.16, rounded to 3) was applied to PODs that used $BW^{3/4}$ scaling 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 using an uncertainty factor of 3. For PODs that did not use $BW^{3/4}$ such as early-life effects, an interspecies uncertainty factor, UF_A, of 10 was applied (U.S. EPA, 2011).

A subchronic-to-chronic uncertainty factor, UF_S, differs depending on the exposure duration. For studies of 16- to 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 could have been reached by 16–26 weeks. The 104-week kidney data, however, are confounded due to age-associated factors, so this comparison might not be completely reliable. Additionally, some but not all markers of kidney toxicity appear more severely affected by ETBE at 2 years compared with observations at 16–26 weeks (e.g., histopathology, BUN) (Suzuki et al., 2012; JPEC, 2010a). Thus, a UF_S of 3 was applied for studies of 16- to 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 to all PODs derived because 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 kidney weight and a

10% extra risk of urothelial hyperplasia were selected assuming that they represent minimal biologically significant response levels.

A database uncertainty factor, UF_D, of 1 was applied to all PODs. The ETBE oral toxicity data set includes a 2-year toxicity study in rats (Suzuki et al., 2012; JPEC, 2010a), a 26-week toxicity study in rats (Miyata et al., 2013), prenatal developmental toxicity studies in rats and rabbits (Aso et al., 2014; Asano et al., 2011), and both single- and multigeneration reproductive studies and developmental studies in rats {Fujii, 2010, 1248027;Gaoua, 2004, 87678;Gaoua, 2004, 87676}. The ETBE data set does not indicate immunotoxicity (Banton et al., 2011; Li et al., 2011). Additionally, the available mouse study observed less severe effects than those in rats, suggesting that mice are less sensitive than rats. Although most of the studies are in rats, the ETBE oral database adequately covers all major systemic effects, including reproductive and developmental effects, and the available evidence does not raise concern that additional studies would likely lead to identification of a more sensitive endpoint or a lower POD. Furthermore, the effects observed in inhalation studies support the noncancer effects observed in the oral studies. Therefore, an uncertainty factor for the database, UF_D, of 1 was applied.

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

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

Endpoint and Reference	POD_{HED} (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; 2-year Suzuki et al. (2012); JPEC (2010a)	14.5	$BMDL_{10\%}$	3	10	1	1	1	30	5×10^{-1}
Increased absolute kidney weight; male rat; 2-year Suzuki et al. (2012); JPEC (2010a)	29.0	NOAEL	3	10	1	1	1	30	1×10^{0}
Increased absolute kidney weight; female rat; 2-year Suzuki et al. (2012); JPEC (2010a)	28.8	$BMDL_{10\%}$	3	10	1	1	1	30	1×10^{0}
Increased absolute kidney weight; male rat; 26-week JPEC (2008c); Miyata et al. (2013)	27.6	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{-1}
Increased absolute kidney weight; female rat; 26-week JPEC (2008c); Miyata et al. (2013)	13.7	$BMDL_{10\%}$	3	10	1	3	1	100	1×10^{-1}

Endpoint and Reference	POD_{HED} (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; P0 male rat; 18-week Gaoua (2004b)	22.6	$BMDL_{10\%}$	3	10	1	3	1	100	2×10^{-1}
Increased absolute kidney weight; male rat; 16-week Fujii et al. (2010)	33.4	$BMDL_{10\%}$	3	10	1	3	1	100	3×10^{-1}
Increased absolute kidney weight; female rat; 17-week Fujii et al. (2010)	217	$BMDL_{10\%}$	3	10	1	3	1	100	2×10^{0}

