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Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol)

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ABBREVIATIONS

AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
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
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertain factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
U.S.	United States

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PREFACE

This Toxicological Review critically reviews the publicly available studies on *tert*-butyl alcohol (*tert*-butanol) 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 is the first IRIS assessment for this chemical.

Toxicological Reviews for *tert*-butanol and ethyl *tert*-butyl ether (ETBE) were developed simultaneously because they have several overlapping scientific aspects.

- *tert*-Butanol is one of the primary metabolites of ETBE, and some of the toxicological effects of ETBE are attributed to *tert*-butanol. Therefore, data on ETBE are considered informative for the hazard identification and dose-response assessment of *tert*-butanol, and vice versa.
- The scientific literature for the two chemicals includes data on α_{2u}-globulin-related nephropathy; therefore, a common approach was employed to evaluate these data as they relate to the mode of action for kidney effects.
- A combined physiologically based pharmacokinetic (PBPK) model for *tert*-butanol and ETBE in rats was modified to support the dose-response assessments for these chemicals (Salazar et al., 2015).

A public meeting was held in December 2013 to obtain input on preliminary materials for *tert*-butanol, including draft literature searches and associated search strategies, evidence tables, and exposure-response arrays prior to the development of the IRIS assessment. All public comments provided were taken into consideration in developing the draft assessment. The complete set of public comments is available on the docket at http://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2013-0111).

Organ/system-specific reference values are calculated based on kidney and thyroid toxicity data. 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. The findings of this assessment and related documents produced during its development are available on the IRIS website (http://www.epa.gov/iris). 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

tert-Butanol primarily is an anthropogenic substance that is produced in large quantities (HSDB, 2007) from several precursors, including 1-butene, isobutylene, acetyl chloride and dimethylzinc, and *tert*-butyl hydroperoxide. The domestic production volume of *tert*-butanol, including imports, was approximately 4 billion pounds in 2012 (U.S. EPA, 2014).

tert-Butanol has been used as a fuel oxygenate, an octane booster in unleaded gasoline, and a denaturant for ethanol. From 1997 to 2005, the annual *tert*-butanol volume found in gasoline ranged from approximately 4 million to 6 million gallons. During that time, larger quantities were used to make methyl *tert*-butyl ether (MTBE) and ETBE. MTBE and ETBE are fuel oxygenates that were used in the United States prior to 2007 at levels of more than 2 billion gallons annually. Current use levels of MTBE and ETBE in the United States are much lower, but use in Europe and Asia remains strong.[1]

tert-Butanol has been used for a variety of other purposes, including as a dehydrating agent and solvent. As such, it is added to lacquers, paint removers, and nail enamels and polishes. *tert*-Butanol also is used to manufacture methyl methacrylate plastics and flotation devices. Cosmetic and food-related uses include the manufacture of flavors, and, because of its camphor-like aroma, it also is used to create artificial musk, fruit essences, and perfume (HSDB, 2007). It is used in coatings on metal and paperboard food containers (Cal/EPA, 1999) and industrial cleaning compounds, and can be used for chemical extraction in pharmaceutical applications (HSDB, 2007).

Fate and Transport

Soil

tert-Butanol is expected to be highly mobile in soil due to its low affinity for soil organic matter. Rainwater or other water percolating through soil is expected to dissolve and transport most *tert*-butanol present in soil, potentially leading to groundwater contamination. Based on its vapor pressure, *tert*-butanol's volatilization from soil surfaces is expected to be an important dissipation process (HSDB, 2007). As a tertiary alcohol, *tert*-butanol is expected to degrade more slowly in the environment compared to primary (e.g., ethanol) or secondary (e.g., isopropanol) alcohols. In anoxic soil conditions, the half-life of *tert*-butanol is estimated to be months (approximately 200 days). Microbial degradation rates are increased in soils supplemented with nitrate and sulfate nutrients (HSDB, 2007).

[1] http://www.ihs.com/products/chemical/planning/ceh/gasoline-octane-improvers.aspx.

Water

tert-Butanol is expected to volatilize from water surfaces within 2 to 29 days and does not readily adsorb to suspended solids and sediments in water (HSDB, 2007). Biodegradation in aerobic water occurs over weeks to months and in anaerobic aquatic conditions, the biodegradation rate decreases. Bioconcentration of *tert*-butanol in aquatic organisms is low (HSDB, 2007).

Air

tert-Butanol primarily exists as a vapor in the ambient atmosphere. Vapor-phase *tert*-butanol is degraded in the atmosphere by reacting with photochemically produced hydroxyl radicals with a half-life of 14 days (HSDB, 2007).

Occurrence in the Environment

The Toxics Release Inventory (TRI) Program National Analysis Report estimated that more than 1 million pounds of *tert*-butanol has been released into the soil from landfills, land treatment, underground injection, surface impoundments, and other land disposal sources. The TRI program also estimated that 476,266 pounds of *tert*-butanol was released into the atmosphere from fugitive emissions and point sources (U.S. EPA, 2012c). In California, air emissions of *tert*-butanol from stationary sources are estimated to be at least 27,000 pounds per year, based on data reported by the state's Air Toxics Program (Scorecard, 2014). The TRI National Analysis Report estimated 7,469 pounds of *tert*-butanol was released into surface waters from point and nonpoint sources in 2011 (U.S. EPA, 2012c).

tert-Butanol has been identified in drinking water wells throughout the United States (HSDB, 2007). California's Geotracker Database[2] lists 3,496 detections of *tert*-butanol in groundwater associated with contaminated sites in that state since 2011. *tert*-Butanol also has been detected in drinking water wells in the vicinity of landfills (U.S. EPA, 2012c). Additionally, *tert*-Butanol leaking from underground storage tanks could be a product of MTBE and ETBE, which can degrade to form *tert*-butanol in soils (HSDB, 2007). The industrial chemical *tert*-butyl acetate also can degrade to form *tert*-butanol in animals post exposure and in the environment.

Ambient outdoor air concentrations of *tert*-butanol vary according to proximity to urban areas (HSDB, 2007).

General Population Exposure

tert-Butanol exposure can occur in many different settings. Releases from underground storage tanks could potentially result in exposure for people who get their drinking water from wells. Due to its high environmental mobility and resistance to biodegradation, *tert*-butanol has the potential to contaminate and persist in groundwater and soil (HSDB, 2007).

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

Ingestion of contaminated food can be a source of *tert*-butanol exposure through its use as a coating in metallic and paperboard food containers (Cal/EPA, 1999), and *tert*-butanol has been detected in food (HSDB, 2007). Internal exposure to *tert*-butanol also can occur as a result of ingestion of MTBE or ETBE, as *tert*-butanol is a metabolite of these compounds (NSF International, 2003).

Other human exposure pathways include inhalation, lactation and, to a lesser extent, dermal contact. Inhalation exposure can occur due to the chemical's volatility and release from industrial processes, consumer products, and contaminated sites (HSDB, 2007). *tert*-Butanol has been identified in mother's milk (HSDB, 2007). Dermal contact is a viable route of exposure through handling consumer products containing *tert*-butanol (NSF International, 2003).

Assessments by Other National and International Health Agencies

Toxicity information on *tert*-butanol has been evaluated by the National Institute for Occupational Safety and Health (NIOSH, 2007), the Occupational Safety and Health Administration (OSHA, 2006), and the Food and Drug Administration (FDA, 2011a, b). The results of these assessments are presented in Appendix A of the Supplemental Information to this Toxicological Review. Of importance to recognize is that these 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 to IRIS assessments is being revised based on comments received from external peer reviewers and the public, and based on IRIS Program experience with the implementation of systematic review methods. Subsequent drafts of the tert-butanol assessment will include the revised Preamble.

1. Scope of the IRIS Program

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

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

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

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

2. Process for developing and peer-reviewing IRIS assessments

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

Before beginning an assessment, the IRIS program discusses the scope with other EPA programs and regions to ensure that the

assessment will meet their needs. Then a public meeting on problem formulation invites discussion of the key issues and the studies and analytical approaches that might contribute to their resolution.

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

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

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

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

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

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

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

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

3. Identifying and selecting pertinent studies

3.1. Identifying studies

Before beginning an assessment, the EPA conducts a comprehensive search of the primary scientific literature. The literature

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

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

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

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

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

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

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

3.2. Selecting pertinent epidemiologic studies

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

- Cohort studies, case-control studies, and some population-based surveys (for example, NHANES) provide the strongest epidemiologic evidence, especially if they collect information about individual exposures and effects.
- Ecological studies (geographic correlation studies) relate exposures and effects by geographic area. They can provide strong evidence if there are large exposure contrasts between geographic areas, relatively little exposure variation within study areas, and population migration is limited.
- Case reports of high or accidental exposure lack definition of the population at risk and the expected number of cases. They can provide information about a rare effect or about the relevance of analogous results in animals.

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

3.3. Selecting pertinent experimental studies

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

- Studies of oral, inhalation, or dermal exposure involve passage through an absorption barrier and are considered

most pertinent to human environmental exposure.

- Injection or implantation studies are often considered less pertinent but may provide valuable toxicokinetic or mechanistic information. They also may be useful for identifying effects in animals if deposition or absorption is problematic (for example, for particles and fibers).

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

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

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

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

4. Evaluating the quality of individual studies

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

4.1. Evaluating the quality of epidemiologic studies

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

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

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating

epidemiologic studies of these effects (U.S. EPA, 2005a, 1998b, 1996, 1991b).

4.2. Evaluating the quality of experimental studies

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

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

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

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (U.S. EPA, 2005a, 1998b, 1996, 1991b). In multi-generation studies, agents that produce developmental effects at doses that are not toxic to the maternal animal are of special concern. Effects that occur at doses associated with mild maternal toxicity are not assumed to result only from maternal toxicity. Moreover, maternal effects may be reversible, while effects on the offspring may be permanent (U.S. EPA, 1998b, §3.1.2.4.5.4; 1991b, §3.1.1.4),.

4.3. Reporting study results

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

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

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

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

5. Evaluating the overall evidence of each effect

5.1. Concepts of causal inference

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

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

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

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

Specificity of association: As originally intended, this refers to one cause associated with one effect. Current understanding that many agents cause multiple effects and many effects have multiple causes make this a less informative aspect of causation, unless the effect is rare or unlikely to have multiple causes.

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

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

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

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

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

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

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

5.2. Evaluating evidence in humans

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

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

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

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

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

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

5.3. Evaluating evidence in animals

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

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

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

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

Negative or null results do not invalidate positive results in a different experimental system. The EPA regards all as valid observations and looks to explain differing results using mechanistic information (for example, physiologic or metabolic differences across test systems) or methodological

differences (for example, relative sensitivity of the tests, differences in dose levels, insufficient sample size, or timing of dosing or data collection).

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

In evaluating evidence of genetic toxicity:

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

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

5.4. Evaluating mechanistic data

Mechanistic data can be useful in answering several questions.

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

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

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

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

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

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

2) **Is the hypothesized mode of action relevant to humans?** The assessment reviews the key events to identify critical similarities and differences between the test animals and humans. Site concordance is not assumed between animals and humans, though it may hold

for certain effects or modes of action. Information suggesting quantitative differences in doses where effects would occur in animals or humans is considered in the dose-response analysis. Current levels of human exposure are not used to rule out human relevance, as IRIS assessments may be used in evaluating new or unforeseen circumstances that may entail higher exposures.

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

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

For cancer, the assessment evaluates evidence of a mutagenic mode of action to guide extrapolation to lower doses and consideration of susceptible lifestages. Key data include the ability of the agent or a metabolite to react with or bind to DNA, positive results in multiple test systems, or similar properties and structure-activity relationships to mutagenic carcinogens (U.S. EPA, 2005a ,§2.3.5).

5.5. Characterizing the overall weight of the evidence

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

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

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

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

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

Not likely to be carcinogenic to humans: There is robust evidence for concluding that there is no basis for concern. There

may be no effects in both sexes of at least two appropriate animal species; positive animal results and strong, consistent evidence that each mode of action in animals does not operate in humans; or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

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

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

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

Likely to be a causal relationship: Sufficient evidence that a causal relationship is likely, but important uncertainties remain. For example, observational studies show an association but co-exposures are difficult to address or other lines of evidence are limited or inconsistent; or multiple animal studies from different laboratories demonstrate effects and there are limited or no human data.

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

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

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

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

6. Selecting studies for derivation of toxicity values

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

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

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

extrapolation to levels found in the environment.

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

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

7. Deriving toxicity values

7.1. General framework for dose-response analysis

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

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

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

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

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

7.2. Modeling dose to sites of biologic effects

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

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

- Intermittent study exposures are standardized to a daily average over the duration of exposure. For chronic effects, daily exposures are averaged over the lifespan. Exposures during a critical period, however, are not averaged over a longer duration (U.S. EPA, 2005a, §3.1.1; 1991b, §3.2).
- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.

- Oral doses are scaled allometrically using mg/kg$^{3/4}$-day as the equivalent dose metric across species. Allometric scaling pertains to equivalence across species, not across lifestages, and is not used to scale doses from adult humans or mature animals to infants or children (U.S. EPA, 2011; 2005a, §3.1.3).
- Inhalation exposures are scaled using dosimetry models that apply species-specific physiologic and anatomic factors and consider whether the effect occurs at the site of first contact or after systemic circulation (U.S. EPA, 2012a; 1994, §3).

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

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

7.3. Modeling response in the range of observation

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

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

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

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

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

7.4. Extrapolating to lower doses and response levels

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation, typically used for cancer data, considers what is known about modes of action (U.S. EPA, 2005a, §3.3.1 and §3.3.2).

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

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

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

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

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

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

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

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

7.5. Considering susceptible populations and lifestages

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

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

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

3) In the absence of chemical-specific data, the EPA has developed *age-dependent adjustment factors* for early-life exposure to potential carcinogens that have a mutagenic mode of action. There is evidence of early-life susceptibility to

various carcinogenic agents, but most epidemiologic studies and cancer bioassays do not include early-life exposure. To address the potential for early-life susceptibility, the EPA recommends (U.S. EPA, 2005b, §5):

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

7.6. Reference values and uncertainty factors

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

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

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

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

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

Adverse-effect level to no-observed-adverse-effect level. If a point of departure is based on a lowest-observed-adverse-effect level, the assessment must infer a dose where such effects are not expected. This can be a matter of great uncertainty, especially if there is no evidence available at lower doses. A factor of 10 is applied to account for the uncertainty in making this inference. A factor other than 10 may be used, depending on the magnitude and nature of the response and the shape of the dose-response curve (U.S. EPA, 2002, §4.4.5; 1998b, §4.2; 1996, §4; 1994, §4.3.9.1; 1991b, §3.4).

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

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

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

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

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

7.7. Confidence and uncertainty in the reference values

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

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

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

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

These criteria are consistent with guidelines for systematic reviews that evaluate the quality of evidence. These also focus on whether further research would be likely to change confidence in the estimate of effect (Guyatt et al., 2008b).

All assessments discuss the significant uncertainties encountered in the analysis. The EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a, §3.6). For example, the discussion distinguishes model

uncertainty (lack of knowledge about the most appropriate experimental or analytic model) and parameter uncertainty (lack of knowledge about the parameters of a model). Assessments also discuss human variation (interpersonal differences in biologic susceptibility or in exposures that modify the effects of the agent).

August 2013

EXECUTIVE SUMMARY

Summation of Occurrence and Health Effects

tert-Butanol does not occur naturally; it is produced by humans for multiple purposes, such as a solvent for paints, a denaturant for ethanol and several other alcohols, a dehydrating agent, and in the manufacture of flotation agents, fruit essences, and perfumes. *tert*-Butanol also is a primary metabolite of methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE). Exposure to *tert*-butanol primarily occurs through breathing air containing *tert*-butanol vapors and consuming contaminated water or foods. Exposure can also occur through direct skin contact.

Animal studies demonstrate that chronic oral exposure to *tert*-butanol is associated with kidney and thyroid effects. Developmental effects (e.g., reduced fetal viability) have been observed in short-term exposure to high levels of *tert*-butanol (via oral or inhalation exposure) in animals. Neurodevelopmental effects also have been observed, but results were inconsistent. No chronic inhalation exposure studies have been conducted. There is suggestive evidence that *tert*-butanol is carcinogenic to humans based on renal tumors in male rats and thyroid tumors in female mice.

Effects Other Than Cancer Observed Following Oral Exposure

Kidney effects are a potential human hazard of oral exposure to *tert*-butanol. Kidney toxicity was observed in males and females in two strains of rats. Kidney weights were increased in male and female rats after 13 weeks or 15 months of treatment. Histopathological examination in male and female rats observed increased incidence or severity of nephropathy after 13 weeks of oral exposure, increased severity of nephropathy after a 2-year oral exposure, and increased transitional epithelial hyperplasia after 2 years of oral exposure. Additionally, increased suppurative inflammation was noted in females after 2 years of oral exposure. In one strain of mice, the only kidney effect observed was an increase in kidney weight (absolute or relative) in female mice after 13 weeks, but no treatment-related histopathological lesions were reported in the kidneys of male or female mice at 13 weeks or 2 years. A mode of action (MOA) analysis determined that *tert*-butanol exposure induces a male rat-specific α_{2u}-globulin-associated nephropathy. *tert*-Butanol, however, is a weak inducer of α_{2u}-globulin-nephropathy, and is not the sole process contributing to renal tubule nephropathy. Chronic progressive nephropathy (CPN) may also be involved in some of the noncancer effects, but the evidence is inconclusive. Endpoints specifically related to either α_{2u}-globulin-nephropathy or CPN were not considered for kidney hazard identification. Changes in kidney weights, transitional epithelial hyperplasia, suppurative

inflammation, and severity and incidence of nephropathy, however, are considered to result from *tert*-butanol exposure and are appropriate for identifying a hazard to the kidney.

There is suggestive evidence of developmental toxicity following *tert*-butanol exposure. Developmental effects include increased fetal loss, decreased fetal body weight, and increased skeletal variations. At this time, no conclusions were drawn in regard to reproductive system toxicity. There is inadequate information at this time to draw conclusions regarding neurodevelopmental toxicity, liver, and urinary bladder toxicity.

Oral Reference Dose (RfD) for Effects Other Than Cancer

Kidney toxicity, represented by kidney transitional epithelial hyperplasia, was chosen as the basis for the overall oral reference dose (RfD) (see Table ES-1). The chronic study by NTP (1995) and the observed kidney effects were used to derive the RfD. The endpoint of transitional epithelial hyperplasia was selected as the critical effect because it was observed in both rat sexes consistently, 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 16 mg/kg-day. The BMDL was converted to a human equivalent dose using body weight$^{3/4}$ scaling, and the value of 3.84 mg/kg-day was used as the point of departure (POD) for RfD derivation (U.S. EPA, 2011).

The overall RfD was calculated by dividing the POD for kidney transitional epithelial hyperplasia by a composite uncertainty factor (UF) of 30 to account for the extrapolation from animals to humans (3) and for interindividual differences in human susceptibility (10).

Table ES-1. Organ/system-specific RfDs and overall RfD for *tert*-butanol

Hazard	Basis	Point of departure* (mg/kg-day)	UF	Chronic RfD (mg/kg-day)	Study exposure description	Confidence
Kidney	Transitional epithelial hyperplasia	3.8	30	1×10^{-1}	Chronic	High
Overall RfD	**Kidney**	**3.8**	**30**	**1×10^{-1}**	**Chronic**	**High**

*HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011).

Effects Other Than Cancer Observed Following Inhalation Exposure

Kidney effects are a potential human hazard of inhalation exposure to *tert*-butanol. Although no effects were observed in mice, kidney weights were increased in male and female rats following 13 weeks of inhalation exposure. In addition, nephropathy severity increased in male rats. No human studies are available to evaluate the effects of inhalation exposure. As discussed above for oral effects, endpoints specifically related to either α_{2u}globulin nephropathy or CPN were

not considered for kidney hazard identification. Changes in kidney weights and severity of nephropathy, however, are considered a result of *tert*-butanol exposure and are appropriate for identifying a hazard to the kidney.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

Kidney toxicity, represented by transitional epithelial hyperplasia, was chosen as the basis for the inhalation reference concentration (RfC) (see Table ES-2). Although endpoints from a route-specific study were considered, the availability of a physiologically based pharmacokinetic (PBPK) model for *tert*-butanol in rats [modified by Salazar et al. (2015)] allowed for more specific and sensitive equivalent inhalation PODs derived from a route-to-route extrapolation from the PODs of the oral NTP (1995) study. The POD adjusted for the human equivalent concentration (HEC) was 26.1 mg/m^3 based on transitional epithelial hyperplasia.

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

Table ES-2. Organ/system-specific RfCs and overall RfC for *tert*-butanol

Hazard	Basis	Point of departure* (mg/m^3)	UF	Chronic RfC (mg/m^3)	Study exposure description	Confidence
Kidney	Transitional epithelial hyperplasia	26.1	30	9×10^{-1}	Chronic	High
Overall RfC	**Kidney**	**26.1**	**30**	**9×10^{-1}**	**Chronic**	**High**

*Continuous inhalation human equivalent concentration that leads to the same average blood concentration of *tert*-butanol as continuous oral exposure at the BMDL.

Evidence of Human Carcinogenicity

Under the EPA's cancer guidelines (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* for *tert*-butanol. *tert*-Butanol induced kidney tumors in male (but not female) rats and thyroid tumors (primarily benign) in male and female mice following long-term administration in drinking water (NTP, 1995). The potential for carcinogenicity applies to all routes of human exposure.

Quantitative Estimate of Carcinogenic Risk from Oral Exposure

A quantitative estimate of carcinogenic potential from oral exposure to *tert*-butanol was based on the increased incidence of thyroid follicular cell adenomas in female B6C3F$_1$ mice, and thyroid follicular cell adenomas and carcinomas in male B6C3F$_1$ mice (NTP, 1995). 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 *tert*-butanol was considered to have "suggestive evidence of carcinogenic potential," the NTP study was well conducted and quantitative analysis could be useful for providing a sense of the magnitude of potential carcinogenic risk (U.S. EPA, 2005a). A slope factor was derived for thyroid tumors in female and male mice. The modeled *tert*-butanol POD was scaled to HEDs according to EPA guidance by converting the BMDL$_{10}$ on the basis of (body weight)$^{3/4}$ scaling (U.S. EPA, 2011, 2005a). Using linear extrapolation from the BMDL$_{10}$, a human equivalent oral slope factor was derived (slope factor = 0.1/BMDL$_{10}$). The resulting oral slope factor is **5×10^{-4} per mg/kg-day**.

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

No chronic inhalation exposure studies to *tert*-butanol are available. Lifetime exposure to *tert*-butanol has been associated with increased renal tubule adenomas and carcinomas as well as thyroid follicular cell adenomas and carcinomas. As stated above, the rat kidney tumors are unsuitable for quantitative analysis as there is not enough data to determine the relative contribution of α_{2u}-globulin nephropathy and other processes to the overall kidney tumor response. Although the mouse thyroid tumors served as the basis for the oral slope factor, route-to-route extrapolation is not possible for these thyroid effects in mice because the only PBPK model available is for rats. Therefore, no quantitative estimate of carcinogenic risk could be determined for inhalation exposure.

Susceptible Populations and Lifestages for Cancer and Noncancer

In vitro studies suggest that cytochrome P-450 (CYP450) (Cederbaum et al., 1983; Cederbaum and Cohen, 1980), plays a role in the metabolism of *tert*-butanol. No studies, however, have identified the specific CYPs responsible for the biotransformation of *tert*-butanol. Various CYPs are under-expressed in the mouse fetus and neonate (Lee et al., 2011) and decreased in older mice (Lee et al., 2011) and rats (Lee et al., 2008). Decreased ability to detoxify and transport *tert*-butanol out of the body could result in increased susceptibility to *tert*-butanol.

With regard to cancer, differences in lifestage sensitivity to chemically induced thyroid carcinogenesis are unknown (U.S. EPA, 1998a). An increased incidence of thyroid tumors was identified in mice after *tert*-butanol exposure, and human studies have demonstrated that children are more sensitive than adults are to thyroid carcinogenesis resulting from ionizing radiation.

Collectively, there is little evidence on *tert*-butanol itself to identify any populations or lifestages that may be especially susceptible.

Key Issues Addressed in Assessment

An evaluation of whether *tert*-butanol caused α_{2u}-globulin-associated nephropathy was performed. The presence of α_{2u}-globulin in the hyaline droplets was confirmed in male rats by α_{2u}-globulin immunohistochemical staining. Linear mineralization and tubular hyperplasia were reported in male rats, although only in the chronic study. Other subsequent steps in the pathological sequence, including necrosis, exfoliation, and granular casts, either were absent or inconsistently observed across subchronic or chronic studies. None of these effects occurred in female rats or in either sex of mice, although these endpoints were less frequently evaluated in these models. Evidence implies an α_{2u}-globulin MOA is operative, although it is relatively weak in response to *tert*-butanol and is not solely responsible for the renal tubule nephropathy observed in male rats. CPN also is instrumental in renal tubule nephropathy, in both male and female rats. Several other effects in the kidney unrelated to α_{2u}-globulin or CPN, however, were observed in female or male rats (U.S. EPA, 1991a), including suppurative inflammation in female rats, transitional epithelial hyperplasia in male and female rats, and increased kidney weights in both sexes of rats (NTP, 1997, 1995). These specific effects are considered the result of *tert*-butanol exposure and therefore, relevant to humans.

Concerning cancer, α_{2u}-globulin accumulation is indicated as relatively weak in response to *tert*-butanol exposure and not the sole mechanism responsible for the renal tubule carcinogenicity observed in male rats. Although CPN and other effects induced by both α_{2u}-globulin processes and *tert*-butanol play a role in renal tubule nephropathy, the evidence indicates that CPN does not induce the renal tubule tumors associated with *tert*-butanol exposure in male rats, suggesting that other, unknown processes contribute to renal tumor development. Based on this analysis of available MOA data, these renal tumors are considered relevant to humans (U.S. EPA, 1991a).

In addition, an increase in the incidence of thyroid follicular cell adenomas was observed in male and female mice in a 2-year drinking water study (NTP, 1995). Thyroid follicular cell hyperplasia was considered a preneoplastic effect associated with the thyroid tumors, and the incidences of follicular cell hyperplasias were elevated in both male and female B6C3F$_1$ mice following exposure. U.S. EPA (1998a) describes the procedures the Agency uses in evaluating potential human cancer hazard and dose-response assessments for chemicals that are animal thyroid carcinogens. The available database is inadequate in four of the five required areas (U.S. EPA, 1998a), suggesting that an antithyroid MOA is not operating in mouse thyroid follicular cell tumorigenesis. No other MOAs for thyroid tumors were identified, and the mouse thyroid tumors are considered relevant to humans (U.S. EPA, 2005a, 1998a).

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

A literature search and screening strategy were used to identify literature characterizing the health effects of *tert*-butanol. This 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 *tert*-butanol, and remaining references were sorted into categories for further evaluation. This section describes the literature search and screening strategy in detail.

The chemical-specific search was conducted in four online scientific databases, including PubMed, Web of Science, Toxline, and TSCATS through May 2015, using the keywords and limits described in Table LS-1. The overall literature search approach is shown graphically in Figure LS-1. An additional seven citations were obtained using additional search strategies described in Table LS-2. After electronically eliminating duplicates from the citations retrieved through these databases, 2,648 unique citations were identified.

The resulting 2,648 citations were screened for pertinence and separated into categories as presented in Figure LS-1 using the title and either abstract or full text, or both, to examine the health effects of *tert*-butanol exposure. The inclusion and exclusion criteria used to screen the references and identify sources of health effects data are provided in Table LS-3.

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

- 200 references were identified as "Sources of Mechanistic and Toxicokinetic Data" and "Sources of Supplementary Health Effects Data"; these included 39 studies describing physiologically based pharmacokinetic (PBPK) models and other toxicokinetic information, 73 studies providing genotoxicity and other mechanistic information, 1 human case report, 74 irrelevant exposure paradigms (including acute, dermal, eye irritation, and injection studies), 6 preliminary toxicity studies, and 7 physical dependency studies. Information from these studies was not extracted into evidence tables; however, these studies were considered as support for assessing *tert*-butanol health effects, for example, evaluation of mode of action and extrapolation of experimental animal findings to humans. Additionally, 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 exposure. 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.

- 63 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.

- 2,373 references were identified as not being pertinent (not on topic) to an evaluation of the health effects of *tert*-butanol 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 *tert*-butanol mixtures were not considered pertinent to the assessment because the separate effects of the gasoline or other chemical components could not be determined. Retrieving a large number of 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.

The complete list of references and the sorting of these materials can be found on the *tert*-butanol project page of the HERO website at https://hero.epa.gov/index.cfm/project/page/project_id/1543.

Selection of Studies for Inclusion in Evidence Tables

To summarize the important information systematically from the primary health effects studies in the *tert*-butanol database, evidence tables were constructed in a standardized tabular format as recommended by NRC (2011). Studies were arranged in evidence tables by effect, species, duration, and design, and not by quality. Of the studies that were retained after the literature search and screen, 12 studies were identified as "Sources of Health Effects Data" and were considered for extraction into evidence tables for hazard identification in Chapter 1. Initial review found two references (Cirvello et al., 1995; Lindamood et al., 1992) to be publications of the NTP (1995) data prior to the release of the final National Toxicology Program (NTP) report. One publication (Takahashi et al., 1993) in the "Supplementary Studies" category also was based on data from the NTP report. The interim publications and the final NTP report differed. The finalized NTP (1995) report was considered the more complete and accurate presentation of the data; therefore, this report was included in evidence tables and Cirvello et al. (1995), Takahashi et al. (1993), and Lindamood et al. (1992) were not. Data from the remaining 10 references in the "Sources of Health Effects Data" category 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 *tert*-butanol absorption and metabolism, pilot studies, and one case report were not included in the evidence tables. Short-term and acute studies (including an 18-day study and a 14-day study by NTP) using oral and inhalation exposures were performed primarily in rats and did not differ qualitatively from the results of the longer studies (i.e., ≥30-day exposure studies). These were grouped as supplementary studies, however, because the database of chronic and subchronic rodent studies was considered sufficient for evaluating chronic health effects of *tert*-butanol 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 may be discussed in the narrative sections of Chapter 1 and are described in sections such as the "Mode of Action Analysis" to augment the discussion or presented in appendices, if they provide additional information.

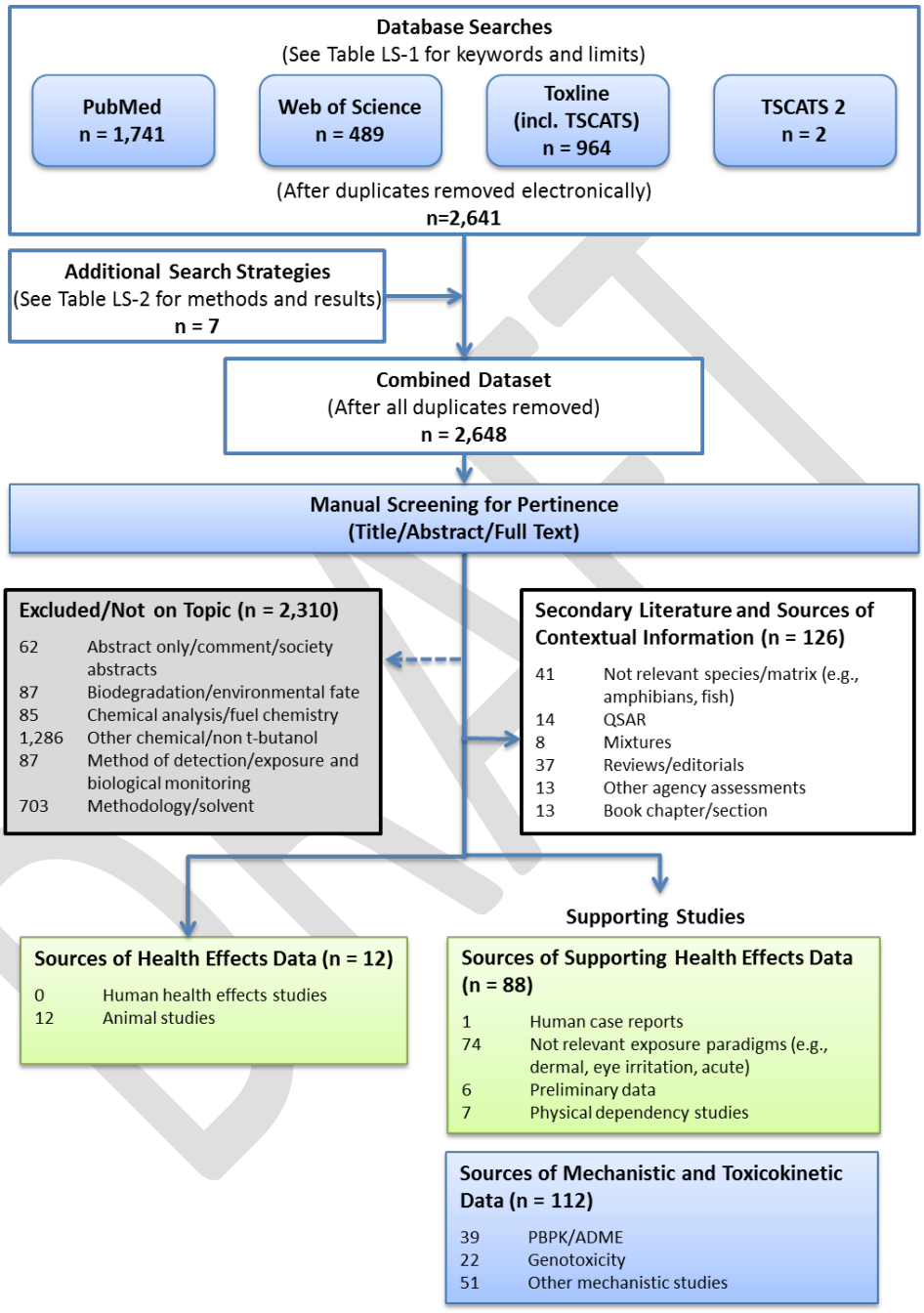


Figure LS-1. Summary of literature search and screening process for *tert*-butanol.

Table LS-1. Details of the search strategy employed for *tert*-butanol

Database (Search Date)	Keywords	Limits
PubMed (12/20/2012) (4/17/2014) (5/13/2015)	*tert*-butanol *OR 75-65-0[rn] OR "t-butyl hydroxide" OR "2-methyl-2-propanol" OR "trimethyl carbinol" OR "t-butyl alcohol" OR tert-butanol OR "tert-butyl alcohol" OR tert-butyl alcohol[mesh]*	None
Web of Science (12/20/2012) (4/17/2014) (5/13/2015)	Topic = *(tert-butanol OR 75-65-0 OR "t-butyl hydroxide" OR "2-methyl-2-propanol" OR "trimethyl carbinol" OR "t-butyl alcohol" OR "tert-butanol" OR "tert-butyl alcohol")*	Refined by: Research Areas = (cell biology OR respiratory system OR microscopy OR biochemistry molecular biology OR gastroenterology OR hepatology OR public environmental occupational health OR oncology OR physiology OR cardiovascular system cardiology OR toxicology OR life sciences biomedicine other topics OR hematology OR pathology OR neurosciences neurology OR developmental biology)
Toxline (includes TSCATS) (1/11/2013) (4/17/2014) (5/13/2015)	*tert*-butanol *OR 75-65-0 [rn] OR t-butyl hydroxide OR 2-methyl-2-propanol OR trimethyl carbinol OR t-butyl alcohol OR tert-butanol OR tert-butyl alcohol OR tert-butyl alcohol*	Not PubMed
TSCATS2 (1/4/2013) (4/17/2014) (5/13/2015)	75-65-0	None

Table LS-2. Summary of additional search strategies for *tert*-butanol

Approach used	Source(s)	Date performed	Number of additional references identified
Manual search of citations from reviews	Review article: McGregor (2010). *Tertiary*-butanol: A toxicological review. Crit Rev Toxicol 40(8): 697-727.	1/2013	5
	Review article: Chen (2005). Amended final report of the safety assessment of *t*-butyl alcohol as used in cosmetics. Int J Toxicol 24(2): 1-20.	1/2013	2
Manual search of citations from reviews conducted	IPCS (1987a). Butanols: Four isomers: 1-butanol, 2-butanol, *tert*-butanol, isobutanol [WHO EHC]. Geneva,	1/2013	None

Approach used	Source(s)	Date performed	Number of additional references identified
by other international and federal agencies	Switzerland: World Health Organization.		
	OSHA (1992). Occupational safety and health guideline for *tert*-butyl alcohol. Cincinnati, OH: Occupational Safety and Health Administration.	1/2013	None

Table LS-3. Inclusion-exclusion criteria

	Inclusion criteria	Exclusion criteria
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, monkey, dog	• Ecological species* • Nonmammalian species*
Exposure	• Exposure is to *tert*-butanol • Exposure is measured in an environmental medium (e.g., air, water, diet) • Exposure via oral, inhalation, or dermal routes	• Study population is not exposed to *tert*-butanol • Exposure to a mixture only (e.g., gasoline containing *tert*-butanol) • Exposure via injection (e.g., intravenous) • Exposure pattern less relevant to chronic health effects (e.g., acute)
Outcome	• Study includes a measure of one or more health effect endpoints, including effects on the nervous, musculoskeletal, cardiovascular, immune, hematological, endocrine, respiratory, urinary, and gastrointestinal systems; reproduction; development; liver; kidney; eyes; skin; and cancer • Physical dependency studies where withdrawal symptoms were evaluated after removal of *tert*-butanol treatment	
Other		Not on topic, including: • Abstract only, editorial comments were not considered further because study was not potentially relevant • Bioremediation, biodegradation, or environmental fate of *tert*-butanol, 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 *tert*-butanol • Use of *tert*-butanol as a solvent or methodology for testing unrelated to *tert*-butanol

	Inclusion criteria	Exclusion criteria
		• Not chemical specific: Studies that do not involve testing of *tert*-butanol • Foreign language studies that were not considered further because, based on title or abstract, judged not potentially relevant • QSAR studies

*Studies that met this exclusion criterion were not considered a source of health effects data or supplementary health effects data/mechanistic and toxicokinetic data, but were considered as sources of contextual information.

Database Evaluation

For this draft assessment, 12 references reported on experimental animal studies that comprised the primary sources of health effects data; no studies were identified that evaluated humans exposed to *tert*-butanol (e.g., cohort studies, ecological studies). The animal studies were evaluated using the study quality considerations outlined in the Preamble, 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. As stated in the Preamble, studies were evaluated to identify the suitability of the study 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 *tert*-butanol 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 and statistical power to detect dose-related differences or trends
- Ascertainment of survival, vital signs, disease or effects, and cause of death
- Control of other variables that could influence the occurrence of effects

Additionally, several general considerations, presented in Table LS-4, were used in evaluating the animal studies. 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. Discussion of study strengths and limitations were 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 experimental animal studies, comprised entirely of studies performed in rats and mice, were associated with drinking water, oral gavage, liquid diets (i.e., maltose/dextrin), and inhalation exposures to *tert*-butanol. With the exception of neurodevelopmental studies, these sources were conducted according to Organisation for Economic Co-operation and Development Good Laboratory Practice (GLP) guidelines, presented extensive histopathological data, or clearly presented their methodology; thus, these studies are considered high quality. These studies include 2-year bioassays using oral exposures in rats and mice; two subchronic drinking water studies in rats and one in mice; an inhalation subchronic study in rats and mice; a reevaluation of the NTP (1995) rat data; two oral developmental studies; two inhalation developmental studies; and a single one-generation reproductive study that also evaluates other systemic effects (Table LS-5). A more detailed discussion of any methodological concerns that were identified precedes each endpoint evaluated in the hazard identification section. Overall, the experimental animal studies of *tert*-butanol involving repeated oral or inhalation exposure were considered to be of acceptable quality, and whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to *tert*-butanol.

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

Methodological feature	Considerations (relevant information extracted into evidence tables)
Test animal	Suitability of the species, strain, sex, and source of the 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 the 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 the 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 database

Study category	Study duration, species/strain, and administration method
Chronic	2-year study in F344 rats (drinking water) NTP (1995) 2-year study in B6C3F$_1$ mice (drinking water) NTP (1995)
Subchronic	13-week study in B6C3F$_1$ mice (drinking water) NTP (1995) 13-week study in F344 rats (drinking water) NTP (1995) 13-week study in F344 rats (inhalation) NTP (1997) 13-week study in B6C3F$_1$ mice (inhalation) NTP (1997) 10-week study in Wistar rats (drinking water) Acharya et al. (1997), Acharya et al. (1995)
Reproductive	One-generation reproductive toxicity study in Sprague-Dawley rats (gavage) Lyondell Chemical Co. (2004)
Developmental	Developmental study (GD 6–20) in Swiss Webster mice (diet) Daniel and Evans (1982) Developmental study (GD 6–18) in CBA/J mice (drinking water) Faulkner et al. (1989) Developmental study (GD 6–18) in C57BL/6J mice (drinking water) Faulkner et al. (1989) Developmental study (GD 1–19) in Sprague-Dawley rats (inhalation) Nelson et al. (1989)
Neurodevelopmental	Neurodevelopmental study (GD 6–20) in Swiss Webster mice (diet) Daniel and Evans (1982) Neurodevelopmental study (GD 1–19) in Sprague-Dawley rats (inhalation) Nelson et al. (1991)

1. HAZARD IDENTIFICATION

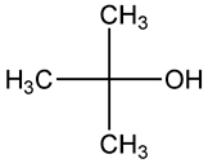
1.1. Overview of Chemical Properties and Toxicokinetics

1.1.1. Chemical Properties

tert-Butanol is a white crystalline solid or colorless, highly flammable liquid (above 25.7°C) with a camphor-like odor (NIOSH, 2005; IPCS, 1987a). *tert*-Butanol contains a hydroxyl chemical functional group; is miscible with alcohol, ether, and other organic solvents; and is soluble in water (IPCS, 1987a). Selected chemical and physical properties of *tert*-butanol are presented in Table 1-1.