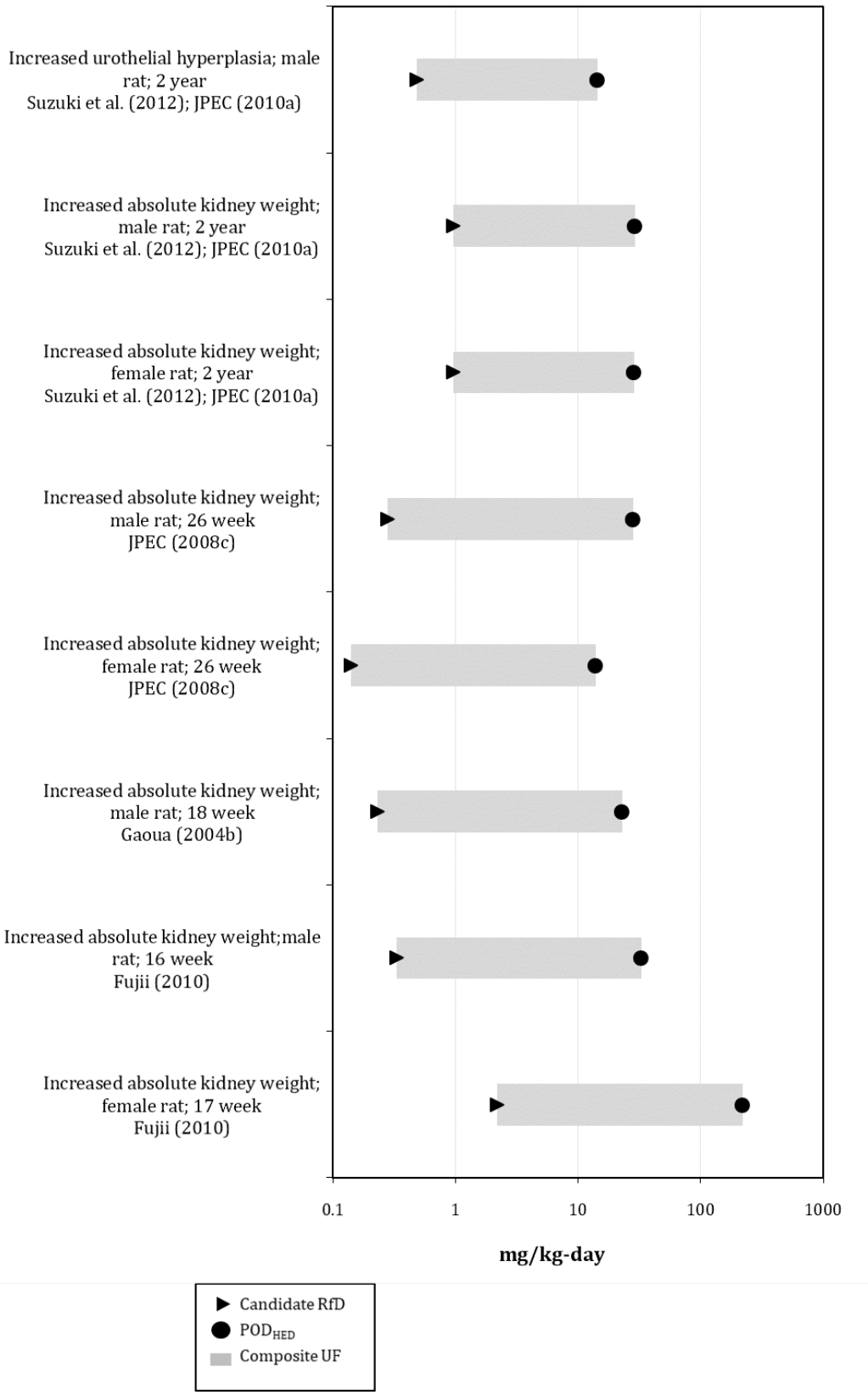


Figure 2-1. Candidate values with corresponding POD and composite UF.

2.1.4. Derivation of Organ/System-Specific Reference Doses

Table 2-3 distills the candidate values from Table 2-2 into a single value for each organ or system. Organ- or system-specific RfDs are useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Kidney Toxicity

For ETBE, candidate values were derived for increases in urothelial hyperplasia or absolute kidney weight in male or female rats, spanning a range from 1×10^{-1} to 2×10^{0} mg/kg-day, for an overall 20-fold range. Selection of a point estimate considered multiple aspects, including study design and consistency across estimates. As stated previously, reference values based on lifetime exposure are preferred over subchronic exposures. The only candidate reference values based on data from a 2-year oral study are those for increased absolute kidney weight in female rats and urothelial hyperplasia in male rats (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). While urothelial hyperplasia in male rats was the most sensitive candidate value, this endpoint was not observed in female rats or mice of either sex whereas increased kidney weights were observed in multiple studies in rats of both sexes and in mice. A lower candidate value based on increased kidney weights in female rats was derived from a smaller (n=15), shorter duration study (26 weeks; JPEC 2008c), however, based on comparison of BMD values (204 vs 224 mg/kg-d) and composite UFs (30 vs 100), this lower value reflects greater variability and uncertainty in the data. The composite UF for the candidate value based on increased kidney weight in female rats treated for 2 years was the lowest uncertainty, providing greater certainty in the selection of this candidate value. In addition, the candidate values from female rats are not potentially confounded by α_{2u}-globulin related processes.

Collectively, these observations indicate that the most appropriate basis for a kidney-specific RfD would be the increased absolute kidney weight in female rats from the 2-year oral study (Suzuki et al., 2012; JPEC, 2010a). Therefore, the candidate value for increased absolute kidney weight in female rats (**1×10^{0} mg/kg-day**) was selected as the kidney-specific reference dose for ETBE. Confidence in this RfD is high. The candidate value is derived from a well-conducted GLP study, involving approximately 50 animals per group, assessing a wide range of kidney endpoints. In addition, the POD is based on benchmark dose modeling, with the POD within the range of tested doses (e.g., not requiring extrapolation well beyond the experimental range), and the reference value is associated with less relative uncertainty (as illustrated by the smaller composite UF). Furthermore, several additional studies demonstrate quantitatively similar PODs and candidate values for kidney effects (see Table 2-2 and Figure 2-1).

Table 2-3. Organ/system-specific RfDs and overall RfD for ETBE

Effect	Basis	RfD (mg/kg-day)	Study exposure description	Confidence
Kidney	Increased absolute kidney weight in female rats Suzuki et al. (2012); JPEC (2010a)	1×10^0	Chronic	High
Overall RfD	**Kidney**	**1×10^0**	**Chronic**	**High**

2.1.5. Selection of the Overall Reference Dose

For ETBE, kidney effects were identified as the strongest hazard and carried forward for dose-response analysis; thus, only one organ/system-specific reference dose was derived. Therefore, the kidney-specific RfD of **1×10^0 mg/kg-day** is the overall RfD for ETBE. This value is based on increased absolute kidney weight in female rats exposed to ETBE.

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). Decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD. In the case of ETBE, no specific potential for early lifestage susceptibility to ETBE exposure was identified, as discussed in Section 1.3.3.

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, 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 (Suzuki et al., 2012; JPEC, 2010a) 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 the existing evidence base does not raise concerns that additional studies would be likely to lead to the identification of a more sensitive endpoint. Furthermore, multiple studies demonstrate quantitatively similar PODs and candidate values for kidney effects (see Table 2-2 and Figure 2-1), providing additional support. Reflecting high confidence in the principal study and high confidence in the database, confidence in the RfD is high.

2.1.7. Previous IRIS Assessment

No previous oral assessment for ETBE is available in IRIS.

2.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The inhalation 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 UF values generally applied to reflect limitations of the data used.

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

Kidney effects were identified as a potential human hazard of ETBE exposure based on studies in experimental animals (summarized in Section 1.3.1). These studies were evaluated using general study quality characteristics as discussed in Section 6 of the Preamble and in Section 1.1.1; see also U.S. EPA (2002) 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 generally preferred over animal studies as the basis for reference values 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 small number of 2-hour inhalation studies at doses up to 208.9 mg/m^3 (Nihlén et al., 1998b; Vetrano, 1993). These studies were not considered for dose-response assessment because they are of acute duration and investigated toxicokinetics.

The database for ETBE includes inhalation studies and data sets that are potentially suitable for use in deriving inhalation reference values. Specifically, effects associated with ETBE exposure in animals include observations of organ weight and histological changes in the kidney in chronic and subchronic studies in male and female rats.

Kidney Toxicity

Evidence exists supporting kidney effects following ETBE exposure in rats, including organ weight changes, histopathology (urothelial hyperplasia and exacerbation of CPN), and altered serum biomarkers (creatinine, BUN, cholesterol). The most consistent, dose-related findings across multiple studies, in both sexes were for kidney weight changes and increased CPN severity. In the case of kidney weight changes, one chronic and numerous subchronic studies investigated this endpoint following inhalation exposure (Suzuki et al., 2012; Hagiwara et al., 2011; Fujii et al., 2010; JPEC, 2010b, 2008b, c; Gaoua, 2004b; Medinsky et al., 1999). A 2-year study by inhalation (Saito et al., 2013; JPEC, 2010b) exposure reported increased urothelial hyperplasia in male rats only, and increased kidney weight and CPN severity in both sexes. Increased kidney weights from the 13-week studies were also considered for dose-response analysis (Saito et al., 2013; JPEC, 2010b).

In the Saito et al. (2013) 2-year inhalation study, male and female F344 rats (50/sex/dose group) were exposed to concentrations of 0, 2,090, 6,270, or 20,900 mg/m^3 (JPEC, 2010b). Increased incidence of urothelial hyperplasia was only observed in males and significantly

increased at 6,270 and 20,900 mg/m^3. Increased severity of CPN was significantly increased in males and females at 20,900 mg/m^3. Absolute kidney weight was significantly increased in males at concentrations ≥ 2,090 mg/m^3 and in females at ≥ 6,270 mg/m^3.

In the JPEC (2008b) 13-week whole-body inhalation study, male and female Crl:CD(SD) rats were exposed to concentrations of 0, 627, 2,090, 6,270, or 20,900 mg/m^3 for 6 hours/day, 5 days/week. Significant increases in absolute kidney weights occurred in male rats exposed to 6,270 or 20,900 mg/m^3 ETBE compared with controls, while changes in female rats were not statistically significant, and were not modeled.

In the Medinsky et al. (1999) 13-week whole-body inhalation study, male and female F344 rats were exposed to concentrations of 0, 2,090, 7,320, or 20,900 mg/m^3 for 6 hours/day, 5 days/week. Kidney weights were increased at the highest two doses in both male and females. Slight, but statistically significant, increases in various clinical chemistry parameters were observed; however, these effects were reported to be of uncertain toxicological significance and were not modeled.