Table 1-1. Physicochemical properties and chemical identity of *tert*-butanol

Characteristic	Information	Reference
Chemical name	*tert*-Butanol	HSDB (2007)
Synonyms/Trade names	*t*-Butyl alcohol; *tert*-Butanol; *tert*-Butyl alcohol; *t*-Butyl hydroxide; 1,1-Dimethylethanol; NCI-C55367; 2-Methyl-2-propanol; *tertiary* Butanol; Trimethyl carbinol; Trimethyl methanol; *t*-butyl alcohol; TBA	HSDB (2007) IPCS (1987b)
Chemical formula	$C_4H_{10}O$	HSDB (2007)
CASRN	75-65-0	HSDB (2007)
Molecular weight	74.12	HSDB (2007)
Melting point	25.7°C	HSDB (2007)
Boiling point	82.41°C	HSDB (2007)
Vapor pressure	40.7 mm Hg @ 25°C	HSDB (2007)
Density/Specific gravity	0.78581	HSDB (2007)
Flashpoint	11°C (closed cup)	HSDB (2007)
Water solubility at 25°C	1×10^6 mg/L	HSDB (2007)
Octanol/Water Partition Coefficient (Log K_{OW})	0.35	HSDB (2007)
Henry's Law Constant	9.05×10^{-6} atm-m^3/mole	HSDB (2007)
Odor threshold	219 mg/m^3	HSDB (2007)
Conversion factors	1 ppm = 3.031 mg/m^3 1 mg/m^3 = 0.324 ppm	IPCS (1987b)

Characteristic	Information	Reference
Chemical structure		HSDB (2007)

1.1.2. Toxicokinetics

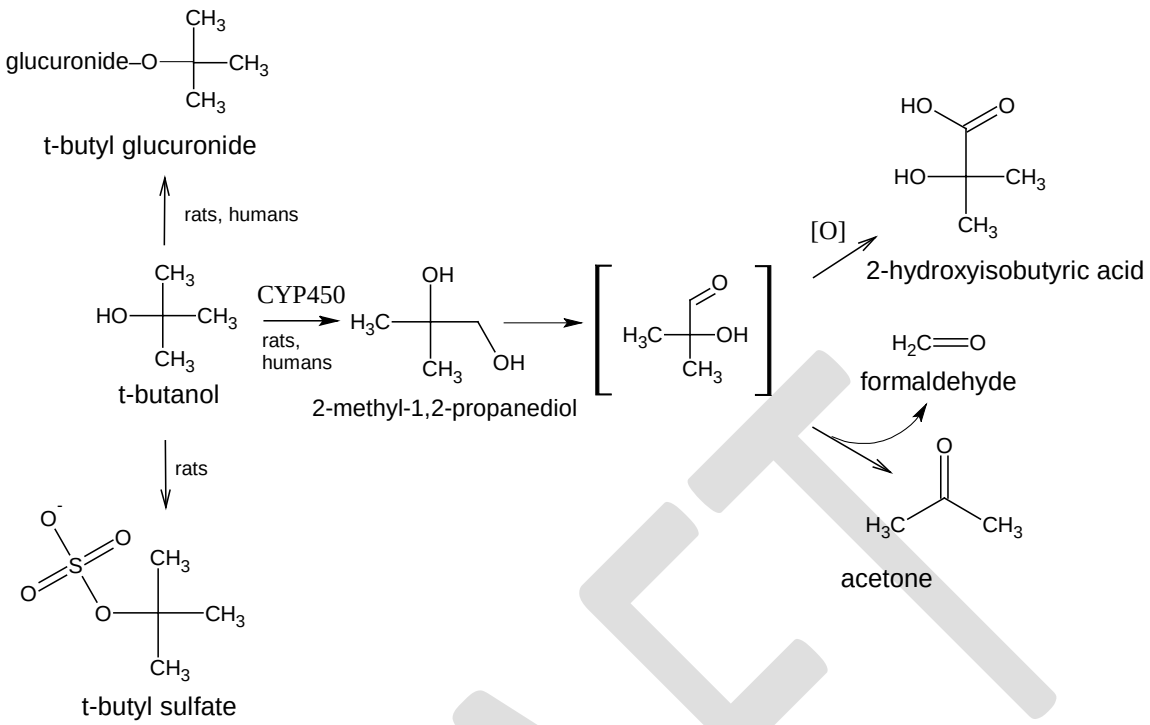
tert-Butanol is rapidly absorbed following exposure by oral and inhalation routes (see Appendix B, Section B.1.1). Studies in experimental animals indicate that 99% of the compound was absorbed after oral administration. Comparable blood levels of *tert*-butanol and its metabolites have also been observed after acute oral or inhalation exposures in rats (ARCO, 1983). In another study (Faulkner et al., 1989), blood concentrations indicated that absorption was complete at 1.5 hours following oral gavage doses of *tert*-butanol in female mice.

tert-Butanol is distributed throughout the body following oral, inhalation, and i.v. exposures (Poet et al., 1997; Faulkner et al., 1989; ARCO, 1983). Following exposure to *tert*-butanol in rats, *tert*-butanol was found in kidney, liver, and blood, with male rats retaining more *tert*-butanol than female rats (Williams and Borghoff, 2001).

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

Human data on the excretion of *tert*-butanol comes from studies of methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE) (Nihlén et al., 1998a, b). The half-life of *tert*-butanol in urine following MTBE exposure was 8.1 ± 2.0 hours (average of the 90.1- and 757-mg/m^3 MTBE doses); the half-life of *tert*-butanol in urine following ETBE exposure was 7.9 ± 2.7 hours (average of 104- and 210-mg/m^3 ETBE doses). These studies reported urinary levels of *tert*-butanol (not including downstream metabolites) to be less than 1% of administered MTBE or ETBE concentrations (Nihlén et al., 1998a, b). Amberg et al. (2000) also observed a similar half-life of 9.8 ± 1.4 hours after human exposure to ETBE of 170 mg/m^3. The half-life for *tert*-butanol in rat urine was 4.6 ± 1.4 hours at ETBE levels of 170 mg/m^3.

A more detailed summary of *tert*-butanol toxicokinetics is provided in Appendix B, Section B.1.



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

Figure 1-1. Biotransformation of *tert*-butanol in rats and humans.

1.1.3. Description of Toxicokinetic Models

No physiologically based pharmacokinetic (PBPK) models have been developed specifically for administration of *tert*-butanol. Some models have been used to study *tert*-butanol as the primary metabolite after oral or inhalation exposure to MTBE or ETBE. The most recent 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 more-detailed summary of the toxicokinetic models is provided in Appendix B, Section B.1.5.

A PBPK model for *tert*-butanol was modified by adapting previous models for MTBE and *tert*-butanol (Leavens and Borghoff, 2009; Blancato et al., 2007). The addition of a sequestered blood compartment for *tert*-butanol substantially improved the model fit. The alternative modification of changing to diffusion-limited distribution between blood and tissues also improved the model fit, but was considered less biologically plausible. Physiological parameters and partition coefficients were obtained from published measurements. The rate constants for *tert*-butanol metabolism and elimination were from a published PBPK model of MTBE with a *tert*-butanol subcompartment (Blancato et al., 2007). Additional model parameters were estimated by calibrating to data sets for i.v., oral, and inhalation exposures as well as repeated dosing studies for *tert*-butanol. Overall, the model produced acceptable fits to multiple rat time-course datasets of *tert*-butanol blood levels following either inhalation or oral gavage exposures.

1.1.4. Chemicals Extensively Metabolized to *tert*-Butanol

tert-Butanol is a metabolite of other compounds, including ETBE, MTBE, and *tert*-butyl acetate. Some of the toxicological effects observed in these compounds are attributed to *tert*-butanol. There are no assessments by national or international health agencies for ETBE. Animal studies demonstrate that chronic exposure to ETBE is associated with noncancer kidney effects, including increased kidney weights in male and female rats accompanied by increased chronic progressive nephropathy (CPN), urothelial hyperplasia (in males), and increased blood concentrations of total cholesterol, blood urea nitrogen, and creatinine (Saito et al., 2013; Suzuki et al., 2012). In these studies, increased liver weight and centrilobular hypertrophy also were observed in male and female rats exposed to ETBE. Liver adenomas and carcinomas were increased in male rats following 2-year inhalation exposure (Saito et al., 2013).

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 data on carcinogenicity in animals. ATSDR reported that inhalation exposure was associated with 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 1997, EPA's Office of Water concluded that MTBE is carcinogenic to animals and poses a potential carcinogenic potential to humans based on an increased incidence of Leydig cell adenomas of the testes, kidney tumors, lymphomas, and leukemia in exposed rats (U.S. EPA, 1997). In 1998, the International Agency for Research on Cancer (IARC) found "limited evidence" of MTBE carcinogenicity in animals and placed MTBE in Group 3 (i.e., not classifiable as to carcinogenicity in humans) (IARC, 1999). IARC reported that oral exposure in rats resulted in testicular tumors in males and lymphomas and leukemias (combined) in females; inhalation exposure in male rats resulted in renal tubule adenomas; and inhalation exposure in female mice resulted in hepatocellular adenomas (IARC, 1999).

No assessments by national or international agencies or chronic studies for *tert*-butyl acetate are available.

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 *tert*-butanol can affect kidneys in humans or animals. The database examining kidney effects following *tert*-butanol exposure contains eight studies from 5 references performed in rats or mice (Lyondell Chemical Co., 2004; Acharya et al., 1997; NTP, 1997; Acharya et al., 1995; NTP, 1995), and a reevaluation of the rat data from NTP (1995), published by Hard et al. (2011); no human data are

available. Studies using short-term and acute exposures that examined kidney effects are not included in the evidence tables; they are discussed in the text, however, if they provide data to inform mode of action (MOA) or hazard identification. *tert*-Butanol exposure resulted in kidney effects after both oral (drinking water) and inhalation exposure in both sexes of rats (Table 1-1, Table 1-2, Figure 1-1, and Figure 1-2); studies are arranged in the evidence tables first by effect, then by route, and then duration.

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 somewhat complicated by the common occurrence of age-related, spontaneous lesions characteristic of chronic progressive nephropathy (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 largely unknown (see further discussion below).

Kidney weight. Changes in kidney weight (absolute and relative to body weight) were observed in male and female F344 rats following exposures of 13 weeks (oral and inhalation) (NTP, 1997) and 15 months (oral) (NTP, 1995). Lyondell Chemical Co. (2004) also reported increases in absolute and relative kidney weight in Sprague-Dawley rats administered *tert*-butanol orally for approximately 10 weeks (tabular data presented in Supplemental Information to this Toxicological Review). Changes were observed in both male and female rats, which exhibited strong dose-related increases in absolute kidney weight (Spearman's rank coefficient > 0.78) following either oral or inhalation exposures (Figure 1-3). Of the oral (Figure 1-4 and inhalation (Figure 1-5) mouse studies, only inhalation exposure in female mice induced a strong dose-related increase (Spearman's rank coefficient = 0.9) in absolute kidney weights.

Measures of relative, as opposed to absolute, organ weight are sometimes preferred because they account for changes in body weight that might influence changes in organ weight (Bailey et al., 2004), although potential impact should be evaluated. For *tert*-butanol, body weight in exposed animals noticeably decreased at the high doses relative to controls in the oral 13-week and 2-year studies NTP (1995). In this case, the decreased body weight of the animals affects the relative kidney weight measures, resulting in an artificial exaggeration of changes. Thus, absolute weight was determined the more reliable measure of kidney weight change for this assessment. Additionally, a recent analysis indicates that increased 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). Although relative and absolute kidney weight data are both presented in exposure-response arrays (and in evidence tables in Supplemental Information), the absolute measures were considered more informative for determining *tert*-butanol hazard potential.

Kidney histopathology. Treatment-related histopathological changes were observed in the kidneys of male and female F344 rats following 13-week and 2-year oral exposures (NTP, 1995) and male F344 rats following a 13-week inhalation exposure (NTP, 1997). Similarly, male Wistar rats exposed for approximately 10 weeks exhibited an increase in histopathological kidney lesions (Acharya et al., 1997; Acharya et al., 1995). B6C3F$_1$ mice, however, did not exhibit histopathological changes when exposed for 13 weeks and 2 years via the oral route (NTP, 1995) and 13 weeks via the inhalation route (NTP, 1997). More specific details on the effects observed in rats, reported by NTP (1997, 1995) and Acharya et al. (1997); (1995) are described below.

Nephropathy and severity of nephropathy were reported in male and female rats in the 13-week oral studies (NTP, 1995). The nephropathy was characterized as "…a spontaneous background lesion…typically consist[ing] of scattered renal tubules lined by basophilic regenerating tubule epithelium." (NTP, 1995). NTP (1995) noted that the increase in severity of nephropathy was related to *tert*-butanol and "characterized by an increase in the number and size of foci of regeneration." The severity of nephropathy increased, compared with controls, in the 13-week male rats, which exhibited nephropathy in 94% of all exposed animals and 70% of controls. Conversely, lesion severity was unchanged in the females, although nephropathy incidence significantly increased with *tert*-butanol exposure. In the 13-week inhalation study (NTP, 1997), nephropathy was present in all but two male rats, including controls. NTP (1997) characterized the reported chronic nephropathy in control male rats as "1 to 3 scattered foci of regenerative tubules per kidney section. Regenerative foci were characterized by tubules with cytoplasmic basophilia, increased nuclear/cytoplasmic ratio, and occasionally thickened basement membranes and intraluminal protein casts." In exposed groups, the severity generally increased from minimal to mild with increasing dose as "evidenced by an increased number of foci." No treatment-related kidney histopathology was reported in the female rats exposed through inhalation (NTP, 1997).

In the 2-year oral study by NTP (1995), nephropathy was reported at 15 months and 2 years. The NTP (1995) characterization of nephropathy following chronic exposure included multiple lesions: "thickened tubule and glomerular basement membranes, basophilic foci of regenerating tubule epithelium, intratubule protein casts, focal mononuclear inflammatory cell aggregates within areas of interstitial fibrosis and scarring, and glomerular sclerosis." At 15 months, male and female rats (30/30 treated; 10/10 controls) had nephropathy, and the severity scores ranged from minimal to mild. At 2 years, male and female rats (149/150 treated; 49/50 controls) also had nephropathy, and although the severity was moderate in the control males and minimal to mild in the control females, severity increased with *tert*-butanol exposure in both sexes (NTP, 1995).

The lesions collectively described by NTP (1997, 1995) as nephropathy and noted to be common spontaneous lesions in rats, are consistent with CPN. The effects characterized as CPN are related to age and not considered histopathological manifestations of chemically induced toxicity

[see U.S. EPA (1991a), p. 35 for further details and a list of the typical observable histopathological features of CPN]. These lesions, however, are frequently exacerbated by chemical treatment (NTP, 1997), as evidenced by the dose-related increases in severity of the nephropathy compared to female and male rat controls. The chemical-related changes in nephropathy severity are included in the consideration of hazard potential.

NTP (1995) observed other kidney lesions, described as being associated with nephropathy but diagnosed separately. Renal mineralization is defined by NTP (1995) as "focal mineral deposits primarily at the corticomedullary junction." This mineralization is distinct from linear mineralization, which is considered a lesion characteristic of α_{2u}-globulin nephropathy (for further discussion of this particular lesion, see *Mode of Action Analysis—Kidney Effects*). The mineralization is characterized as distinct linear deposits along radiating medullary collecting ducts. An increased incidence of linear mineralization was limited to exposed males in the 2-year oral study (NTP, 1995).

Renal mineralization was observed in essentially all female rats at all reported treatment durations. A dose-related, increased incidence of mineralization was reported in male rats at the 13-week, 15-month, and 2-year oral evaluations (NTP, 1995). NTP (1995) describes focal, medullary mineralization as being associated with CPN but notes that focal mineralization is "usually more prominent in untreated females than in untreated males," which is consistent with the widespread appearance of this lesion in females. This description, however, is inconsistent with the observation in this and other databases that age-related nephropathy (i.e., CPN) is generally more prevalent and more severe in male rats compared to females (U.S. EPA, 1991a). The association of mineralization with CPN is unclear, considering the lack of spontaneous lesions in the control and low-dose groups of 13-week males and the dose-response relationships the *tert*-butanol-exposed males exhibited in the 13-week (NTP, 1997, 1995) and 2-year studies (NTP, 1995). Furthermore, due to the overwhelming presence of mineralization in the control and treated female rats, the contribution, if any, of *tert*-butanol to the formation of this lesion in females could not be determined. Thus, the mineralization could be related to both aging of the animals and *tert*-butanol exposure.

Two other histological kidney lesions observed in male and female rats are suppurative inflammation and transitional epithelial hyperplasia. These lesions were observed in the 2-year oral NTP (1995) study. Although NTP (1995) describes these lesions as related to the nephropathy (characterized above as common and spontaneous, and considered CPN), that suppurative inflammation and transitional epithelial hyperplasia exhibited incidence patterns different from those reported for nephropathy is notable. Incidence of suppurative inflammation in female rats was low in the control group and increased with dose, with incidences ≥24% in the two highest dose groups, compared with controls. In comparison, 20% of the control males exhibited suppurative inflammation, and the changes in incidence were not dose related (incidences ranging from 18 to 36%). The data for males suggest that CPN plays a role in the induction of suppurative

inflammation; considering the responses in the females, however, the effect appears to be predominantly treatment related. Suppurative inflammation was not observed in the animals of the 13-week oral (NTP, 1995) or inhalation study (NTP, 1997), which both reported nephropathy (as CPN), providing further support that this lesion is not specifically related to the nephropathy.

Transitional epithelial hyperplasia was observed in both male and female rats exposed orally (NTP, 1995). In the control males, 50% of the animals exhibited transitional epithelial hyperplasia and the incidence and severity increased with dose. Only the mid- and high-dose females, however, exhibited dose-related increases in incidence and severity of transitional epithelial hyperplasia; this lesion was not reported in the control or low-dose females. NTP (1995) described transitional epithelial hyperplasia as increased layers of the transitional epithelial lining of the renal pelvis; study authors noted no progression of this hyperplastic lesion to neoplasia. The relatively high background in male controls (i.e., 50%) suggests some potential influence, other than *tert*-butanol treatment, on this effect. The absence of this effect in female control and low-dose animals and the dose-related increases in both males and females, however, indicate that similar to the suppurative inflammation, the transitional epithelial hyperplasia is predominantly treatment related. Transitional epithelial hyperplasia should not be confused with another lesion noted at the 2-year evaluation, renal tubule hyperplasia, which was considered preneoplastic (for further details regarding this type of hyperplasia, see the discussion under kidney tumors below).

Additional histopathological changes, including increased tubular degeneration, degeneration of the basement membrane of the Bowman's capsule, diffused glomeruli, and glomerular vacuolation were noted in a 10-week study in male Wistar rats (Acharya et al., 1997; Acharya et al., 1995). A decrease in glutathione in the kidney accompanied these changes, which the study authors noted as potentially indicative of oxidative damage. Acharya et al. (1997); Acharya et al. (1995) used one dose and a control group and did not report incidences. The increased tubule degeneration and glomerular vacuolation could be characterized as tubular atrophy and glomerular hyalinization, respectively, consistent with CPN; however, without quantitative information, examining the differences between the control and treated animals to determine if CPN plays a role in development of these effects is not possible. Although based on the noted appearance of the effects in the treated animals compared with controls, the effects likely are treatment related.

Serum or urinary biomarkers informative of kidney toxicity were not measured in the studies discussed above. Some changes occurred in urinalysis parameters (e.g., decreased urine volume and increased specific gravity), accompanied by reduced water consumption, and thus might not be related to an effect of kidney function (NTP, 1995).

Kidney tumors. The kidney is also a target organ for cancer effects (Table 1-3, Figure 1-1). Male F344 rats had an increased incidence of combined renal tubule adenomas or carcinomas in the 2-year oral bioassay (Hard et al., 2011; NTP, 1995). The increase in tumors from control was similar in the low- and high-dose groups and highest in the mid-dose group. Overall, tumor increases were statistically significant in trend testing, which accounted for mortality ($p \leq 0.018$).

Mortality increased with increasing exposure (p = 0.001); increased mortality alone, however, does not account for the highest tumor incidence occurring at the middle dose.

Increases in incidence and severity of renal tubule hyperplasia also were observed in male rats. NTP (1995) stated that "[t]he pathogenesis of proliferative lesions of renal tubule epithelium is generally considered to follow a progression from hyperplasia to adenoma to carcinoma (Hard, 1986)." Similarly, EPA considered the renal tubule hyperplasia to be a preneoplastic effect associated with the renal tubule tumors. Renal tubule hyperplasia was found in one high-dose female (NTP, 1995); no increase in severity was observed. This effect in females, which was not considered toxicologically significant, is not discussed further. Two renal tubular adenocarcinomas in male mice also were reported (NTP, 1995), one each in the low- and high-dose groups, but were not considered by NTP to be "biologically noteworthy changes"; thus the tumors in mice are not discussed further.

A Pathology Working Group, sponsored by Lyondell Chemical Company, reevaluated the kidney changes in the NTP 2-year study to determine if additional histopathological changes could be identified to inform the MOA for renal tubule tumor development (Hard et al., 2011). In all cases, working group members were blinded to treatment groups, and used guidelines published by Hard and Wolf (1999) and refinements reported by (Hard and Seely, 2006); Hard and Seely (2005) and Hard (2008). The group's report and analysis by Hard et al. (2011) confirmed the NTP findings of renal tubule hyperplasia and renal tubule tumors in male rats at 2 years. In particular, they reported similar overall tumor incidences in the exposed groups. Hard et al. (2011), however, reported fewer renal tubule adenomas and carcinomas in the control group than in the original NTP study. As a result, all treated groups had statistically significant increases in renal tubule adenomas and carcinomas (combined) when compared to controls. Additionally, Hard et al. (2011) considered fewer tumors to be carcinomas than did the original NTP study. Results of both NTP (1995) and the reanalysis by Hard et al. (2011) are included in Table 1-3 and Figure 1-1.