2.2.2. Methods of Analysis

No biologically based dose-response models are available for ETBE. In this situation, dose-response models thought to be consistent with underlying biological processes were evaluated to determine how best to model the dose-response relationship empirically in the range of the observed data. Consistent with this approach, all models available in EPA's BMDS were evaluated. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), the BMC and the 95% BMCL were estimated using BMR to represent a minimal, biologically significant level of change. As noted in Section 2.1.2, a 10% relative change from the control mean (relative deviation; RD) was used as a BMR for absolute kidney weight, and a BMR of 10% extra risk was considered appropriate for the quantal data on incidences of urothelial hyperplasia. When modeling was feasible, the estimated BMCLs were used as points of departure (PODs); the PODs are summarized in Table 2-4. Further details including the modeling output and graphical results for the model selected for each endpoint are found in Appendix C of the Supplemental Information to this Toxicological Review.

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 (ADJ) 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 modeled benchmark concentration from the animal exposures the inhalation studies (JPEC, 2008b; Medinsky et al., 1999){JPEC, 2010, 1517421} 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 an HEC 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 extra-respiratory effects were observed. 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 Supplemental 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 2-4 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD (POD_{HEC}) for each inhalation data set discussed above.

Table 2-4. 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; 2-year Saito et al. (2013); JPEC (2010b)	Male F344 rats	Gamma	10%	2,734	1,498	268	265
Increased CPN severity; 2-year Saito et al. (2013); JPEC (2010b)	Male and female F344 rats	NOAEL[d]: 6270 mg/m^3				1,120	1,110
Increased absolute kidney weight; 2-year Saito et al. (2013); JPEC (2010b)	Female F344 rats	NOAEL[d,e]: 6270 mg/m^3 6% ↑ in kidney weight				1,120	1,110

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; 13- week JPEC (2008b)	Male Sprague-Dawley rats	NOAEL[d]: 627 mg/m^3 10% ↑ in kidney weight				112	111
Increased absolute kidney weight; 13-week JPEC (2008b)	Female Sprague-Dawley rats	Linear	10% RD	28,591	16,628	2,969	2,946
Increased absolute kidney weight; 13-week Medinsky et al. (1999)	Male F344 rats	Hill	10% RD	6,968	2,521	450	447
Increased absolute kidney weight; 13-week Medinsky et al. (1999)	Female F344 rats	Exponential (M4)	10% RD	5,610	3,411	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 hr) × (days exposed per week ÷ 7 days).
[c]POD_{HEC} calculated by adjusting the POD_{ADJ} by the DAF (=0.992) for a Category 3 gas (U.S. EPA, 1994).
[d]NOAEL was used due to lack of suitable model fit (see Appendix C).
[e]Absolute kidney weight was increased 5, 6, and 18% at 2090, 6270, and 20,900 mg/m^3. A NOAEL was selected based on the dose closest to a 10% change in order to more closely approximate a minimally biologically significant change.

2.2.3. Derivation of Candidate Values

In 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 (U.S. EPA, 2002).

An interspecies uncertainty factor, UF_A, of 3 ($10^{0.5}$ = 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. Furthermore, the magnitude of change in absolute kidney weights appeared to increase in male and female rats exposed for 26 weeks compared with 13–18 weeks, when results across oral and inhalation exposures were evaluated based upon internal blood concentrations (see Figure 1-2), suggesting that toxicity would be expected to increase with exposure durations greater than 13 weeks. Therefore, a UF_S of 10 was applied for studies of 13 weeks. A UF_S of 1 was applied to 2-year studies.

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied to all PODs derived because 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 kidney weight and a 10% extra risk of histological lesions were selected assuming that they represent minimal biologically significant response levels. In some cases, the data were not amenable to modeling, thus a NOAEL was selected to represent a minimal biologically significant response.

A database uncertainty factor, UF_D, of 1 was applied to all PODs. The ETBE inhalation toxicity database includes a 2-year toxicity study in rats (Saito et al., 2013; JPEC, 2010b) and 13-week toxicity studies in mice and rats (JPEC, 2008b; Medinsky et al., 1999). Generally, if the RfD or RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproduction study is missing, or a factor of 10 if both are missing {U.S. EPA, 2002, 88824}. There are no developmental or multi-generation reproductive studies by the inhalation route; however, the oral studies of prenatal developmental toxicity in rats and rabbits (Aso et al., 2014; Asano et al., 2011), and single- and multi-generation reproductive toxicity and developmental toxicity in rats (Fujii et al., 2010; Gaoua, 2004a, b) are available to inform the inhalation database. In addition, systemic effects are anticipated to be similar via oral or inhalation exposure to ETBE, first pass effects are not indicated by the available data, and no evidence is available to suggest that untransformed ETBE would have a significant role in toxicity. Similarly, the oral ETBE data set does not indicate immunotoxicity and differences in outcome would not be anticipated for inhalation exposures (Banton et al., 2011; Li et al., 2011). Although most of the studies are in rats, the available mouse study observed effects that were less severe than those in rats, suggesting that mice are not more sensitive than rats. The ETBE inhalation database, supported by the information from the oral database, adequately covers all major systemic effects, including reproductive, developmental, immunological and neurological effects, and the available evidence does not raise concern that additional studies would likely lead to identification of a more sensitive endpoint or a lower POD. Therefore, a database UF_D of 1 was applied.

Table 2-5 is a continuation of Table 2-4, and summarizes the application of UF values 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, UF values, and PODs, with each bar corresponding to one data set described in Table 2-4 and Table 2-5.

Table 2-5. Effects and corresponding derivation of candidate values

Endpoint (Sex and species) and Reference	POD_{HEC} (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; 2-year Saito et al. (2013); JPEC (2010b)	265	$BMCL_{10\%}$	3	10	1	1	1	30	9×10^0
Increased CPN severity; male and female rats; 2-year Saito et al. (2013); JPEC (2010b)	1,110	NOAEL	3	10	1	1	1	30	4×10^1
Increased absolute kidney weight; female rats; 2-year Saito et al. (2013); JPEC (2010b)	1,110	NOAEL	3	10	1	1	1	30	4×10^1
Increased absolute kidney weight; male rat; 13-week JPEC (2008b)	111	NOAEL	3	10	1	10	1	300	4×10^{-1}
Increased absolute kidney weight; female rat; 13-week JPEC (2008b)	2,946	$BMCL_{10\%}$	3	10	1	10	1	300	1×10^1
Increased absolute kidney weight; male rat; 13-week Medinsky et al. (1999)	447	$BMCL_{10\%}$	3	10	1	10	1	300	2×10^0
Increased absolute kidney weight; female rat; 13-week Medinsky et al. (1999)	604	$BMCL_{10\%}$	3	10	1	10	1	300	2×10^0

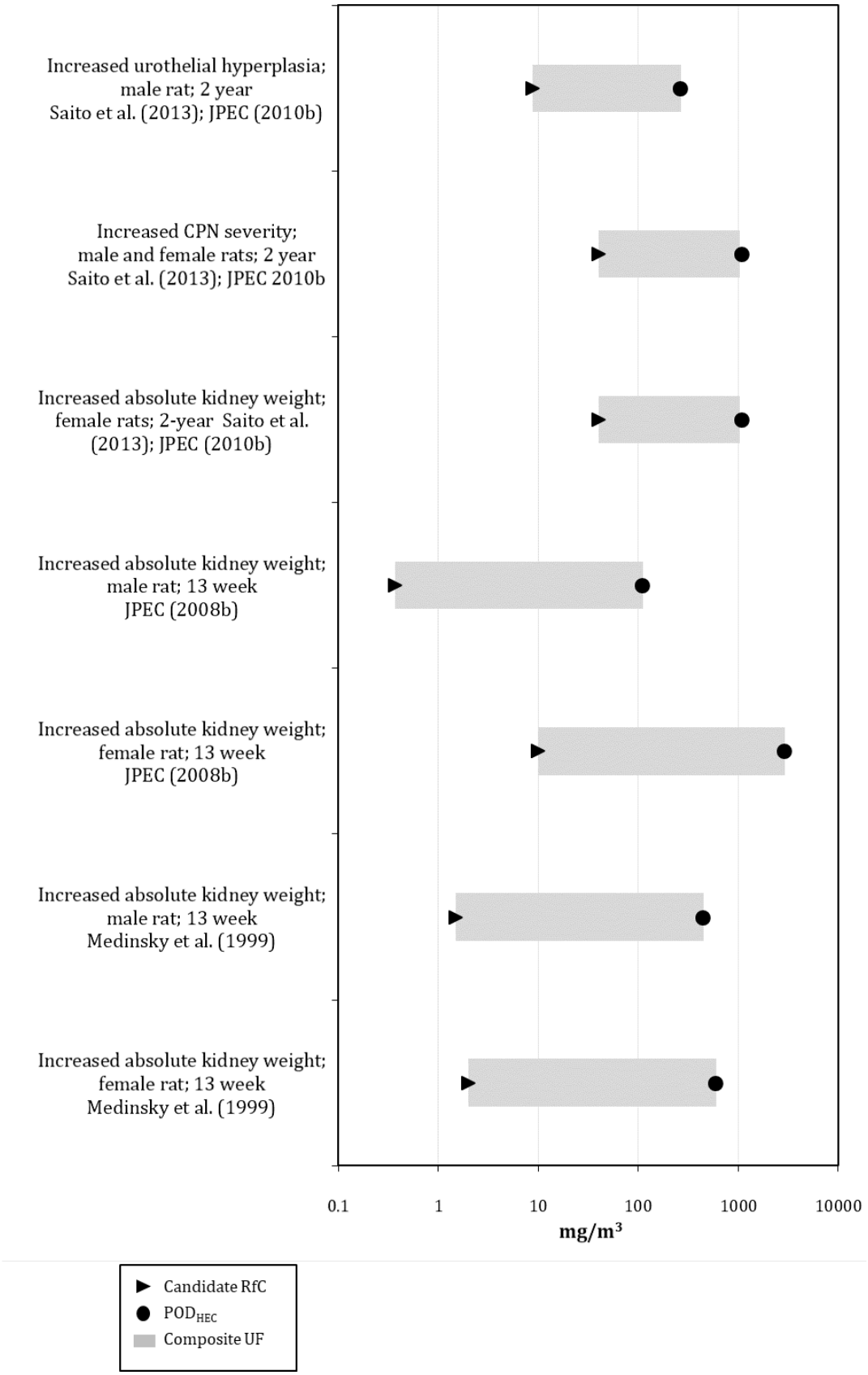


Figure 2-2. Candidate values with corresponding POD and composite UF.