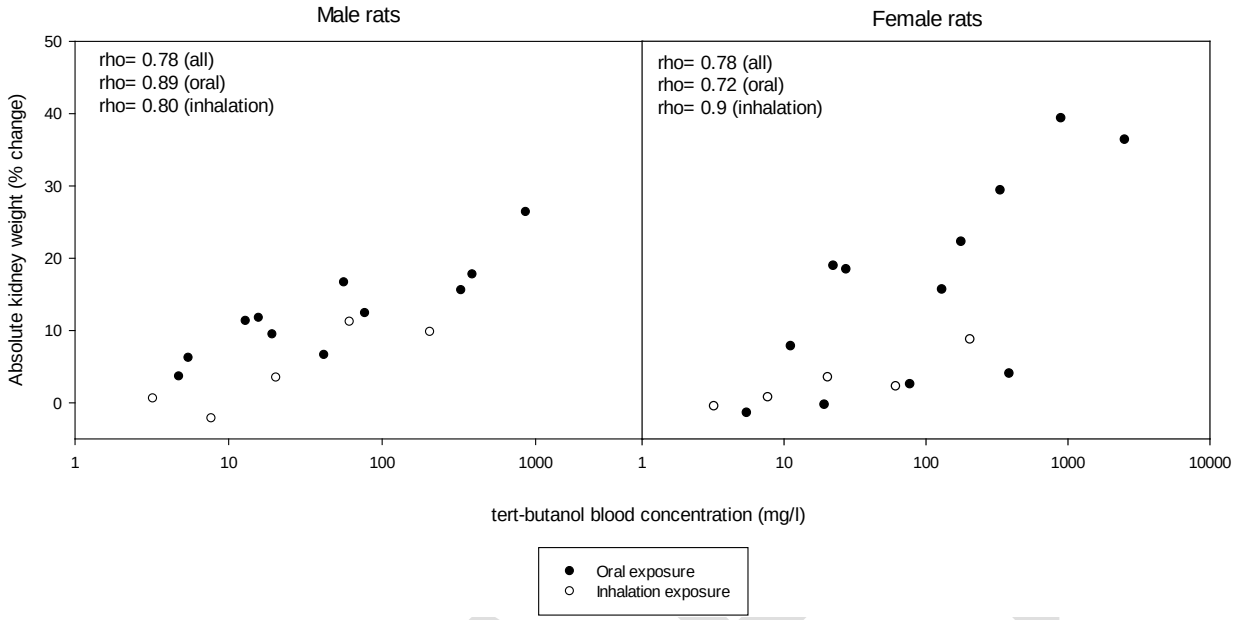


Figure 1-2. Comparison of absolute kidney weight change in male and female rats across oral and inhalation exposure based on internal blood concentration. Spearman rank correlation coefficient (rho) was calculated to evaluate the direction of a monotonic association (e.g., positive value = positive association)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

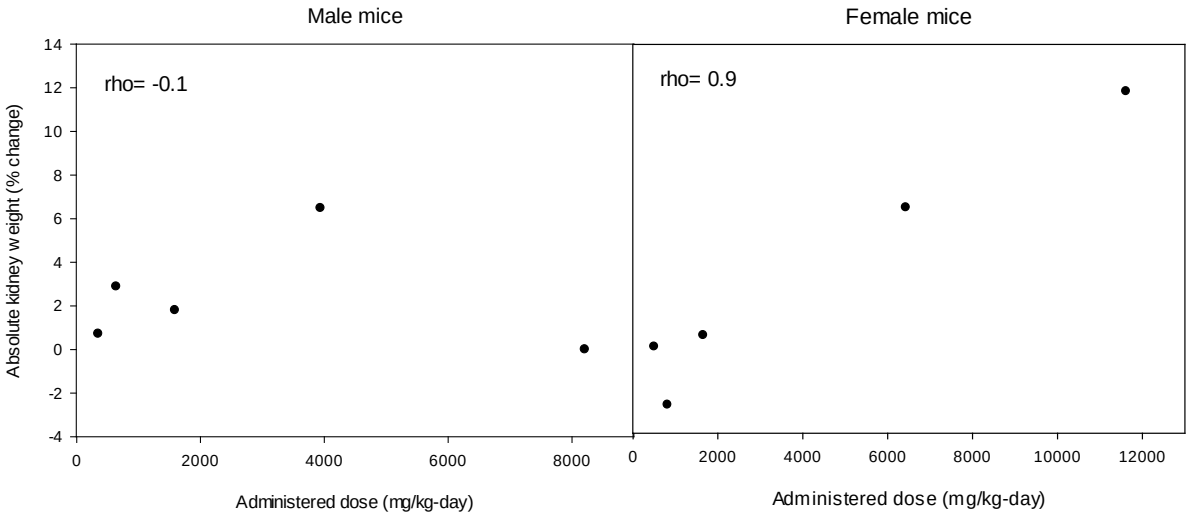


Figure 1-3. Comparison of absolute kidney weight change in male and female mice following oral exposure based on administered concentration. Spearman rank correlation coefficient (rho) was calculated to evaluate the direction of a monotonic association (e.g., positive value = positive association) and the strength of association.

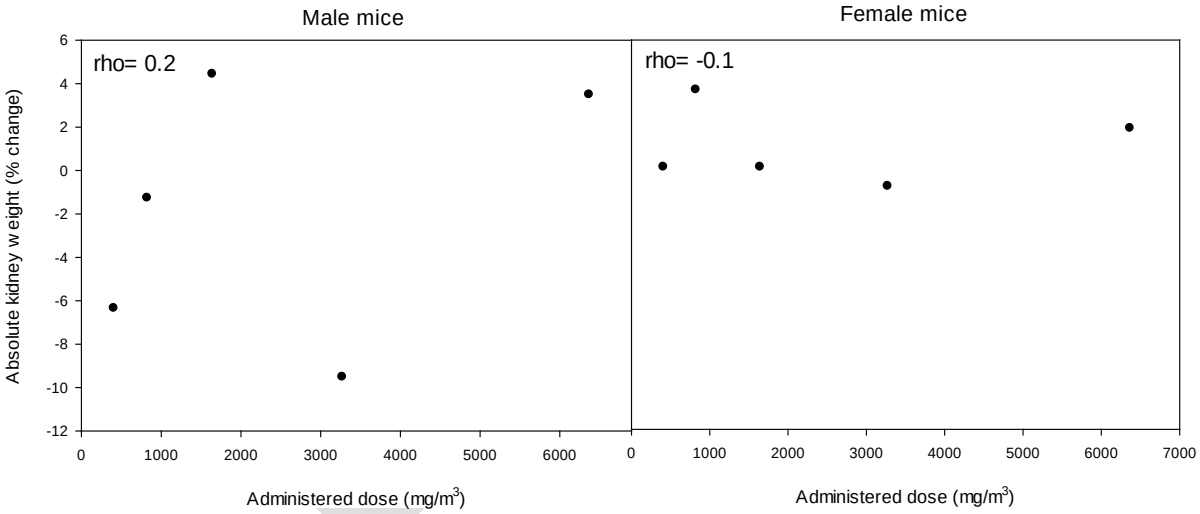


Figure 1-4. Comparison of absolute kidney weight change in male and female mice following inhalation exposure based on administered concentration. Spearman rank correlation coefficient (rho) was calculated to evaluate the direction of a monotonic association (e.g., positive value = positive association) and the strength of association.

Table 1-2. Changes in kidney histopathology in animals following exposure to *tert*-butanol

Reference and study design	Results
Acharya et al. (1997) Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	↑ tubular degeneration, degeneration of the basement membrane of the Bowman's capsule, diffused glomeruli, and glomerular vacuolation (no incidences reported) ↓ kidney glutathione (~40%)*
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Incidence (severity): **Males** Dose (mg/kg-d) / Mineralization[b] / Nephropathy[c] 0 / 0/10 / 7/10 (1.0) 230 / 0/10 / 10/10 (1.6*) 490 / 2/10 (1.5) / 10/10 (2.6*) 840 / 8/10*(1.4) / 10/10 (2.7*) 1,520 / 4/10*(1.0) / 10/10 (2.6*) 3,610[a] / 4/10*(1.0) / 7/10 (1.1) **Females** Dose (mg/kg-d) / Mineralization[b] / Nephropathy[c] 0 / 10/10 (1.7) / 2/10 (1.0) 290 / 10/10 (2.0) / 3/10 (1.0) 590 / 10/10 (2.0) / 5/10 (1.0) 850 / 10/10 (2.0) / 7/10* (1.0) 1,560 / 10/10 (2.0) / 8/10* (1.0) 3,620[a] / 6/10 (1.2) / 7/10* (1.0)
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kgd F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kgd 13 weeks	Study authors indicated no treatment-related changes in kidney histopathology (histopathological data not provided for the 13-week study)

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>NTP (1995)

F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months interim)
Drinking water (0, 1.25, 2.5, 5, 10 mg/mL)
M: 0, 90, 200, 420[a] mg/kg-d
F: 0, 180, 330, 650[a] mg/kg-d
2 years</td><td>Incidence (severity):
Males
<table><tr><th>Dose (mg/kg-d)</th><th>Mineralization[b] (interim)</th><th>Mineralization[b] (terminal)</th><th>Linear mineralization[b] (terminal)</th></tr><tr><td>0</td><td>1/10 (1.0)</td><td>26/50 (1.0)</td><td>0/50</td></tr><tr><td>90</td><td>2/10 (1.0)</td><td>28/50 (1.1)</td><td>5/50* (1.0)</td></tr><tr><td>200</td><td>5/10 (1.8)</td><td>35/50 (1.3)</td><td>24/50* (1.2)</td></tr><tr><td>420[a]</td><td>9/10* (2.3)</td><td>48/50* (2.2)</td><td>46/50* (1.7)</td></tr></table><table><tr><th>Dose (mg/kg-d)</th><th>Transitional epithelial hyperplasia</th><th>Nephropathy[c] severity</th></tr><tr><td>0</td><td>25/50 (1.7)</td><td>3.0</td></tr><tr><td>90</td><td>32/50 (1.7)</td><td>3.1</td></tr><tr><td>200</td><td>36/50* (2.0)</td><td>3.1</td></tr><tr><td>420[a]</td><td>40/50* (2.1)</td><td>3.3*</td></tr></table>Females<table><tr><th>Dose (mg/kg-d)</th><th>Mineralization[b] Interim</th><th>Mineralization[b] Terminal</th><th>Inflammation (suppurative) incidence</th></tr><tr><td>0</td><td>10/10 (2.8)</td><td>49/50 (2.6)</td><td>2/50</td></tr><tr><td>180</td><td>10/10 (2.9)</td><td>50/50 (2.6)</td><td>3/50</td></tr><tr><td>330</td><td>10/10 (2.9)</td><td>50/50 (2.7)</td><td>13/50*</td></tr><tr><td>650[a]</td><td>10/10 (2.8)</td><td>50/50 (2.9)</td><td>17/50*</td></tr></table><table><tr><th>Dose (mg/kg-d)</th><th>Transitional epithelial hyperplasia</th><th>Nephropathy[c] severity</th></tr><tr><td>0</td><td>0/50</td><td>1.6</td></tr><tr><td>180</td><td>0/50</td><td>1.9*</td></tr><tr><td>330</td><td>3/50 (1.0)</td><td>2.3*</td></tr><tr><td>650[a]</td><td>17/50*(1.4)</td><td>2.9*</td></tr></table></td></tr>
<tr><td>NTP (1995)

B6C3F$_1$ mouse; 60/sex/treatment
Drinking water (0, 5, 10, or 20 mg/mL)
M: 0, 540, 1,040, or 2,070[a] mg/kg-d</td><td>No treatment-related changes in kidney related histopathology observed</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>F: 0, 510, 1,020, or 2,110 mg/kg-d
2 years</td><td></td></tr>
<tr><td>NTP (1997)

F344/N rat; 10/sex/treatment
Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3)
(dynamic whole-body chamber)
6 hr/d, 5 d/wk
13 weeks
Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported</td><td>Male

<table>
<tr><th>Concentration (mg/m^3)</th><th>Incidence of chronic nephropathy[d]</th><th>Average severity of chronic nephropathy</th></tr>
<tr><td>0</td><td>9/10</td><td>1.0</td></tr>
<tr><td>406</td><td>8/10</td><td>1.4</td></tr>
<tr><td>824</td><td>9/10</td><td>1.4</td></tr>
<tr><td>1,643</td><td>10/10</td><td>1.6</td></tr>
<tr><td>3,273</td><td>10/10</td><td>1.9</td></tr>
<tr><td>6,368</td><td>10/10</td><td>2.0</td></tr>
</table>
Females: no treatment-related changes in kidney related histopathology observed
Severity categories: 1= minimal, 2= mild. No results from statistical tests reported</td></tr>
<tr><td>NTP (1997)

B6C3F$_1$ mouse; 10/sex/treatment
Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3)
(dynamic whole-body chamber)
6 hr/d, 5 d/wk
13 weeks
Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported</td><td>No treatment-related changes in kidney related histopathology observed</td></tr>
</table>

* Statistically significant $p \leq 0.05$ as determined by the study authors.

[a] The high-dose group had an increase in mortality.

[b] Mineralization defined in NTP (1995) as focal mineral deposits, primarily at the corticomedullary junction. Linear mineralization was defined as foci of distinct linear deposits along radiating medullary collecting ducts; linear mineralization not observed in female rats.

[c] Nephropathy defined in NTP (1995) as lesions including thickened tubule and glomerular basement membranes, basophilic foci of regenerating tubule epithelium, intratubule protein casts, focal mononuclear inflammatory cell aggregates within areas of interstitial fibrosis and scarring, and glomerular sclerosis.
[d] Nephropathy characterized in NTP (1997) as scattered foci of regenerative tubules (with cytoplasmic basophilia, increased nuclear/cytoplasmic ratio, and occasionally thickened basement membranes and intraluminal protein casts).

Note: Conversions from drinking water concentrations to mg/kg-day performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.

Table 1-3. Changes in kidney tumors in animals following exposure to *tert*-butanol

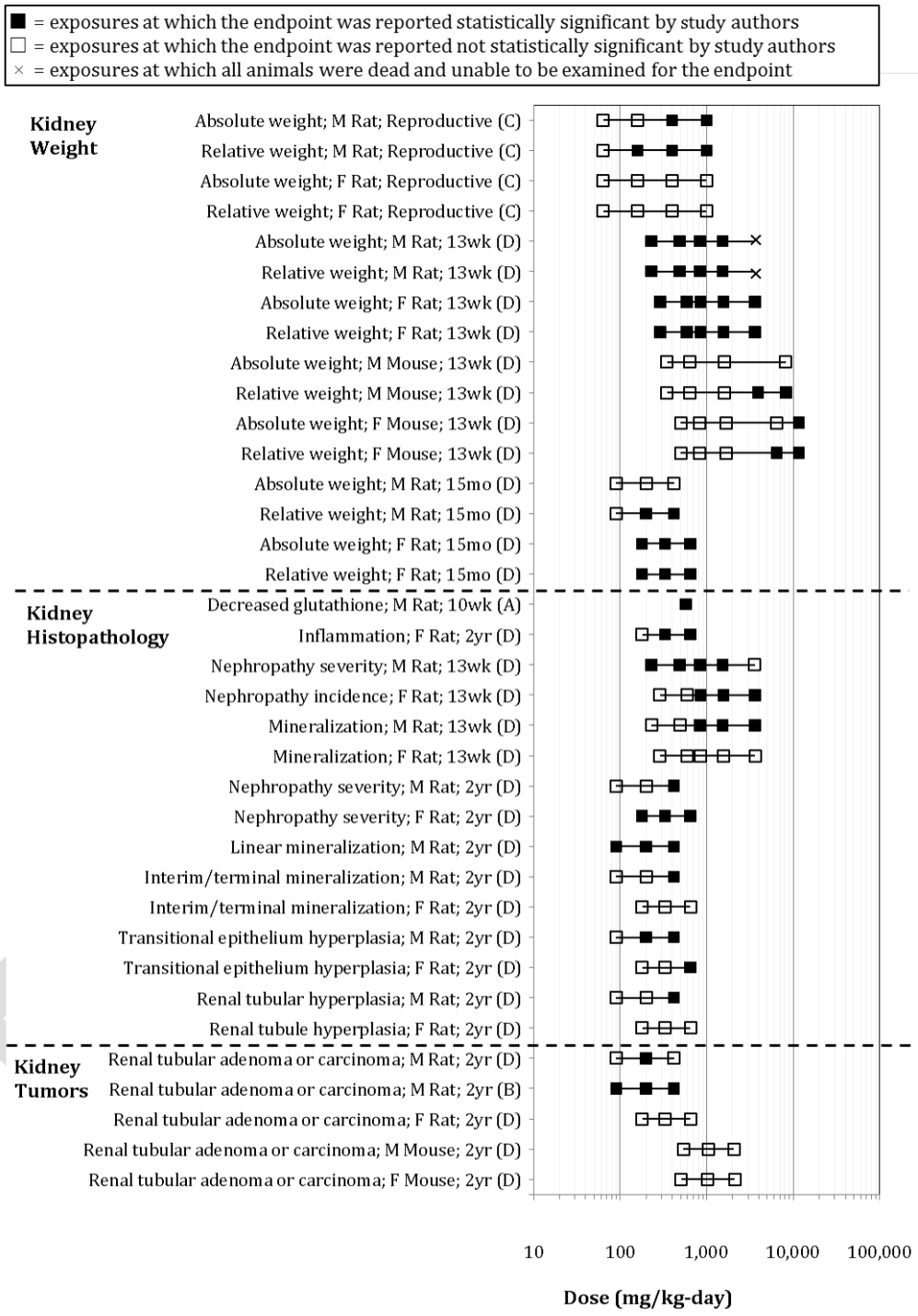
Reference and study design	Results			
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	**Male**			
	Dose (mg/kg-d)	Renal tubule hyperplasia (standard and extended evaluation combined)	Renal tubule adenoma (single)	Renal tubule adenoma (multiple)
	0	14/50 (2.3)	7/50	1/50
	90	20/50 (2.3)	7/50	4/50
	200	17/50 (2.2)	10/50	9/50*
	420[a]	25/50* (2.8)	10/50	3/50
	Dose (mg/kg-d)	Renal tubule carcinoma	Renal tubule adenoma (single or multiple) or carcinoma	
	0	0/50	8/50	
	90	2/50	13/50	
	200	1/50	19/50*	
	420[a]	1/50	13/50	
	Female			
	Dose (mg/kg-d)	Renal tubule hyperplasia	Renal tubule adenoma (single)	Renal tubule adenoma (multiple)
	0	0/50	0/50	0/50
	180	0/50	0/50	0/50
	330	0/50	0/50	0/50
	650[a]	1/50 (1.0)	0/50	0/50

Reference and study design	Results
	Dose (mg/kg-d) / Renal tubule carcinoma / Renal tubule adenoma (single or multiple) or carcinoma 0 / 0/50 / 0/50 180 / 0/50 / 0/50 330 / 0/50 / 0/50 650[a] / 0/50 / 0/50 Based on standard and extended evaluations (combined). Results do not include the animals sacrificed at 15 months.
Hard et al. (2011) Reanalysis of the slides from male rats (all slides in controls and high-dose groups of males and females, and slides from all other males with renal tumors) in the NTP (1995) study (see above)	**Male** Dose (mg/kg-d) / Renal tubule adenoma (single) / Renal tubule adenoma (multiple) / Renal tubule carcinoma / Renal tubule adenoma (single or multiple) or carcinoma 0 / 3/50 / 1/50 / 0/50 / 4/50 90 / 9/50 / 3/50 / 1/50 / 13/50* 200 / 9/50 / 9/50 / 0/50 / 18/50* 420 / 9/50 / 3/50 / 1/50 / 12/50*
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	No increases in kidney-related tumors. Two renal tubule adenocarcinomas, one in the low-dose and one in the high-dose groups, were observed in male mice. These tumors were not considered treatment related.

* Statistically significant $p \leq 0.05$ as determined by the study authors.

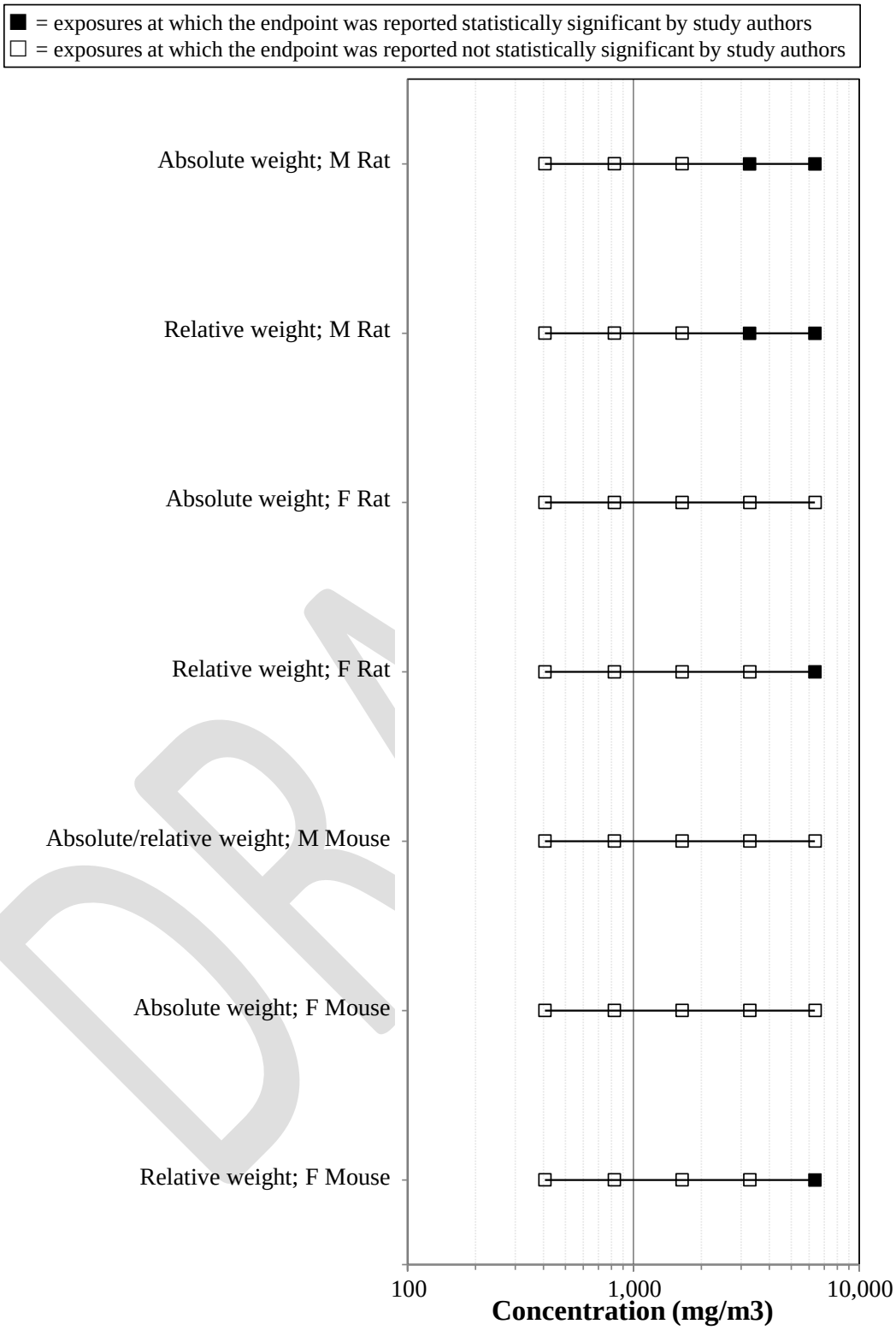
[a] The high-dose group had an increase in mortality.