2.2.4. Derivation of Organ/System-Specific Reference Concentrations

Table 2-6 distills the candidate values from Table 2-5 into a single value for the kidney. Organ- or system-specific reference values can 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 values were derived for increased kidney weight in both sexes of rats, and urothelial hyperplasia in males, spanning a range from 4×10^{-1} to 4×10^{1} mg/m^3, for an overall 100-fold range. While the increase in urothelial hyperplasia in male rats resulted in the lowest candidate value, this endpoint was observed only in male rats and thus the biological relevance of this lesion is more uncertain than the kidney endpoints observed in both sexes. The candidate RfC for increased absolute kidney weight in female rats (**4×10^{1} mg/m^3**) was ultimately selected as the kidney-specific RfC for ETBE, consistent with the selection of the kidney-specific RfD (see Section 2.1.4). As discussed in Section 2.1.4, this lesion is a general indicator of kidney toxicity, which has been observed in both sexes of rats (and to a lesser extent in mice). This RfC is identical to the candidate RfC for the increased severity of CPN in male and female rats, however in this case the kidney weight was considered a more certain indication of kidney toxicity. Confidence in this kidney-specific RfC is medium. The candidate value is derived from a well-conducted study, involving a sufficient number of animals per group, including both sexes, and assessing a wide range of kidney endpoints, however, the inability to model the selected endpoint resulted in some reduction in confidence.

Table 2-6. Organ-/system-specific RfCs and overall RfC for ETBE

Effect	Basis	RfC (mg/m^3)	Study exposure description	Confidence
Kidney	Increased absolute kidney weight in female rats Saito et al. (2013); JPEC (2010b)	4×10^{1}	Chronic	Medium
Overall RfC	**Kidney**	**4×10^{1}**	**Chronic**	**Medium**

2.2.5. Selection of the Overall Reference Concentration

For ETBE, kidney effects were identified as the primary hazard; thus, a single organ-/system-specific RfC was derived. Therefore, the kidney-specific RfC of **4×10^{1} mg/m^3** is selected as the overall RfC, representing an estimated exposure level below which deleterious effects from ETBE exposure are not expected to occur.

The overall RfC 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). Decisions concerning averaging exposures over time for comparison with the RfC should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could lead

to an increased level of concern, , even if average levels over the full exposure duration were less than or equal to the RfC. In the case of ETBE, no specific potential for early lifestage susceptibility to ETBE exposure was identified, as discussed in Section 1.3.3.

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 medium. Confidence in the principal study, Saito et al. (2013); JPEC (2010b), is medium This study was well conducted, following GLP guidelines that involved a sufficient number of animals per group (including both sexes), and assessed a wide range of tissues and endpoints, however, the inability to model the POD resulted in some reduction in confidence. Confidence in the database is high; the available studies evaluated a comprehensive array of endpoints, and that additional studies would lead to identification of a more sensitive endpoint is not indicated. Reflecting medium confidence in the principal study and high confidence in the database, overall confidence in the RfC for ETBE is medium.

2.2.7. Previous IRIS Assessment

No previous inhalation assessment for ETBE is available in 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 for ETBE. To derive the RfD and RfC, the UF approach (U.S. EPA, 2000, 1994) was applied to a POD based on kidney toxicity in rats treated chronically. UFs were applied to the PODs to account for extrapolating from an animal bioassay to human exposure and for the likely existence of a diverse human population of varying susceptibility. Default approaches are used for these extrapolations, 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, and the PODs were calculated from data on the effects of ETBE reported by studies in rats. The database for ETBE exposure includes three lifetime bioassays in rats, several reproductive/developmental studies in rats and rabbits, several subchronic studies in rats and mice, and immunotoxicity assays.

Although the database is adequate for reference value derivation, some uncertainty associated with the database remains, such as the lack of chronic studies in a species other than rats (e.g., mice), the lack of developmental/reproductive inhalation studies, and limited or no information available regarding effects in humans or animals with deficient ALDH2 activity.

The toxicokinetic and toxicodynamic differences for ETBE between the animal species from which the POD was derived and humans are unknown. 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,

male rats appear more susceptible than females to ETBE toxicity. The underlying mechanistic basis of this apparent difference, however, is not understood (except in the case of kidney effects, in which effects in males are likely exacerbated by α2u-globulin nephropathy). Most importantly, which animal species and sexes are more comparable to humans is unknown.