Note: Conversions from drinking water concentrations to mg/kg-d performed by study authors.



Sources: (A) Acharya et al. (1997); (1995); (B) Hard et al. (2011)*; (C) Lyondell Chemical Co. (2004) (D) NTP (1995); *reanalysis of NTP (1995).

Figure 1-5. Exposure response array for kidney effects following oral exposure to *tert*-butanol.



Source: NTP (1997).

Figure 1-6. Exposure-response array of kidney effects following inhalation exposure to *tert*-butanol (13-week studies, no chronic studies available).

Mode of Action Analysis—Kidney Effects

a) α_{2u}-Globulin-Associated Renal Tubule Nephropathy and Carcinogenicity

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 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-associated renal tubule nephropathy and carcinogenicity occurring in male rats are presumed not to be relevant for assessing human health hazards (U.S. EPA, 1991a), evaluating the data to determine if 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 *tert*-butanol exposure was evaluated using the U.S. EPA (1991a) Risk Assessment Forum Technical panel report, *Alpha$_{2u}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat*. This report provides specific guidance for evaluating renal tubule tumors in male rats 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.

Studies in the *tert*-butanol database evaluated and reported effects on the kidney, providing some evidence to evaluate this MOA. Additionally, several studies were identified that specifically evaluated the role of α_{2u}-globulin in *tert*-butanol-induced renal tubule nephropathy and carcinogenicity (Borghoff et al., 2001; Williams and Borghoff, 2001; Takahashi et al., 1993). Because the evidence reported in these studies is specific to α_{2u}-globulin accumulation, it is presented in this section; it was not included in the animal evidence tables in the previous section.

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 papillae 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 1-7).

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 is occurring in male rats and is involved in renal tubule tumor development must be determined. Second, whether the renal effects in male rats exposed to *tert*-butanol are solely due to the α_{2u}-globulin process also must be determined.

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

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

2) the protein 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 1-7.

The available data relevant to this first question are summarized in Table 1-4, Figures 1-8 and 1-9, and are evaluated below.

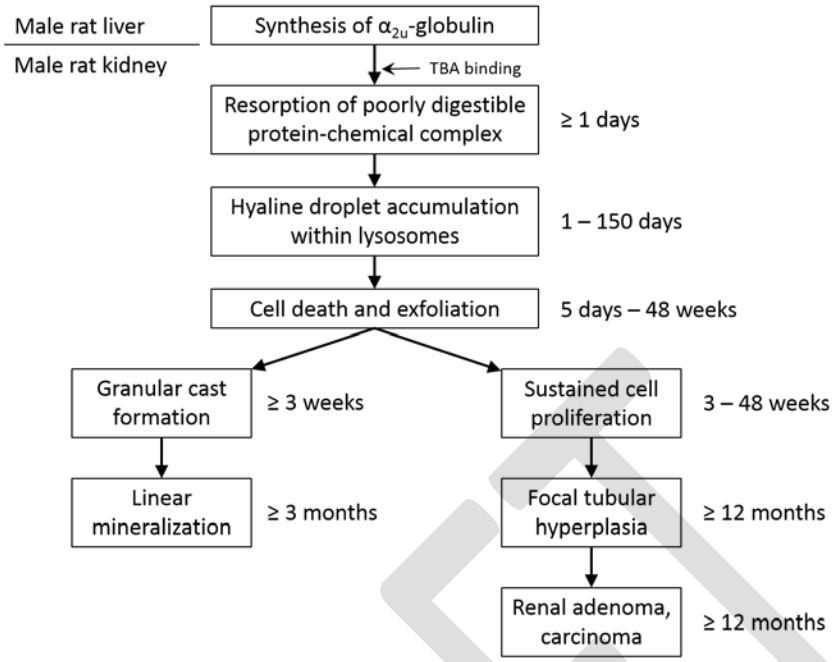


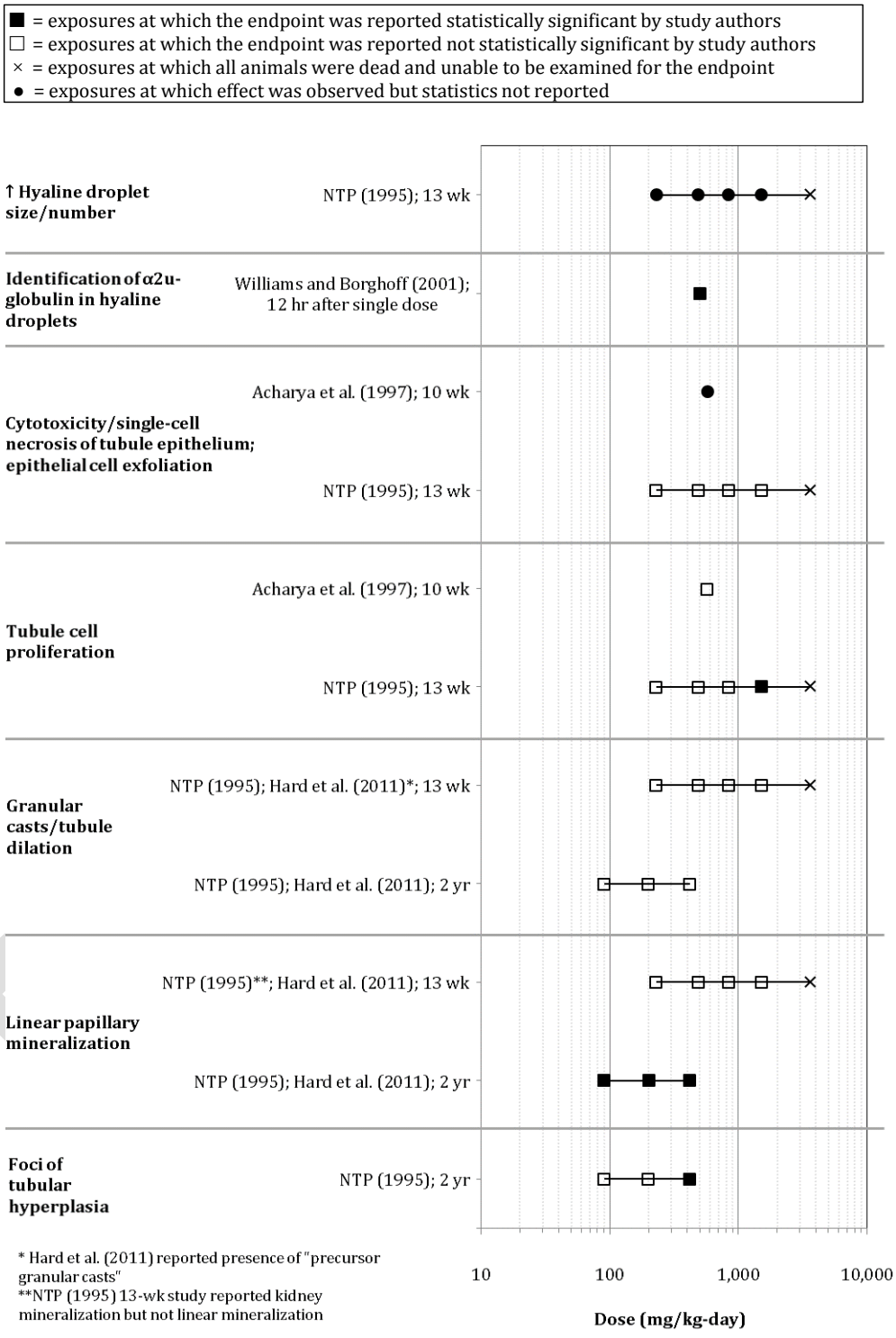
Figure 1-7. Temporal pathogenesis of α_{2u}-globulin-associated nephropathy in male rats. α_{2u}-Globulin synthesized in the livers of male rats is delivered to the kidney, where it can accumulate in hyaline droplets and be retained by epithelial cells lining the S2 (P2) segment of the proximal tubules. Renal pathogenesis following continued *tert*-butanol exposure and increasing droplet accumulation can progress step-wise from increasing epithelial cell damage, death and dysfunction leading to the formation of granular casts in the corticomedullary junction, linear mineralization of the renal papillae, and carcinogenesis of the renal tubular epithelium. Adapted from Swenberg and Lehman-McKeeman (1999) and U.S. EPA (1991a).

Table 1-4. Summary of data on the α_{2u}-globulin process in male rats exposed to *tert*-butanol

Duration	Dose	Results	Comments	Reference
*1) **Hyaline droplets are increased in size and number***				
10 d (inhalation)	0, 758, 1,364, 5,304 mg/m^3	+	stat sig at 5,304 mg/m^3; stat sig trend	Borghoff et al. (2001)
13 wk (inhalation)	0, 3,273, 6,368 mg/m^3	–		NTP (1997)[a]
13 wk (oral)	0, 230, 490, 840, 1,520, 3,610 mg/kg-d	(+)	observed in all but highest dose group	NTP (1995)
*2) **The protein in the hyaline droplets is α_{2u}globulin***				
10 d (inhalation)	0, 758, 1,364, 5,304 mg/m^3	+	stat sig at 5,304 mg/m^3; stat sig trend	Borghoff et al. (2001)
12 h (elapsed time following single oral dose)	0, 500 mg/kg	+		Williams and Borghoff (2001)
*3) **Several (but not necessarily all) additional steps in the pathological sequence are present in male rats, such as:***				
a) Subsequent cytotoxicity and single-cell necrosis of tubule epithelium, with exfoliation of degenerate epithelial cells				
10 wk (oral)	0, 575 mg/kg-d	(+)	degeneration of renal tubules reported	Acharya et al. (1997)
13 wk (oral)	0, 230, 490, 840, 1,520, 3,610 mg/kg-d	–		NTP (1995)
b) Sustained regenerative tubule cell proliferation (NOTE: The positive studies below reported cell proliferation but did not observe necrosis or cytotoxicity; therefore, it cannot be assumed that the results indicate regenerative proliferation is occurring.)				
10 wk (oral)	0, 575 mg/kg-d	–		Acharya et al. (1997)
10 d (inhalation)	0, 758, 1,364, 5,304 mg/m^3	+	stat sig at all doses; stat sig trend	Borghoff et al. (2001)
13 wk (oral)	0, 230, 490, 840, 1,520, 3,610 mg/kg-d	+	elevated at 840 mg/kg-d; stat sig at 1,520 mg/kg-d	NTP (1995)
c) Development of intraluminal granular casts from sloughed cellular debris, with consequent tubule dilation				
13 wk (oral)	0, 230, 490, 840, 1,520, 3,610 mg/kg-d	–; (+)[b]		NTP (1995); Hard et al. (2011)[c]
2 yr (oral)	0, 90, 200, 420 mg/kg-d	–		NTP (1995); Hard et al. (2011)[d]

Duration	Dose	Results	Comments	Reference
d) Linear mineralization of tubules in the renal papilla				
13 wk (oral)	0, 230, 490, 840, 1,520, 3,610 mg/kg-d	–		NTP (1995); Hard et al. (2011)[c]
2 yr (oral)	0, 90, 200, 420 mg/kg-d	+; (+)	all doses stat sig	NTP (1995); Hard et al. (2011)[d]
e) Foci of tubular hyperplasia				
2 yr (oral)	0, 90, 200, 420 mg/kg-d	+	stat sig trend at all doses; stat sig at 420 mg/kg-d	NTP (1995)

+ = Statistically significant change reported in one or more treated groups.
(+) = Effect was reported in one or more treated groups, but statistics not reported.
– = No statistically significant change reported in any of the treated groups.
[a] NTP (1997) did not observe any effects consistent with α_{2u}-globulin nephropathy.
[b] Precursors to granular casts reported.
[c] Reanalysis of hematoxylin and eosin-stained kidney sections from all male control and 1,520 mg/kg-d groups, as well as a representative sample of kidney sections stained with Mallory Heidenhain stain, from the 13-wk study from NTP (1995).
[d] Reanalysis of slides for all males in the control and 420 mg/kg-day dose group and all animals with renal tubule tumors from 2-yr NTP (1995). Protein casts reported, not granular casts.



*Hard et al. (2011) reported presence of "precursor granular casts."
**NTP (1995) 13-wk study reported kidney mineralization but not linear mineralization.

Figure 1-8. Exposure-response array for effects potentially associated with α_{2u}-globulin renal tubule nephropathy and tumors in male rats after oral exposure to *tert*-butanol.

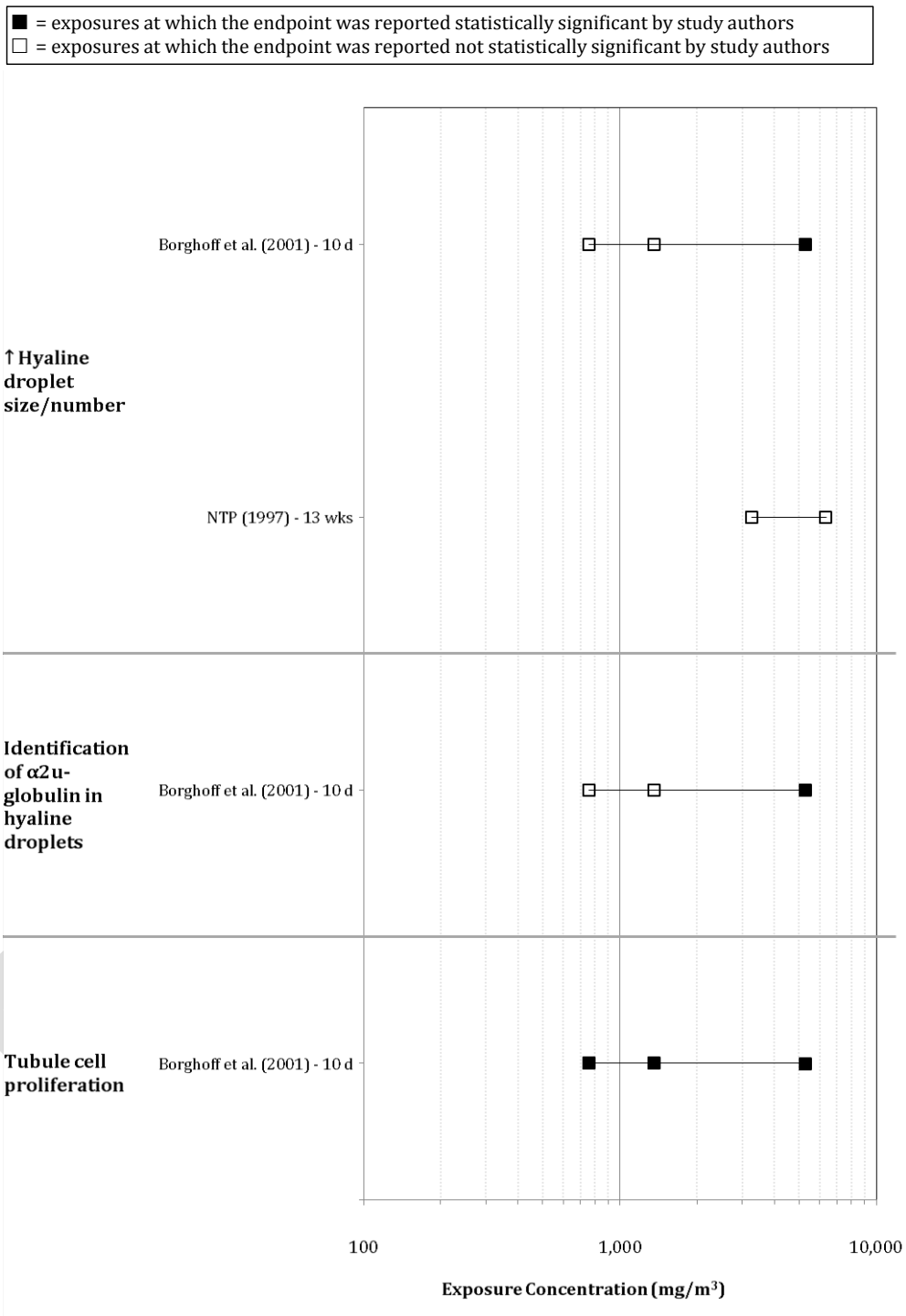


Figure 1-9. Exposure-response array for effects potentially associated with α_{2u}-globulin renal tubule nephropathy and tumors in male rats after inhalation exposure to *tert*-butanol.

Question One: Is the α_{2u}globulin process occurring in male rats exposed to tert-butanol?

(1) The first criterion to consider is whether hyaline droplets are increased in size and number in male rats. As noted above, the excessive accumulation of hyaline droplets can appear quickly, within 1 or 2 days, and persist throughout chronic exposures, although the severity begins to decline around 5 months (U.S. EPA, 1991a). A statistically significant positive trend in the accumulation of large protein droplets with crystalloid protein structures was observed in kidneys of male rats exposed to inhalation concentrations of 758, 1,364, and 5,304 mg/m^3 *tert*-butanol for 6 hr/day for 10 days (Borghoff et al., 2001). These droplets were small and minimally present in control male rats and were not observed in female rats. Similarly, data from the 13-week NTP oral study (NTP, 1995; Takahashi et al., 1993; Lindamood et al., 1992) demonstrated an increase in the accumulation of hyaline droplets. The lowest dose of 230 mg/kg-day had minimal hyaline droplet formation compared to controls, although the next three doses (490, 840, and 1,520 mg/kg-day) had a higher accumulation of droplets with angular, crystalline structures that was similar in incidence and severity among these dose groups. No droplets were observed in female rats or in mice.

NTP (1997), however, found no difference between the control and treatment groups stained for hyaline droplet formation in male rats exposed to 0, 3,273, or 6,368 mg/m^3 *tert*-butanol via inhalation for 13 weeks; in fact, this study did not report any other lesions that could be specifically associated with α_{2u}-globulin nephropathy in male rats. These results from NTP (1997), which are inconsistent with the findings of both Borghoff et al. (2001) and NTP (1995), do not appear to be due to differences in dose. Comparison of the oral and inhalation studies on the basis of *tert*-butanol blood concentration (see Supplemental Information) showed that a 13-week exposure in the range of the NTP (1995) doses of 490–840 mg/kg-day leads to the same average blood concentration as 6-hr/day, 5 day/week inhalation exposures to 3,273–6,368 mg/m^3. The absence of similar histopathological findings in the 13-week inhalation NTP (1997) study compared to those reported in the two oral studies is not understood, but might be indicative of the strength of *tert*-butanol to induce, consistently, α_{2u}-globulin nephropathy. The results from the two other studies (Borghoff et al., 2001; NTP, 1995) indicate that hyaline droplets increase in size and number in male rats following *tert*-butanol exposures. 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. Accumulated hyaline droplets with an α_{2u}-globulin etiology can be confirmed by using immunohistochemistry to identify the α_{2u}-globulin protein. Two short-term studies measured α_{2u}-globulin immunoreactivity in the hyaline droplets of the renal proximal tubular epithelium (Borghoff et al., 2001; Williams and Borghoff, 2001). Following 10 days of inhalation exposure, Borghoff et al. (2001) did not observe an exposure-related increase in α_{2u}-globulin using immunohistochemical staining. When using an enzyme-linked immunosorbent assay (ELISA), a more sensitive method of detecting α_{2u}-globulin, however, a statistically significant positive

correlation of α_{2u}-globulin concentration with dose of *tert*-butanol (determined by correlating with cell proliferation labeling indices) was observed, with accumulation of α_{2u}-globulin protein statistically significant by pairwise comparison only in the highest dose group. No positive staining for α_{2u}-globulin was observed in exposed female rats. In a follow-up study, Williams and Borghoff (2001) used a single gavage dose of 500 mg/kg [selected on the basis of results by NTP (1995) for induction of hyaline droplet accumulation], and reported a statistically significantly higher renal concentration of α_{2u}-globulin (by ELISA) in treated male rats than in controls 12 hours after exposure. Further, equilibrium dialysis methods determined that the binding of *tert*-butanol to α_{2u}-globulin was reversible. These data indicate the presence of α_{2u}-globulin in *tert*-butanol-treated male rats, although requiring a more sensitive method of detection of α_{2u}-globulin than is typically used could indicate that *tert*-butanol is not a strong inducer of α_{2u}-globulin accumulation. Therefore, the available data are sufficient to fulfill the second criterion for α_{2u}-globulin present in the hyaline droplets, but suggest weak induction of α_{2u}-globulin by *tert*-butanol.

(3) The third criterion considered is whether several (but not necessarily all) additional events in the histopathological sequence associated with α_{2u}-globulin nephropathy appear in male rats in a manner consistent with the understanding of α_{2u}-globulin pathogenesis. Evidence of cytotoxicity and single-cell necrosis of the tubule epithelium subsequent to the excessive accumulation of hyaline droplets, with exfoliation of degenerate epithelial cells, should be observable after five days of continuous exposure, peaking at 19 days [reviewed in U.S. EPA (1991a)]. The formation and accumulation of granular casts from the exfoliated cellular debris would follow, causing tubule dilation at the junction of the S3 (P3) segment of the proximal tubule and the descending thin loop of Henle, and the commencement of compensatory cell proliferation within the S2 (P2) segment, both occurring after three weeks of continuous exposure. Following chronic exposures, this regenerative proliferation could result in focal tubular hyperplasia, and eventually progress to renal adenoma and carcinoma (Figure 1-7).

Several of these steps were observed following *tert*-butanol exposure in male rats, most notably linear papillary mineralization and foci of tubular hyperplasia, consistent with the expected disease progression. Some lack of consistency and dose-related concordance, however, was evident across the remaining steps in the histopathological sequence. First, the accumulation of hyaline droplets and the concentrations of α_{2u}-globulin in the hyaline droplets at doses that induced significant tumor formation in male rats were not significant. Next, necrosis or cytotoxicity was absent and only precursors to granular casts at stages well within the expected timeframe of detectability were present. Finally, a 13-week inhalation study found no evidence of α_{2u}-globulin nephropathy (NTP, 1997), despite evaluating exposure concentrations predicted to result in similar blood *tert*-butanol levels as for the 13-week oral study (NTP, 1995), which reported increases in droplet accumulation and sustained regenerative tubule cell proliferation. A detailed evaluation and analysis of all the evidence relevant to this criterion follows.

Detailed evaluation of the available evidence supporting the third criterion

Single cell death and exfoliation into the renal tubules was inconsistently observed. Single cell death or necrosis was not associated with *tert*-butanol exposure in male rat kidneys after 10 or 13 weeks (Acharya et al., 1997; NTP, 1995). Acharya et al. (1997) reported degeneration of renal tubules, one pathological consequence of single cell necrosis, however, in male rats exposed to *tert*-butanol in drinking water for 10 weeks. As renal tubule epithelial cell death and epithelial degeneration should occur as early as 5 days post exposure and persist for up to 48 weeks (Swenberg and Lehman-McKeeman, 1999; Short et al., 1989), the lack of consistency in these observations could be the result of both weak induction of α_{2u}-globulin and a lack of later examinations.

a. Sustained regenerative cell proliferation also was not observed. Acharya et al. (1997) did not observe *tert*-butanol-induced proliferation following 10 weeks of oral exposure, but renal tubule proliferation was observed following another chemical exposure (trichloroacetic acid) in the same study. Therefore, the inference is that *tert*-butanol treatment did not induce regenerative tubule cell proliferation in male rats from this study. Borghoff et al. (2001) reported a dose-related increase in epithelial cell proliferation within the proximal tubule as measured by BrdU labeling indices in all male rats exposed to *tert*-butanol via inhalation for 10 days. The study did not report cytotoxicity, however, which, combined with the early time point makes unlikely that the cell proliferation was compensatory. NTP (1995) also observed increased cell proliferation in the renal tubule epithelium following 13-week oral exposures in male rats [only male rats were studied in the retrospective analysis by Takahashi et al. (1993) reported in NTP (1995)]. Proliferation was elevated at 840–1,520 mg/kg-day, a range higher than the single 575-mg/kg-day dose eliciting no such proliferative effect (Acharya et al., 1997), as described above. NTP (1995) reported, however, that no necrosis was observed, suggesting the proliferation was not regenerative.

b. Granular cast formation was not observed, although one study noted precursors to cast formation. NTP (1995) did not observe the formation of granular casts or tubular dilation; however, Hard et al. (2011) reanalyzed the 13-week oral NTP data from male rats treated with 0 or 1,520 mg/kg-day and identified precursors to granular casts in 5/10 animals in the treated group. The significance of these granular cast precursors, described as sporadic basophilic tubules containing cellular debris, is unknown, because 13 weeks of exposure is within the expected timeframe of frank formation and accumulation of granular casts (≥3 weeks). Granular cast formation, however, might not be significantly elevated with weak inducers of α_{2u}-globulin (Short et al., 1986), which is consistent with the reported difficulty in measuring α_{2u}-globulin in hyaline droplets associated with *tert*-butanol exposure.

c. Linear mineralization of tubules within the renal papillae was consistently observed in male rats. This lesion typically appears at chronic time points, occurring after exposures of 3 months up to 2 years (U.S. EPA, 1991a). Consistent with this description, 2-year oral exposure to *tert*-butanol induced a dose-related increase in linear mineralization, but not following 13-week exposure [(NTP, 1995); Table 1-2].

d. Renal tubule hyperplasia was observed in the only available 2-year study. 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). A dose-related increase in renal tubule hyperplasia was observed in male rats following 2-year oral exposures (NTP, 1995). By comparison, renal tubule hyperplasia was observed in only one high-dose female.

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. Therefore, observations of temporal and dose-response concordance for these effects are informative for drawing conclusions on causation.

As mentioned above, most steps in the sequence of α_{2u}-globulin nephropathy are observed at the expected time points following exposure to *tert*-butanol. Accumulation of hyaline droplets was observed early, at 12 hours following a single bolus exposure (Williams and Borghoff, 2001), and at 10 days (Borghoff et al., 2001) or 13 weeks (NTP, 1995) following continuous exposure; α_{2u}-Globulin was identified as the protein in these droplets (Borghoff et al., 2001; Williams and Borghoff, 2001). Lack of necrosis and exfoliation might be due to the weak induction of α_{2u}-globulin and a lack of later examinations. Granular cast formation was not reported by any of the available studies, which could also indicate weak α_{2u}-globulin induction. Regenerative cell proliferation, which was not observed, is discussed in more detail below. Observations of the subsequent linear mineralization of tubules and focal tubular hyperplasia fall within the expected timeframe of the appearance of these lesions. Overall, no explicit inconsistencies are present in the temporal appearance of the histopathological lesions associated with α_{2u}-globulin nephropathy; however, the dataset would be bolstered by measurements at additional time points to lend strength to the MOA evaluation.

Inconsistencies do occur in the dose-response among lesions associated with the α_{2u}-globulin nephropathy progression. Hyaline droplets were induced in the proximal tubule of all surviving male rats in the 13-week NTP oral study (NTP, 1995; Takahashi et al., 1993; Lindamood et al., 1992), although the incidence at the lowest dose was minimal, while the incidence at the three higher doses was more prominent. These results are discordant with the tumor results, given that all treated groups of male rats in the NTP 2-year oral bioassay had increased kidney tumor incidence, including the lowest dose of 90 mg/kg-day [according to the reanalysis by Hard et al. (2011)]. This lowest dose was less than the 230 mg/kg-day in the 13-week oral study that had only minimal hyaline droplet formation. Furthermore, although the incidence of renal tubule hyperplasia had a dose-related increase (NTP, 1995), a corresponding dose-related increase in the severity of tubular hyperplasia did not result. Severity of tubule hyperplasia was increased only at the highest dose, which was not consistent with renal tumor incidence.

Although the histopathological sequence has data gaps, such as the lack of observable necrosis or cytotoxicity or granular casts at stages within the timeframe of detectability, overall, a

sufficient number of steps (e.g., linear papillary mineralization, foci of tubular hyperplasia) were observed to fulfill the third criterion.

Summary and Conclusions for Question One:

Oral exposure to male F344 rats resulted in an increased incidence of renal tubule tumors in a 2-year oral bioassay (Hard et al., 2011; NTP, 1995). Several histopathological observations in exposed male rats were consistent with an α_{2u}-globulin MOA. This evidence includes the increased size and number of hyaline droplets and the accumulated α_{2u}-globulin protein in the hyaline droplets. Additionally, several subsequent steps in the histopathological sequence were observed. Overall, available data are sufficient for all three required criteria, suggesting that the α_{2u}-globulin process is operative. Although the evidence indicates a role for α_{2u}-globulin accumulation in the etiology of kidney tumors induced by exposure to *tert*-butanol in male rats, that *tert*-butanol is a weak inducer of α_{2u}-globulin is plausible, considering the limited histopathological observations and uncertainty regarding the temporal and dose concordance of the lesions.

Question Two: Are the renal effects in male rats exposed to tert-butanol solely due to the α_{2u}-globulin process?

If the α_{2u}-globulin process is operative, U.S. EPA (1991a) identifies a second question that must be answered regarding whether the renal effects are (a) solely due to the α_{2u}-globulin process, (b) a combination of the α_{2u}-globulin process and other carcinogenic processes, or (c) primarily due to other processes. U.S. EPA (1991a) states that additional data can help inform whether the α_{2u}-globulin process is the sole contributor to renal tubule tumor development in male rats. These additional considerations are highlighted and discussed, where possible, in detail below.

Hypothesis-testing of the α_{2u}-globulin sequence of effects and structure-activity relationships that might suggest the chemical belongs in a different class of suspected carcinogens: No data are available to evaluate these considerations.

Biochemical information regarding binding of the chemical to the α_{2u}-globulin protein: Williams and Borghoff (2001) report that *tert*-butanol reversibly and noncovalently binds to α_{2u}-globulin in the kidneys of male rats. This provides additional support to the involvement of the α_{2u}-globulin process.

Presence of sustained cell replication in the S2 (P2) segment of the renal tubule at doses used in the cancer bioassay and a dose-related increase in hyperplasia of the renal tubule: Sustained cell division in the proximal tubule of the male rat is consistent with, although not specific to, the α_{2u}-globulin process. Cell proliferation was observed in two studies [13-week, NTP (1995) and 10-day, Borghoff et al. (2001)] but whether the proliferation was compensatory is unknown, as cytotoxicity was not observed in these studies. Although the data do not support sustained cell division occurring subsequent to cytotoxic cell death, renal tubule hyperplasia in male rats was reported after 2 years of exposure (NTP, 1995). Thus, although some evidence of

sustained cell replication is available, it does not specifically support α_{2u}-globulin protein accumulation.

Covalent binding to DNA or other macromolecules, suggesting another process leading to tumors and genotoxicity (α_{2u}-globulin-inducers are essentially nongenotoxic): One study (Yuan et al., 2007) observed a dose-related increase in *tert*-butanol-DNA adducts in liver, kidney, and lung of mice administered a single low dose of *tert*-butanol (≤1 mg/kg) in saline via gavage (see Appendix B.3 in Supplemental Information for further details). An extremely sensitive method of detection was used (accelerator mass spectrometry), but the DNA adduct species were not identified, and no validation of these results has been identified in the literature. The few studies available to assess the genotoxic potential of *tert*-butanol primarily are negative, although a few studies report DNA damage induced by oxidative stress. DNA damage induced by oxidative stress is consistent with the decreased levels of glutathione in male rat kidneys reported by Acharya et al. (1995) after 10 weeks of *tert*-butanol exposure. This type of genetic damage would not necessarily preclude a role for α_{2u}-globulin, but not enough information is available to determine whether oxidative stress could initiate or promote kidney tumors in concert with α_{2u}-globulin accumulation in male rat kidneys.

Nephrotoxicity not associated with the α_{2u}-globulin process or CPN, suggesting the possibility of other processes leading to renal tubule nephrotoxicity and carcinogenicity: Nephropathy reported in the 13-week oral and inhalation and 2-year oral studies was considered CPN, but these effects were exacerbated by treatment with *tert*-butanol. At 13 weeks (NTP, 1997, 1995) and 2 years (NTP, 1995), oral and inhalation exposure increased the severity of nephropathy in male rats (NTP, 1995). Similarly, the severity of nephropathy was increased in females at 2 years, but only the incidence of nephropathy was increased in females following a 13-week oral exposure (NTP, 1995). Increased incidences of suppurative inflammation and kidney transitional epithelial hyperplasia were observed in female rats orally exposed to *tert*-butanol for 2 years. Although NTP (1995) characterized these endpoints as associated with CPN, the low background incidence in the controls combined with the dose-related increase in incidences indicate that these effects were not related to an age-associated, spontaneous induction of nephropathy. At 2 years, the male rats also exhibited dose-related increases in focal mineralization and transitional epithelial hyperplasia, although the background incidence in the controls was high (i.e., approximately 50%) (NTP, 1995). Neither endpoint in males can be attributed to CPN or α_{2u}-globulin.

Kidney weights also were increased in male and female rats in the 13-week oral and inhalation evaluations (NTP, 1997, 1995) and 15-month oral evaluation (NTP, 1995). The dose-related increases observed in both male and female rats suggest that the kidney weight changes are indicative of treatment-related molecular processes primarily unrelated to either α_{2u}-globulin protein accumulation or CPN. The exacerbation of CPN and the appearance of kidney effects in female (i.e., suppurative inflammation, transitional epithelial hyperplasia) and male rats (i.e., focal mineralization, transitional epithelial hyperplasia) that are not attributed to CPN or α_{2u}-globulin indicate that *tert*-butanol induces renal tubule nephrotoxicity partially independently of

α_{2u}-globulin. The evidence that other processes might be responsible for the renal tubule nephrotoxicity thereby decreases the likelihood that α_{2u}-globulin accumulation is solely responsible for the renal tubule tumors.

Positive tubule tumor responses in female rats and other species implying that α_{2u}-globulin-related processes alone do not account for the renal tubule tumor response: No increase in renal tubule tumor incidence was reported in *tert*-butanol-exposed female rats or mice compared with concurrent controls. Renal tubule tumors were observed only in male rats, providing support for an α_{2u}-globulin process in tumor development.

Summary and Conclusions for Question Two:

Although the evidence suggests that *tert*-butanol induces α_{2u}-globulin nephropathy, the data indicate that *tert*-butanol is a weak inducer of α_{2u}-globulin and that this process is not solely responsible for the renal tubule nephropathy and carcinogenicity observed in male rats. The lack of compensatory cell proliferation in male rats and evidence of nephrotoxicity in female rats suggest that other processes, in addition to the α_{2u}-globulin process, are operating. Furthermore, the accumulation of hyaline droplets and the induction of renal tubule hyperplasia were affected at higher doses compared to those inducing renal tubule tumors. Collectively, these data suggest that *tert*-butanol induces the α_{2u}-globulin pathway at high doses (>420 mg/kg-day), which results in tumor formation. Other, unknown pathways, however, could be operative at lower doses (<420 mg/kg-day), which contribute to renal tumor induction.

b) Chronic Progressive Nephropathy and Renal Carcinogenicity

There is scientific disagreement regarding the extent to which CPN can be characterized as a carcinogenic MOA suitable for analysis under the EPA's cancer guidelines. Proponents of CPN as an MOA have developed an evolving series of empirical criteria for attributing renal tubule tumors to CPN. Hard and Khan (2004) proposed criteria for concluding that a chemical is associated with renal tubule tumors through an interaction with CPN. Hard et al. (2013) slightly revised and restated their criteria for considering exacerbation of CPN as an MOA for renal tubule tumors in rats. Table 1-5 lists these sets of proposed empirical criteria for attributing renal tubule tumors to CPN.

Table 1-5. Proposed empirical criteria for attributing renal tumors to CPN

• First and foremost, the chemical must have been shown to exacerbate CPN to very advanced stages of severity, especially end-stage kidney disease, in comparison to control rats in a 2-year carcinogenicity study. • The tumors should occur in very low incidence and, for the most part, be minimal grade lesions conforming to small adenomas or lesions borderline between atypical tubule hyperplasia (ATH) and adenoma. • Such tumors should be associated only with the highest grades of CPN severity. • The tumors and any precursor foci of ATH must be restricted to CPN-affected parenchyma and are usually observed only toward the end of the 2-year studies. • Careful microscopic examination of renal parenchyma not involved in the CPN process should reveal no evidence of compound-induced cellular injury or other changes that would suggest alternative modes of action. Source: Hard and Khan (2004)	• Lack of genotoxic activity based on overall evaluation of in vitro and in vivo data. • Tumor incidence is low, usually <10%. • Tumors are found toward the end of 2-year studies. • Lesions are usually ATH or adenomas (carcinomas can occasionally occur). • Chemical exacerbates CPN to most advanced stages, including end-stage kidney disease. • ATH and tumors occur in rats with advanced CPN and in CPN-affected tissue. • Absence of cytotoxicity in CPN-unaffected tubules, in rats with lower grades of CPN, and in subchronic studies. Source: Hard et al. (2013)

Hard et al. (2013) maintain knowing the detailed etiology or underlying mechanism for CPN is not necessary. Instead, identifying increased CPN with its associated increase in tubule cell proliferation as the key event is adequate. Nonetheless, Hard et al. (2013) also postulated a sequence of key events for renal tumorigenesis involving exacerbation of CPN:

- Exposure to chemical (usually at high concentrations);
- Metabolic activation (if necessary);
- Exacerbated CPN, including increased number of rats with end-stage renal disease;
- Increased tubule cell proliferation because more kidney is damaged due to CPN exacerbation;
- Hyperplasia; and
- Adenoma (infrequently carcinoma).

In contrast to Hard et al. (2013); Hard and Khan (2004),Melnick et al. (2013); Melnick et al. (2012) concluded, based on an analysis of 60 NTP studies, no consistent association exists between exacerbated CPN and the incidence of renal tubule tumors in rats. Without a consistent association

and an understanding of its key events, they maintain that determining the human relevance of processes that might be occurring in rats is not possible. An earlier analysis of 28 NTP studies (Seely et al., 2002) found a slight but statistically significant increase in CPN severity in animals with renal tubule tumors, without determining that this relationship is causal. They suggested that the number of tumors due to chemically exacerbated CPN would be few.

Evaluation of the MOA Proposed by Hard et al. (2013)

Setting aside the question of whether CPN is (Hard et al., 2013; Hard and Khan, 2004) or is not (Melnick et al., 2013; Melnick et al., 2012) an MOA suitable for analysis, this section provides an analysis of the mechanistic data pertinent to CPN. EPA's cancer guidelines (U.S. EPA, 2005a) define a framework for judging whether available data support a hypothesized MOA; the analysis in this section follows the structure presented in the cancer guidelines.

Description of the hypothesized MOA. Under the EPA framework, toxicokinetic studies are important for identifying the active agent, but toxicokinetic events per se are not key events of an MOA. Thus, the EPA analysis of the MOA proposed by Hard et al. (2013) begins with (1) exacerbated CPN, including increased number of rats with end-stage renal disease, and proceeds via (2) increased tubule cell proliferation, (3) hyperplasia, and (4) adenoma, or infrequently, carcinoma.

Strength, consistency, specificity of association. The relationship between exacerbated CPN and renal tumors is moderate to strong in male rats in the NTP (1995) study. According to the NTP (1995) analysis, the mean CPN grades (same as "severity of nephropathy" reported by NTP) presented on a scale 1–4 for male rats with renal tumors were 3.5, 3.6, 3.7, and 3.4 at doses 0, 1.25, 2.5, and 5 mg/mL. The mean CPN grades for male rats without renal tumors were 2.9, 2.8, 2.8, and 3.2 for the same dose groups. The reanalysis of the NTP data by Hard et al. (2011) yielded similar numbers. The relationship between CPN and renal tumors, however, is neither consistent nor specific in the NTP (1995) study: No female rats developed renal tumors regardless of the presence of relatively low-grade or relatively high-grade CPN. For example, in female rats surviving more than 700 days, the mean CPN grades were 1.7 and 3.2 at doses of 0 and 10 mg/mL, respectively, but no tumors developed in either group.

Dose-response concordance. The dose-response relationships for CPN, renal tubule hyperplasia, and renal tubule tumors somewhat differ. According to the NTP (1995) analysis, at doses of 0, 1.25, 2.5, and 5 mg/mL, the mean CPN grades for all male rats were 3.0, 3.1, 3.1, and 3.3; the incidences of renal tubule hyperplasia (standard and extended evaluation combined) were 14/50, 20/50, 17/50, and 25/50; and the incidences of renal tubule adenomas or carcinomas were 8/50, 13/50, 19/50, and 13/50 (Table 1-3). The reanalysis by Hard et al. (2011) reported similar tumor incidences (4/50, 13/50, 18/50, and 12/50), except that four fewer rats in the controls and one fewer rat in the group exposed to 2.5 mg/mL had tumors. The lower control incidence observed in this reanalysis accentuates the differences in these dose-response relationships. In examining the various lesions at the mid-dose—the dose with the greatest increase in renal tubule

tumors in male rats—a minor increase (14/50 in controls versus 17/50 in the mid-dose group) in renal tubule hyperplasia incidence was observed, with a marginal change in CPN severity (i.e., group average of 3.0 to 3.1). That a minor increase in hyperplasia and marginal increase in CPN severity would be associated with significant tumor induction seems inconsistent. Furthermore, CPN severity is nearly as great in the female rats, yet no females developed tumors, as noted above.

Temporal relationship. The severity of CPN progressed over time. According to the NTP (1995) analysis, the mean CPN grades in the 13-week study of male rats were 1.0, 1.6, 2.6, 2.7, 2.6, and 1.1 at doses of 0, 2.5, 5, 10, 20, and 40 mg/mL. At the 15-month interim evaluation of the 2-year study, the mean CPN grades were 2.4, 2.8, 2.7, and 2.6 at doses of 0, 1.25, 2.5, and 5 mg/mL and at 2 years, increased to 3.0, 3.1, 3.1, and 3.3. Similarly, the severity of neoplastic lesions increased at the end of life. At the 15-month interim evaluation, only two rats had developed renal tubule hyperplasia and one other had a renal tubule adenoma; at 2 years, the incidences of these two lesions were much higher in all dose groups (see previous paragraph). These results are consistent with CPN as an age-related disease and with hyperplasia and tumors appearing near the end of life.

Biological plausibility and coherence. In general, the relationship between exacerbated CPN and renal tubule tumors in male rats appears plausible and coherent. Some patterns in the dose-response relationships for CPN, hyperplasia, and tumors are discrepant. Perhaps more importantly, the patterns also are discrepant for the relationships between CPN grades and renal tubule tumors in male and female rats. In addition, the increased incidences in renal tubule tumors in all exposed male rats exceed the 10% criterion proposed by Hard et al. (2013) (Table 1-5), even more so when making comparisons with the lower control tumor incidence from the Hard et al. (2011) reanalysis.

Conclusions about the hypothesized CPN-related MOA

As recommended by EPA's cancer guidelines (U.S. EPA, 2005a), conclusions about the hypothesized MOA can be clarified by answering three questions presented below.