Overall, the ETBE data are insufficient to conclude that the α_{2u}-globulin process is operative; however, key noncancer effects related to α_{2u}-globulin (including exacerbation of CPN in male rats) were observed and considered not appropriate for hazard identification and, therefore, not suitable for dose-response consideration. Instead, candidate values in female rats were prioritized to avoid confounding by α_{2u}-globulin processes. Only one subchronic study is available in mice, which are not affected by α_{2u}-globulin. This study seemed to indicate male mice were more sensitive to kidney weight changes than female mice, although these changes did not reach statistical significance {Bond, 1996, 74002}{Medinsky, 1999, 10740}. Therefore, there is uncertainty regarding whether other factors (unrelated to α_{2u}-globulin) may increase the susceptibility of male rats to ETBE related kidney effects.

2.3. RAL SLOPE FACTOR FOR CANCER

The oral slope factor (OSF) is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. The OSF can be multiplied by an estimate of lifetime exposure (in mg/kg-day) to estimate the lifetime cancer risk.

As noted in Section 1.3.2, EPA concluded that there is "inadequate evidence of carcinogenic potential" for oral exposure to ETBE since the two available chronic oral bioassays for ETBE were negative in rats {Maltoni, 1999, 87642}{JPEC, 2010, 1517477}{Suzuki, 2012, 1433129}, and no chronic oral bioassays are available in mice. Furthermore, PBPK analysis indicated the absence of a consistent dose-response relationship for liver tumors when compared across oral and inhalation exposures; therefore, a route to route extrapolation was not performed and no oral slope factor is derived.

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 inhalation exposure can be derived. Quantitative risk estimates can 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 1.3.2, there is "suggestive evidence of carcinogenic potential" for inhalation exposure to 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 each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates.

In the case of ETBE, an inhalation unit risk is derived. A description of the carcinogenicity data is presented in the discussions of biological considerations for cancer dose-response analysis (see Section 1.3.2). Briefly, a well conducted study demonstrated a significant, positive exposure-response for hepatocellular adenomas and carcinomas in male rats. While the majority of liver tumors occurred at the high dose, statistical tests conducted by the study authors found significant dose-response trend by both the Peto test (incidental tumor test) and the Cochran-Armitage test. The available data do not demonstrate that the liver tumors are the result of excessive toxicity in male rats rather than the carcinogenicity of ETBE. Although decreased body weight gain and survival was noted in the high dose males and females, the study authors did not detect changes to the animals' general condition (e.g. abnormal behavior or clinical signs) associated with ETBE. In addition, the study provided no indication that altered to toxicokinetics was responsible for the significantly increased incidence of liver tumors in male rats. Considering these data, along with the uncertainty associated with the suggestive nature of the weight of evidence, quantitative analysis of the tumor data may be useful for providing a sense of the magnitude of potential carcinogenic risk (including workers and consumers). Therefore, the hepatocellular adenomas and carcinomas in male rats were considered for dose response modeling and calculation of a quantitative risk estimate. Because of the suggestive nature of the tumorigenic response (U.S. EPA, 2005a), there is increased uncertainty in this risk estimate, and this is noted below where relevant.

2.4.2. Dose-Response Analysis—Adjustments and Extrapolation Methods

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical be determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (U.S. EPA, 2005a). Within the observed range, the preferred approach is to use modeling to incorporate a wide range of data into the analysis, such as through a biologically based model, if supported by substantial data. Without a biologically based model, as in the case of ETBE, a standard model is used to curve-fit the data and to estimate a POD. EPA uses the multistage model in IRIS dose-response analyses for cancer (Gehlhaus et al., 2011) because it parallels the multistage carcinogenic process and fits a broad array of dose-response patterns.

The second step, extrapolation to lower exposures from the POD, considers what is known about the modes of action for each effect. As above, a biologically based model is preferred (U.S. EPA, 2005a). Otherwise, linear low-dose extrapolation is recommended if the MOA of carcinogenicity is mutagenic or has not been established (U.S. EPA, 2005a). For ETBE, the mode(s)

of carcinogenic action for liver tumors has not been established (see Section 1.3.2). Therefore, linear low-dose extrapolation was used to estimate human carcinogenic risk.

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 the lower end of the observed data, 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 uses 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 (Table 2-7) estimated from the inhalation study (Saito et al., 2013; JPEC, 2010b) was adjusted to reflect continuous exposure by multiplying it by (6 hours/day) ÷ (24 hours/day) and (5 days/week) ÷ (7 days/week) as follows:

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

The approach to determine the HEC accounts for the extrarespiratory 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 extrarespiratory effects were observed. The values reported in the literature for these parameters include a blood:air partition coefficient of 11.6 for rats, L_A (Kaneko et al., 2000) and a blood:air partition coefficient for humans of 11.7, L_H (Nihlén et al., 1995). This allowed a $BMCL_{HEC}$ to be derived as follows:

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

2.4.3. Inhalation Unit Risk Derivation

The POD estimate based on the male rat liver tumor data (Saito et al., 2013; JPEC, 2010b) is summarized in Table 2-7779. 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 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 2-7.