(a) *Is the hypothesized MOA sufficiently supported in the test animals?* Exacerbated CPN leading to renal tubule tumors in male rats late in life appears to have some support. There is lack of consistency, however, between males and females and in the dose-response relationships between CPN, hyperplasia, and adenomas. These inconsistencies make difficult attributing all renal tumors to either CPN or to α_{2u}-globulin-related nephropathy (see previous section on α_{2u}-globulin), raising the likelihood of another, yet unspecified MOA.

(b) *Is the hypothesized MOA relevant to humans?* There is scientific disagreement on this question. Hard et al. (2013); Hard et al. (2009)cite several differences in pathology between rat CPN and human nephropathies in their arguments that CPN-related renal tumors in rats are not relevant to humans. On the other hand, Melnick et al. (2013); Melnick et al. (2012) argue that the etiology of CPN and the mechanisms for its exacerbation by chemicals are unknown and fail to meet fundamental principles for defining an MOA and for evaluating human relevance. This issue is unresolved.

(c) *Which populations or lifestages can be particularly susceptible to the hypothesized MOA?* There are no indications of a human population or lifestage that is especially susceptible to tumors induced through exacerbated CPN.

In summary, considering discrepant patterns in the dose-response relationships for CPN, hyperplasia, and renal tubule tumors and the lack of relationships between CPN grades and renal tubule tumors in male and female rats, together with the lack of a generally accepted MOA for CPN, the renal tubule tumors in rats cannot be attributed to CPN.

This position can be reconciled with that of Melnick et al. (2013); Melnick et al. (2012), who argued against dismissing renal tubule tumors in rats that can be related to exacerbated CPN. It also can be reconciled with Hard et al. (2013), who, while maintaining that these tumors are not relevant to humans, also allow that there is no generally accepted MOA for CPN akin to that for α_{2u}-globulin-related nephropathy. Hard et al. (2013) made this statement after reporting on the collective experience of national and international health agencies worldwide with the use of CPN as an MOA. Of 21 substances that exacerbated CPN and caused renal tumors, most were multisite carcinogens, and other tumor sites contributed to the evaluations. Only two assessments explicitly considered CPN as a renal tumor mechanism. One was the assessment of ethylbenzene by the German Federal Institute for Occupational Safety and Health, in which the agency concluded that the kidney tumors were associated with the high, strain-specific incidence of CPN that is unknown for humans [as discussed in Hard et al. (2013)]. The other was the IRIS assessment of tetrahydrofuran, for which EPA found the evidence insufficient to conclude that the kidney tumors are mediated solely by the hypothesized MOAs (U.S. EPA, 2012d). Hard et al. (2013) attributed these different conclusions to either different data for the two chemicals or the lack of a generally accepted MOA akin to α_{2u}-globulin-related nephropathy.

Relevant to this last point, IARC (1999) developed a consensus statement that listed considerations for evaluating α_{2u}-globulin-related nephropathy in rats, which was based on the work of 22 scientists, including three who were co-authors of Hard et al. (2013) and two who were co-authors of Melnick et al. (2013); Melnick et al. (2012). A similar broad-based consensus that defines a sequence of key events for exacerbated CPN, distinguishes it more clearly from α_{2u}-globulin-related nephropathy, and evaluates its relevance to humans would be helpful in advancing the understanding of these issues.

Overall Conclusions on MOA for Kidney Effects

tert-Butanol increases α_{2u}globulin deposition and hyaline droplet accumulation in male rat kidneys, as well as several of the subsequent steps in that pathological sequence. These data provide sufficient evidence (albeit minimal) that the α_{2u}globulin process is operating, although based on further analysis this chemical appears to be a weak inducer of α_{2u}globulin-nephropathy and this induction is not the sole contributor to renal tubule nephropathy and carcinogenicity. CPN and the exacerbation of CPN (likely due to both α_{2u}-globulin and *tert*-butanol) play a role in renal tubule nephropathy. Although CPN was indicated in the induction of renal tubule nephropathy, the

available evidence indicates that it does not induce the renal tubule tumors in male rats. Additionally, several endpoints indicate renal tubule nephrotoxicity and increased kidney weights related to *tert*-butanol exposure cannot be explained by the α_{2u}globulin or CPN processes. Collectively, the evidence indicates other, unknown processes contribute to renal tubule nephrotoxicity and carcinogenicity.

Integration of kidney effects

Kidney effects (increases in nephropathy, severity of nephropathy, hyaline droplets, linear mineralization, suppurative inflammation, transitional epithelial hyperplasia, mineralization, and kidney weight) were observed, predominantly in male and female rats across the multiple *tert*-butanol studies. The available evidence indicates that multiple processes induce the noncancer kidney effects. The group of lesions generally reported as "nephropathy," is related to CPN. Because this disease is considered to be spontaneous and age-related in rats, the endpoints associated with CPN would not be relevant to humans for purposes of hazard identification. Additionally, two endpoints in male rats (hyaline droplets, linear mineralization) are components of the α_{2u}-globulin process. U.S. EPA (1991a) states that if the α_{2u}-globulin process is occurring in male rats, the renal tubule effects associated with this process in male rats would not be relevant to humans for purposes of hazard identification. In cases such as these, the characterization of human health hazard for noncancer kidney toxicity would rely on effects not specifically associated with CPN or the α_{2u}-globulin-process in male rats.

Several other noncancer endpoints resulted from *tert*-butanol exposure and are appropriate for consideration of a kidney hazard, specifically: suppurative inflammation in female rats, transitional epithelial hyperplasia in male and female rats, severity of nephropathy in male and female rats, incidence of nephropathy in female rats, incidence of mineralization in male rats, and increased kidney weights in rats but not mice. Based on dose-related increases in these noncancer endpoints in rats, kidney effects are a potential human hazard of *tert*-butanol exposure. The hazard and dose-response conclusions regarding these noncancer endpoints associated with *tert*-butanol exposure are discussed further in Section 1.3.1.

The carcinogenic effects observed following *tert*-butanol exposure include increased incidences of renal tubule hyperplasia (considered a preneoplastic effect) and tumors in male rats. EPA concluded that the three criteria were met to indicate that an α_{2u}-globulin process is operating. Because renal tubule tumors in male rats did not arise solely due to the α_{2u}-globulin process and some of the tumors are attributable to other carcinogenic processes, such tumors remain relevant

for purposes of hazard identification U.S. EPA (1991a).[3] The hazard and dose-response conclusions regarding the renal tubule hyperplasia and tumors associated with *tert*-butanol exposure are further discussed as part of the overall weight of evidence for carcinogenicity in Section 1.3.2.

1.2.2. Thyroid Effects

Synthesis of Effects in Thyroid

The database on thyroid effects following *tert*-butanol exposure contains no human data, two oral subchronic and two oral chronic studies (one of each duration in rats and in mice) (NTP, 1995), and two inhalation subchronic studies (one in rats and one in mice) (NTP, 1997). Studies employing short-term and acute exposures that examined thyroid effects are not included in the evidence table; they are discussed, however, in the text if they provide data informative of MOA or hazard identification. No gross thyroid effects were reported in the 13-week evaluations of mice or rats following oral or inhalation exposure (NTP, 1997, 1995), and therefore subchronic studies were not included in the evidence table. The two available chronic studies are arranged in the evidence table by effect and then by species. The design, conduct, and reporting of each study were reviewed, each study was considered adequate to provide information pertinent to this assessment (Table 1-6 and Figure 1-10).

Thyroid effects, specifically follicular cell hyperplasia and adenomas, were observed in mice of both sexes after 2 years of oral exposure via drinking water (NTP, 1995). NTP (1995) noted that "[p]roliferation of thyroid gland follicular cells is generally considered to follow a progression from hyperplasia to adenoma and carcinoma." Similarly, EPA considered the thyroid follicular cell hyperplasia to be a preneoplastic effect associated with the thyroid tumors. Both male and female mice exhibited a dose-related increase in the incidence of hyperplasia, and the average severity across all dose groups was minimal to mild with scores ranging from 1.2 to 2.2 (out of 4). Increased incidence of adenomas were also observed in the *tert*-butanol-treated mice, with the only carcinoma observed in high-dose males. No treatment-related thyroid effects were reported in rats of either sex following 2 years of oral exposure (NTP, 1995).

Although the tumor response in male mice showed a statistically significant increasing trend (Cochran-Armitage trend test, $p = 0.041$) (analysis performed by EPA using the mortality-adjusted rates), the response was non-monotonic, with a slightly lower response at the high-dose

[3] When the α_{2u}-globulin process is occurring, U.S. EPA (1991a) states that one of the following conclusions will be made: (a) if renal tumors in male rats are attributable solely to the α_{2u}-globulin process, such tumors will not be used for human cancer hazard identification or for dose-response extrapolations; (b) if renal tumors in male rats are not linked to the α_{2u} globulin process, such tumors are an appropriate endpoint for human hazard identification and are considered, along with other appropriate endpoints, for quantitative risk estimation; or (c) if some renal tumors in male rats are attributable to the α_{2u}-globulin process and some are attributable to other carcinogenic processes, such tumors remain relevant for purposes of hazard identification, but a dose-response estimate based on such tumors in male rats should not be performed unless there is enough information to determine the relative contribution of each process to the overall renal tumor response.

level than at the mid-dose level. The reason for the non-monotonicity is unclear, although it could be related to the increased mortality in the high-dose group (17/60 animals survived compared with 27/60 animals in the control group). The decreased survival of male mice might have affected the thyroid tumor incidences because animals could have died before tumors could develop. High mortality in the high-dose group occurred before tumors appeared; about 40% of the high-dose males died before the first tumor (a carcinoma) appeared in this group at week 83. By comparison, only ~10% of the control group had died by this time, and the single tumor in the control group was observed at study termination. Mortality in the exposed female mice was similar to controls.

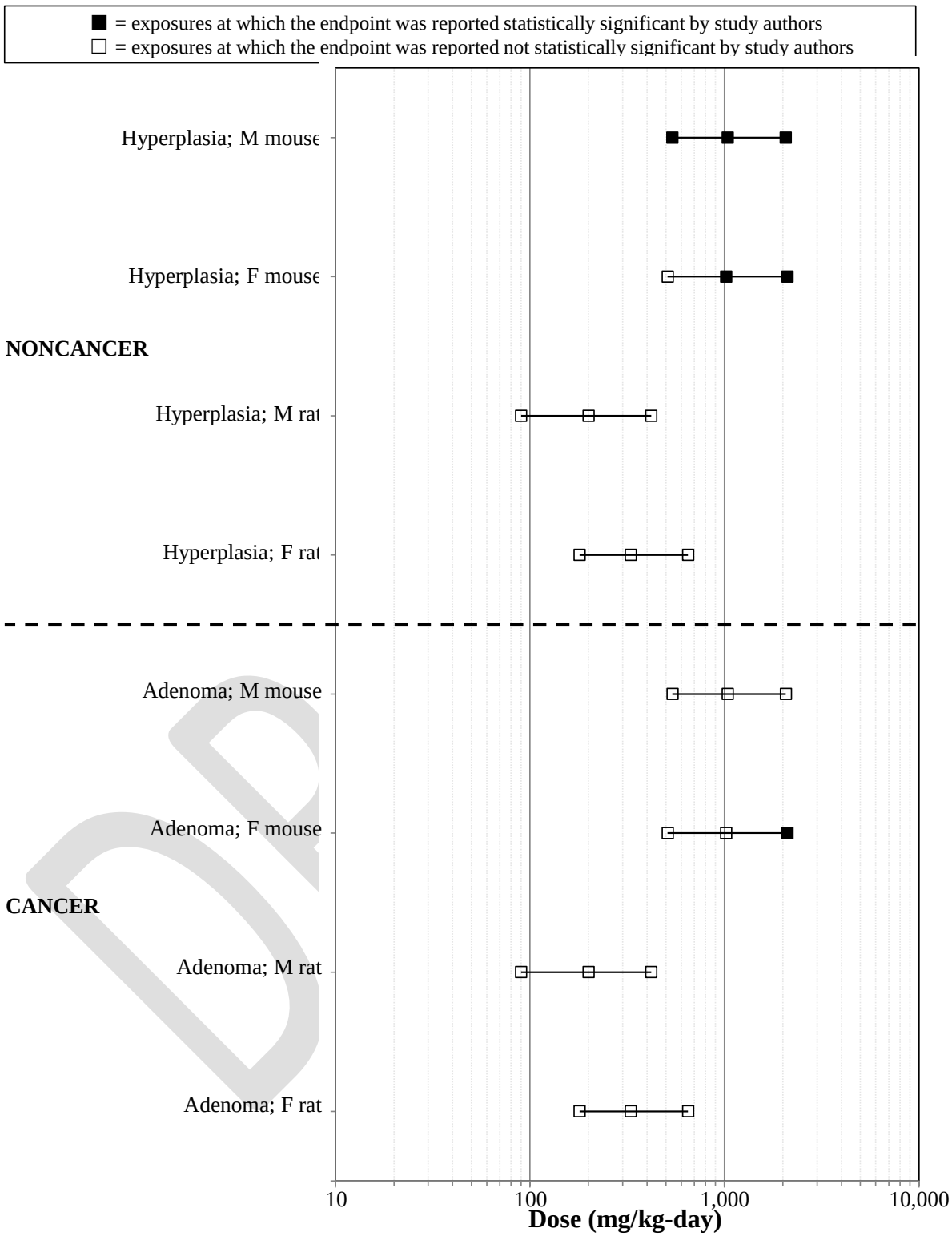
Table 1-6. Evidence pertaining to thyroid effects in animals following oral exposure to *tert*-butanol

Reference and study design	Results			
Follicular cell hyperplasia				
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	Incidence[b]			
	Males		**Females**	
	Dose (mg/kg-d)	Follicular cell hyperplasia	Dose (mg/kg-d)	Follicular cell hyperplasia
	0	3/50	0	0/50
	90	0/49	180	0/50
	200	0/50	330	0/50
	420[a]	0/50	650[a]	0/50
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	Incidence (severity)			
	Males		**Females**	
	Dose (mg/kg-d)	Follicular cell hyperplasia	Dose (mg/kg-d)	Follicular cell hyperplasia
	0	5/60 (1.2)	0	19/58 (1.8)
	540	18/59* (1.6)	510	28/60 (1.9)
	1,040	15/59* (1.4)	1,020	33/59* (1.7)
	2,070[a]	18/57* (2.1)	2,110	47/59* (2.2)
Follicular cell tumors				
NTP (1995)	Incidence[b]			

Reference and study design	Results				
F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	Dose (mg/kg-d)	Follicular cell adenoma	Follicular cell carcinoma		
	Male				
	0	2/50	2/50		
	90	0/49	0/49		
	200	0/50	0/50		
	420[a]	0/50	0/50		
	Female				
	0	1/50	1/50		
	180	0/50	0/50		
	330	1/50	1/50		
	650[a]	0/50	0/50		
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	Incidence				
	Dose (mg/kg-d)	Follicular cell adenoma	Follicular cell carcinoma	Follicular cell adenoma or carcinoma (mortality adjusted rates)[c,d]	Animals surviving to study termination
	Male				
	0	1/60	0/60	1/60 (3.6%)	27/60
	540	0/59	0/59	0/59 (0.0%)	36/60
	1,040	4/59	0/59	4/59 (10.1%)	34/60
	2,070[a]	1/57	1/57	2/57 (8.7%)	17/60
	Female				
	0	2/58	0/58	2/58 (5.6%)	36/60
	510	3/60	0/60	3/60 (8.6%)	35/60
	1,020	2/59	0/59	2/59 (4.9%)	41/60
	2,110	9/59*	0/59	9/59* (19.6%)	42/60

[a]There was a significant decrease in survival in the high-dose group.
[b]Results do not include the animals sacrificed at 15 months.
[c]Mortality-adjusted rates were not calculated by study authors for follicular cell carcinoma. The mortality-adjusted rates for the incidence of adenomas are the same as the combined rates, with the exception of the male high-dose group, where the rate for adenomas alone was 5.9%.
[d]Cochran-Armitage trend test was applied to mortality-adjusted thyroid tumor incidences, by applying the NTP adjusted rates to the observed numbers of tumors to estimate the effective number at risk in each group. For male mice, $p = 0.041$; for female mice, $p = 0.028$.* Statistically significant $p \leq 0.05$ as determined by the study authors.

Note: Conversions from drinking water concentrations to mg/kg-d performed by study authors.



Source: NTP (1995)

Figure 1-10. Exposure-response array of thyroid follicular cell effects following chronic oral exposure to *tert*-butanol. (Note: Only one carcinoma was observed in male mice at the high-dose group.)

Mode of Action Analysis—Thyroid Effects

The MOA responsible for *tert*-butanol-induced thyroid effects has not been the subject of much study. One hypothesis is that *tert*-butanol increases liver metabolism of thyroid hormones, triggering a compensatory increase in pituitary thyroid-stimulating hormone (TSH) production. Such sustained increases in TSH could induce elevated thyroid follicular cell proliferation and hyperplasia and lead to follicular cell adenoma and carcinoma, that, an antithyroid MOA, as identified in U.S. EPA's guidance on the assessment of thyroid follicular cell tumors (U.S. EPA, 1998a).

To determine if the thyroid follicular cell tumors result from a chemically induced antithyroid MOA, U.S. EPA (1998a) requires that the available database demonstrate: (1) increases in thyroid cell growth, (2) thyroid and pituitary hormone changes consistent with the antithyroid MOA, (3) site(s) of the antithyroid action, (4) dose correlation among the various effects, and (5) reversibility of effects in the early stages of disruption. The available evidence pertaining to each of these aspects of antithyroid activity following *tert*-butanol exposure is discussed below.

1) Increases in cell growth (required)

U.S. EPA (1998a) considers increased absolute or relative thyroid weights, histological indicators of cellular hypertrophy and hyperplasia, DNA labeling, and other measurements (e.g., Ki-67 or proliferating cell nuclear antigen expression) to be indicators of increased cell growth. Only a few studies (NTP, 1997, 1995) have evaluated the thyroid by routine histological examination following *tert*-butanol exposure, and none investigated specific molecular endpoints. None of the available long-term studies measured thyroid weight in mice, likely due to the technical limitations involved, and no thyroid effects were attributed to *tert*-butanol exposure in rats treated up to 2 years (NTP, 1997, 1995). Although the short-term female mouse study by Blanck et al. (2010) stated that thyroids were weighed, no results were reported.

An increase in thyroid follicular cell hyperplasia was observed in both female and male mice after a 2-year drinking water exposure to *tert*butanol- (NTP, 1995). The increase was dose dependent in female mice with a slight increase in severity in the highest dose, while male mice experienced a similar magnitude of hyperplasia induction at all doses, with increased severity at the highest dose (NTP, 1995). Thyroid follicular cell hyperplasia was not observed in any mouse study with less than 2 years of exposure: no treatment-related histological alterations in the thyroid of *tert*-butanol-treated (2 or 20 mg/mL) female mice after 3 or 14 days of drinking water exposure (Blanck et al., 2010) were reported, in male or female mice after 13 weeks of drinking water exposure (NTP, 1995) or in male or female mice following 18-day or 13-week inhalation studies (NTP, 1997). The observation of increased hyperplasia in male and female mice after 2 years of exposure is sufficient evidence to support increased thyroid cell growth.

2) Changes in thyroid and relevant pituitary hormones (required)

Evidence of hormonal changes, including decreases in thyroxine (T_4) and triiodothyronine (T_3) and increases in TSH, are required to demonstrate a disruption in the thyroid-pituitary signaling axis (U.S. EPA, 1998a). Blanck et al. (2010) evaluated serum thyroid hormones in mice after 3 or 14 days of exposure to *tert*-butanol. No *tert*-butanol-related effects were observed in T_3, T_4, or TSH levels after 3 days, and although both T_3 and T_4 levels were significantly decreased approximately 10–20% after 14 days of treatment with *tert*-butanol, TSH levels remained unaffected. Similar results were reported with the positive control (phenobarbital). The limited evidence available from this single study suggests that although T_3 and T_4 levels were decreased after 14 days, this perturbation likely was not in excess of the range of homeostatic regulation in female $B6C3F_1$ mice and thus not likely to induce compensatory thyroid follicular cell proliferation. Multiple lines of evidence support this observation: (1) TSH levels were unaffected, indicating that the decrease in T_3 and T_4 levels was not severe enough to stimulate increased TSH secretion by the pituitary; (2) thyroid hyperplasia was not induced in this study, or any others exposing mice for 2.5–13 weeks, suggesting that thyroid proliferation was either not induced by the hormone fluctuations or that any follicular cell proliferation during this period was too slight to be detected by routine histopathological examination; (3) the maximal decrease in T_3 or T_4 hormone levels induced by *tert*-butanol exposure after 14 days (i.e., ~20%) was well within the range of fluctuation in T_3 and T_4 hormone levels reported to occur between the 3- and 14-day control groups [15–40%; (Blanck et al., 2010)]. Although the lower T_3 and T_4 levels following *tert*-butanol were later attributed by the study authors to an increase in liver metabolism (see next section), they could in fact be due to a decrease in thyroid hormone production, resulting from some, as of yet, uninvestigated molecular interactions of *tert*-butanol in the thyroid, pituitary, or hypothalamus.

The absence of information regarding thyroid hormone levels in male mice and lack of molecular studies evaluating exposures >2 weeks in female mice are significant deficiencies in the available database. Together, although small decreases in some thyroid hormone levels have been reported in female mice, the available evidence is inadequate to determine if *tert*-butanol negatively affects the pituitary-thyroid signaling axis in female mice; furthermore, no evidence was available to evaluate this effect in male mice.