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/m^3**, based on the liver tumor response in male F344 rats (Saito et al., 2013; JPEC, 2010b). This unit risk should not be used with continuous exposures exceeding 1,271 mg/m^3 (the POD) because above this level the dose-response relationship is nonlinear. If risk estimates are needed for exposure corresponding to expected overall cancer risks greater than 10%, the full dose-response model as should be consulted.. The slope of the linear extrapolation from the central estimate BMD_{10} is 0.1 ÷ 0.992 × (1,944 mg/kg-day)] = 5×10^{-5} per mg/m^3.

Table 2-7. Summary of the inhalation unit risk derivation

Tumor	Species/Sex	Selected Model	BMR	BMC_{ADJ} (mg/m^3)	POD= $BMCL_{ADJ}$ (mg/m^3)	$BMCL_{HEC}$ (mg/m^3)	Slope factor[a, b] (mg/m^3)$^{-1}$
Hepatocellular adenomas or carcinomas Saito et al. (2013); JPEC (2010b)	Male F344 rat	1° Multistage	10%	1,944	1,271	1,261	8×10^{-5}

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

[b]This value is uncertain because it is based on a determination of *suggestive evidence of carcinogenic potential*; however, the IUR may be useful for some decision purposes such as providing a sense of the magnitude of potential risks or ranking potential hazards (U.S. EPA, 2005a). The uncertainties in the data leading to this suggestive weight of evidence determination for carcinogenicity are detailed in Sections 1.3.2., 2.4.1, and 2.4.4.

2.4.4. Uncertainties in the Derivation of the Inhalation Unit Risk

Uncertainty exists when extrapolating data from animals to estimate potential cancer risks to human populations from exposure to ETBE.

Table 2-8 summarizes several uncertainties that could affect the inhalation unit risk. Although the chronic studies did not report an increase in liver tumorigenesis following oral exposure in rats, no other inhalation studies are available to replicate these findings and none examined other animal models (e.g. mice). In addition, no data in humans are available to confirm a general cancer response or the specific tumors observed in the rat bioassay (Saito et al., 2013; JPEC, 2010b). 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, and no other data (e.g., MOA) supported alternative derivation approaches.

Table 2-8. Summary of uncertainties in the derivation of the inhalation unit risk for ETBE

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of tumor type and relevance to humans: Rat liver tumors are the basis for estimating human cancer risk.	The liver was selected as the target organ (U.S. EPA, 2005a).	An MOA for liver carcinogenicity could not be established, in absence to indicate otherwise rat liver tumors were considered relevant to humans (U.S. EPA, 2005a).
Selection of data set: No other studies are available.	Saito et al. (2013),JPEC (2010b) was selected to derive cancer risks for humans.	Saito et al. (2013), JPEC (2010b) was a well-conducted inhalation study which reported a positive exposure-response trend for liver tumors in male rats. Additional bioassays might add support to the findings or provide results for different (possibly lower) doses.
Selection of dose metric: Alternative could ↑ inhalation unit risk.	Administered concentration was used.	Modeling based on the PBPK-derived internal dose metric of ETBE metabolism decreased the BMCL by 35%. However, PBPK modeling was not used for the dose-response because there was no human model.
Interspecies extrapolation of dosimetry and risk: Alternatives could ↓ or ↑ inhalation unit risk.	The default approach for a Category 3 gas was used.	No data suggest an alternative approach. Although the true human correspondence is unknown, this overall approach is expected to neither overestimate nor underestimate human equivalent risks.
Dose-response modeling: Alternatives could ↓ or ↑ slope factor.	Used multistage dose-response model to derive a BMC and BMCL	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 (U.S. EPA, 2005a).
Statistical uncertainty at POD: ↓ inhalation unit risk 1.4-fold if BMC used as the POD rather than BMCL.	BMCL (preferred approach for calculating 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 ↑ inhalation unit risk 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, discussed in Section 1.3.3. No chemical-specific data are available, however, to determine the extent of enhanced sensitivity due to ETBE-induced carcinogenicity. Beyond ALDH deficiency, no chemical-specific data are available to determine the range of human toxicodynamic variability or sensitivity,

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
		including the susceptibility of children. 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

No previous cancer assessment for ETBE is available in 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, 2005b), either default or chemical-specific age-dependent adjustment factors (ADAFs) are recommended to account for early-life exposure to carcinogens that act through a mutagenic MOA. Because chemical-specific lifestage susceptibility data for cancer are not available, and because the MOA for ETBE carcinogenicity is not known (see Section 1.3.2), application of ADAFs is not recommended.

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