3) Site(s) of antithyroid action (required)

The thyroid and liver are two of several potential sites of antithyroid action, with the liver the most common site of action, where increased microsomal enzyme activity could enhance thyroid hormone metabolism and removal (U.S. EPA, 1998a). Rats are thought to be more sensitive than mice to this aspect of antithyroid activity (Roques et al., 2013; Qatanani et al., 2005; U.S. EPA, 1998a); however, rats exposed to *tert*-butanol for 2 years did not exhibit treatment-related thyroid effects, while mice did. Typically, chronic induction of liver microsomal enzyme activity resulting from repeated chemical exposure would manifest some manner of liver histopathology, such as

hepatocellular hypertrophy or hyperplasia (U.S. EPA, 1998a; NTP, 1995). In a 14-day mechanistic investigation, *tert*-butanol had no effect on liver weight when compared to the control group, but centrilobular hepatocellular hypertrophy was reported in 2/5 livers from high-dose mice (versus 0/6 in control and 0/5 in low-dose mice (Blanck et al., 2010). Relative liver weights increased in male and female mice after 13 weeks of oral exposure (NTP, 1995) to higher doses than those evaluated by Blanck et al. (2010), although absolute liver weight measurements in treated animals showed little change from controls suggesting that the relative measures could have been related to decreases in body weight rather than specific liver effects. Relative (and absolute) liver weights were increased in female mice (only) after 13 weeks of inhalation exposure at the two highest concentrations (NTP, 1997); liver weight was not reported in mice orally exposed for 2 years (NTP, 1995). No increase in mouse hepatocellular hypertrophic or hyperplastic histopathology was reported following 2.5 weeks to 2 years of exposure (NTP, 1997, 1995). In fact, the only liver pathology associated with *tert*-butanol exposure in these studies was an increase in fatty liver in male mice in the high-dose group after 2 years of oral exposure (NTP, 1995). Although increased fatty liver could indicate some non-specific metabolic alteration, the absence of a similar treatment-related effect in livers from female mice, which were sensitive to both thyroid follicular cell hyperplasia and tumor induction, suggests that it might not be related to the thyroid tumorigenesis.

One study evaluated liver enzyme expression and found highly dose-responsive induction of a single phase I cytochrome p450 enzyme (CYP2B10) following 14 days of *tert*-butanol exposure in female mice, with much smaller increases in the expression of another phase I enzyme CYP2B9, and the phase II thyroid hormone-metabolizing enzyme, sulfotransferase 1A1 [(SULT1A1; Blanck et al. (2010)]. CYP2B enzyme induction is commonly used as an indication of constitutive androstane receptor (CAR) activation; CAR can induce expression of a wide range of hepatic enzymes, including several CYPs along with thyroid hormone-metabolizing sulfotransferases (Roques et al., 2013). The only thyroid hormone-metabolizing enzyme induced by *tert*-butanol, however, was SULT1A1, which has been reported to be inducible in a CAR-independent manner in mice (Qatanani et al., 2005). Based on alterations in hepatic phase I and phase II enzyme activities and gene expression, the above data suggest a possible role for increased thyroid hormone clearance in the liver following repeated *tert*-butanol exposure; however, the expression changes in these few enzymes are not supported by any liver histopathological effects in mice exposed for longer durations, so whether this enzyme induction is transient, or simply insufficient to induce liver pathology after >2 weeks of exposure, is unknown. No evidence is available to evaluate the potential for intrathyroidal or any other extrahepatic effects in female mice or for any of these molecular endpoints in male mice; therefore, the available evidence is inadequate to determine if major site(s) of antithyroid action are affected.

4) Dose correlation (required)

Confidence in the disruption of the thyroid-pituitary function is enhanced when dose correlation is present among the hormone levels producing various changes in thyroid

histopathology, including thyroid tumors (U.S. EPA, 1998a). Furthermore, if thyroid hormone levels were affected by liver enzyme induction, confidence would be increased by a concordance among liver effects, thyroid hormone levels, and thyroid pathology. Thyroid hormone levels were evaluated only in female mice exposed to *tert*-butanol; after 2 weeks of exposure, both T_4 and T_3 were decreased with both doses (2 and 20 mg/L), and TSH was unaffected at either dose (Blanck et al., 2010). Liver expression of CYP2B10 was increased in a dose-responsive manner, while SULT1A1 mRNA was induced by 20–30% at both doses (Blanck et al., 2010). As described above, induction of liver microsomal enzyme activity would manifest some manner of liver histopathology (Maronpot et al., 2010; U.S. EPA, 1998a; NTP, 1995). Toxicol Pathol 38:776-795), and consistent with this expected association, centrilobular hepatocellular hypertrophy was reported in 2/5 high-dose mice exposed for 2 weeks (Blanck et al., 2010). No liver histopathology, however, was attributed to *tert*-butanol exposure in female mice exposed for 2.5 weeks to 2 years to comparable *tert*-butanol concentrations (NTP, 1997, 1995). Although liver enzyme levels and activity were not specifically evaluated following subchronic to chronic exposure, the lack of liver pathology suggests a comparable lack of enzyme induction. Conversely, no histopathological alterations were reported in the thyroids of female mice after 2 weeks of oral exposure at doses that elevated some liver enzyme levels (Blanck et al., 2010).

Following 2 years of oral exposure, both follicular cell hyperplasia and follicular cell tumor incidence was increased in mice despite a lack of treatment-related liver pathology (NTP, 1995) (Table 1-6). Any associations relating hormone changes to thyroid pathology or liver enzyme induction are limited due to the inadequate database (described above); the available evidence suggests little concordance among reports of liver, pituitary, and thyroid effects in female mice, and no evidence was available to evaluate these associations in male mice.

5) Reversibility (required)

Chemicals acting via an antithyroid MOA have effects (e.g., increased TSH levels, thyroid follicular cell proliferation) that are reversible after cessation of treatment (U.S. EPA, 1998a). Although increased TSH levels have not been demonstrated following *tert*-butanol exposure, thyroid follicular cell proliferation was observed following chronic exposure. As no studies have evaluated changes in thyroid hormones or thyroid histopathology after cessation of *tert*-butanol treatment, however, the available evidence is inadequate to evaluate reversibility of these effects.

In summary, the available database sufficiently supports only (1) increases in thyroid cell growth. The existing data are inadequate to evaluate (2) thyroid and pituitary hormone changes consistent with the antithyroid MOA, (3) site(s) of the antithyroid action, or (5) reversibility of effects in the early stages of disruption. Although these inadequacies also limit the evaluation of (4) dose correlation among the various effects, the available evidence suggests that little correlation exists among reported thyroid, pituitary, and liver endpoints. Together, the database is inadequate to determine if an antithyroid MOA is operating in mice. In the absence of information to indicate otherwise, the thyroid tumors observed in mice are considered relevant to humans.

Integration of thyroid effects

The thyroid endpoints reported following chronic exposure to *tert*-butanol include increases in follicular cell hyperplasia and tumors in male and female mice. As discussed above, due to inadequacies in four of the five required areas (U.S. EPA, 1998a), the evidence is inadequate to determine if an antithyroid MOA is operating in mice; therefore, the MOA(s) for thyroid tumorigenesis has not been identified. EPA considers the thyroid follicular cell hyperplasia to be an early event in the neoplastic progression of thyroid follicular cell tumors, and no other noncancer effects on the thyroid were observed. Thus, the hazard and dose-response conclusions regarding the thyroid follicular cell hyperplasia and tumors associated with *tert*-butanol exposure are discussed as part of the overall weight of evidence for carcinogenicity in Section 1.3.2.

1.2.3. Developmental Effects

Synthesis of effects related to development

Four studies evaluated developmental effects [three oral or inhalation developmental studies (Faulkner et al., 1989; Nelson et al., 1989; Daniel and Evans, 1982) and a one-generation, oral reproductive study (Lyondell Chemical Co., 2004)] in animals exposed to *tert*-butanol via liquid diet (i.e., maltose/dextrin), oral gavage, or inhalation. No developmental epidemiology studies are available for *tert*-butanol. The animal studies are arranged in the evidence tables by species, strain, and route of exposure. The design, conduct, and reporting of each study were reviewed, and each study was considered adequate to provide information pertinent to this assessment. One study was considered less informative, Faulkner et al. (1989), because it did not provide sufficient information on the dams to determine if fetal effects occurred due to maternal toxicity.

Developmental effects of *tert*-butanol observed after oral exposure (liquid diets or gavage) in several mouse strains and one rat strain include measures of fetal loss or viability (e.g., increased number of resorptions, decreased numbers of neonates per litter) and decreased fetal body weight (Lyondell Chemical Co., 2004; Faulkner et al., 1989; Daniel and Evans, 1982). Daniel and Evans (1982) also observed decreases in body weight gain during post-natal days (PNDs) 2–10; data suggest, however, that this effect might be due to altered maternal behavior or nutritional status. In addition, a single dose study reported a small increase in the incidence of variations of the skull or sternebrae in two mouse strains (Faulkner et al., 1989). Although variations in skeletal development were noted in the study, no malformations were reported. Similar developmental effects were observed after whole-body inhalation exposure in Sprague-Dawley rats for 7 hours/day on gestation days (GDs) 1–19 (Nelson et al., 1989). Fetal effects included dose-related reductions in body weight in male and female fetuses and higher incidence of skeletal variations when analyzed based on individual fetuses (but not on a per litter basis).

In these studies, fetal effects are generally observed at doses that cause toxicity in the dams as measured by clinical signs (e.g., decreased body weight gain, food consumption) (Table 1-7; Figure 1-11; Figure 1-12). As stated in the *Guidelines for Developmental Toxicity Risk Assessment*

(U.S. EPA, 1991b), "an integrated evaluation must be performed considering all maternal and developmental endpoints." "[W]hen adverse developmental effects are produced only at doses that cause minimal maternal toxicity; in these cases, the developmental effects are still considered to represent developmental toxicity and should not be discounted." Although, at doses of "excessive maternal toxicity…information on developmental effects may be difficult to interpret and of limited value." In considering the fetal and maternal toxicity data following *tert*-butanol exposure, the severity of the maternal effects were minimal and therefore the developmental effects in the fetuses should not be discounted (U.S. EPA, 1991b). The observed fetal effects occurred, however, at doses resulting in maternal toxicity across all available studies. Therefore, whether the fetal effects are directly related to *tert*-butanol treatment or are secondary to maternal toxicity remains unclear.

Table 1-7. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d F0 males: 9 weeks beginning 4 weeks prior to mating F0 females: 4 weeks prior to mating through PND21 F1 Males and Females: 7 weeks (throughout gestation and lactation; 1 male and 1 female from each litter was dosed directly from PND 21-28)	Response relative to control Dose (mg/kg-d): 0 / 64 / 160 / 400 / 1000 **Maternal effects** Body weight gain GD 0-20 0 / −3 / −4 / 0 / −16* Food consumption GD 0-20 0 / 0 / 0 / +4 / 0 Body weight gain PND 1-21 0 / +3 / −10 / +3 / +100* Food consumption LD1-14 0 / −2 / −6 / 0 / −16 Live pups/litter response *relative to control* 0 / −9 / −11 / −7 / −33* Dams dosed with 400 or 1000 mg/kg-d showed CNS effects (e.g., ataxia, lethargy) which were undetectable by 4-weeks of exposure in animals exposed to 400 mg/kg-d but not those in the higher dose group. **F1 effects** Viability index (pup survival to PND4) 96.4% / 98.7% / 98.2% / 99.4% / 74.1%* Lactation index (pup survival to PND21) 100% / 100% / 100% / 99.2% / 98.8% Sex ratio (% males) 54.4 / 52.3 / 50.9 / 53.4 / 52.1 Pup weight/litter PND 1 relative to control (%) 0 / +6 / +4 / +7 / −10 Pup weight PND 28 relative to control (%) M: 0 / +2 / 0 / 0 / −12* F: 0 / 0 / −4 / −2 / −8

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Daniel and Evans (1982)

Swiss Webster (Cox) mouse; 15 pregnant dams/treatment
Liquid diet (0, 0.5, 0.75, 1.0%, w/v)
0 (isocaloric amounts of maltose/dextrin), 3,324, 4,879, 6,677 mg/kg-d
GD 6–20</td><td>No statistical analysis was conducted on any of these data

Maternal

Percent change compared to control:

<table>
<tr><th>Dose (mg/kg-d)</th><th>Food consumption (mean g/animal/day)</th><th>Body weight gain</th><th>Number of litters (% pregnant dams)</th></tr>
<tr><td>0</td><td>0</td><td>0</td><td>11 (77%)</td></tr>
<tr><td>3,324</td><td>+2</td><td>−3</td><td>12 (80%)</td></tr>
<tr><td>4,879</td><td>−3</td><td>−19</td><td>8 (53%)</td></tr>
<tr><td>6,677</td><td>−4</td><td>−20</td><td>7 (47%)</td></tr>
</table>

Authors note that lower food consumption in higher tert-butanol dose groups reflects problems with pair feeding and maternal sedation.

Fetal

Percent change compared to control:

<table>
<tr><th>Dose (mg/kg-d)</th><th>Number of neonates/litter</th><th>Fetal body weight on PND 2</th></tr>
<tr><td>0</td><td>0</td><td>0</td></tr>
<tr><td>3,324</td><td>−1</td><td>−7</td></tr>
<tr><td>4,879</td><td>−29</td><td>−19</td></tr>
<tr><td>6,677</td><td>−49</td><td>−38</td></tr>
</table>

Number of stillborn also increased with dose (3, 6, 14, and 20, respectively), but the number of stillborn per litter was not provided. The high dose also caused a delay in eye opening and a lag in weight gain during PND 2–10 (information was only provided in text or figures)</td></tr>
<tr><td>Faulkner et al. (1989)

CBA/J mouse; 7 pregnant females in control, 12 pregnant females in treated
Gavage (10.5 mmoles/kg twice a day);
0 (tap water), 1,556 mg/kg-d
GD 6–18</td><td>Maternal results not reported.

Fetal

<table>
<tr><th></th><th colspan="3">Percent change compared to control:</th><th colspan="2">Incidence:</th></tr>
<tr><th>Dose (mg/kg-d)</th><th>Resorptions/litter</th><th>Live fetuses/ litter</th><th>Fetal weight</th><th>Sternebral variations</th><th>Skull variations</th></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td><td>4/28</td><td>1/28</td></tr>
<tr><td>1,556</td><td>+118*</td><td>−41*</td><td>−4</td><td>7/30</td><td>3/30</td></tr>
</table>

Sternal variations: misaligned or unossified sternebrae
Skull variations: moderate reduction in ossification of supraoccipital bone

Number of total resorptions (10 resorptions/66 implants in controls, 37/94 implants in treated) increased ($p < 0.05$)</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Faulkner et al. (1989)

C57BL/6J mouse; 5 pregnant females in controls, 9 pregnant females treated
Gavage (10.5 mmoles/kg twice a day)
0 (tap water), 1,556 mg/kg-d
GD 6–18</td><td>Maternal results not reported.

Fetal

Percent change compared to control: Incidence:

<table>
<tr><th>Dose (mg/kg-d)</th><th>Resorptions/litter</th><th>Live fetuses/ litter</th><th>Fetal weight</th><th>Sternebral variations</th><th>Skull variations</th></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td><td>5/21</td><td>1/21</td></tr>
<tr><td>1,556</td><td>+428*</td><td>−58*</td><td>−4</td><td>9/16</td><td>7/16</td></tr>
</table>
Sternal variations: misaligned or unossified sternebrae
Skull variations: moderate reduction in ossification of supraoccipital bone

Number of total resorptions (4 resorptions/44 implants in controls, 38/68 implants in treated) increased ($p < 0.05$)</td></tr>
<tr><td>Nelson et al. (1989)

Sprague-Dawley rat; 15 pregnant dams/treatment
Inhalation analytical concentration: 0, 2,200, 3,510, 5,030 ppm (0, 6,669, 10,640, 15,248 mg/m^3), (dynamic whole body chamber)
7 hr/d
GD 1–19</td><td>Maternal: Unsteady gait (no statistical tests reported), dose-dependent ↓ in body weight gain (results presented in figure only), dose-dependent ↓ in food consumption ranging from 7–36% depending on dose and time

Fetal

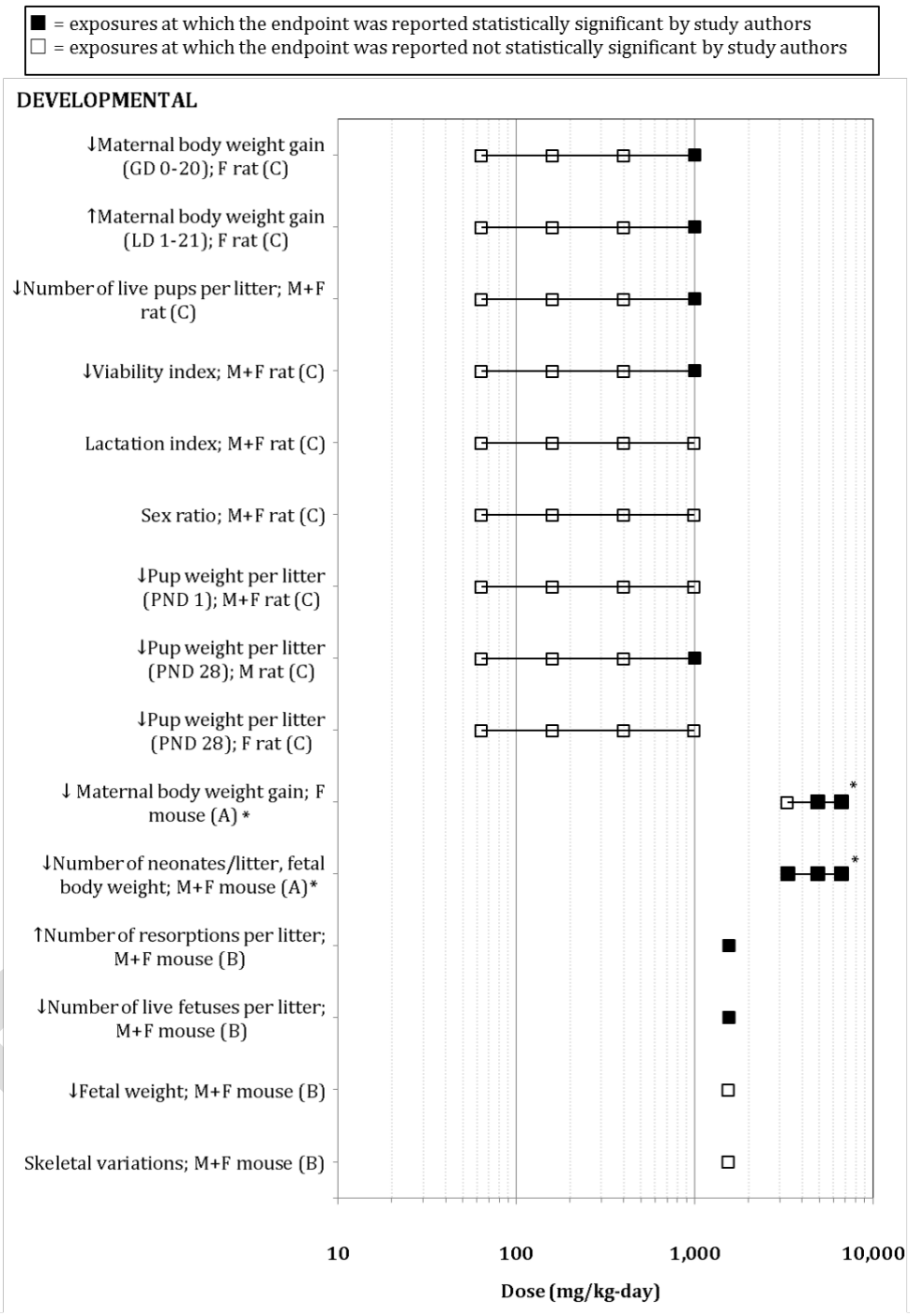
Percent change compared to control (mean ± standard error):

<table>
<tr><th>Dose (mg/m^3)</th><th>Number of live fetuses/litter</th><th>Resorptions per litter</th></tr>
<tr><td>0</td><td>0 (13±2)</td><td>0 (1.1±1.2)</td></tr>
<tr><td>6,669</td><td>0 (13±4)</td><td>+9 (1.2±1.1)</td></tr>
<tr><td>10,640</td><td>+15 (15±2)</td><td>−18 (0.9±1.0)</td></tr>
<tr><td>15,248</td><td>+8 (14±2)</td><td>0 (1.1±0.9)</td></tr>
</table>
Percent change compared to control: Incidence:

<table>
<tr><th>Dose (mg/m^3)</th><th>Fetal weight (males)</th><th>Fetal weight (females)</th><th>Skeletal variation by litter</th><th>Skeletal variation by fetus</th></tr>
<tr><td>0</td><td>0</td><td>0</td><td>10/15</td><td>18/96</td></tr>
<tr><td>6,669</td><td>−9*</td><td>−9*</td><td>14/17</td><td>35/104</td></tr>
<tr><td>10,640</td><td>−12*</td><td>−13*</td><td>14/14</td><td>53/103*</td></tr>
<tr><td>15,248</td><td>−32*</td><td>−31*</td><td>12/12</td><td>76/83*</td></tr>
</table>
Skeletal variation by litter refers to the number of variations observed in the number of litters examined. Skeletal variation by fetus refers to the number of variations observed in the total number of fetuses examined. Fetuses are not categorized by litter.</td></tr>
</table>

* Statistically significant $p \leq 0.05$ as determined by study authors. Conversions from diet concentrations to mg/kg-d performed by study authors. Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.

Note: Percentage change compared to control = (treated value – control value) ÷ control value × 100.



* Study authors did not conduct statistical analysis on these endpoints, but results are determined by EPA to be biologically significant.
Sources: (A) Daniel and Evans (1982); (B) Faulkner et al. (1989); Lyondell Chemical Co. (2004)

Figure 1-11. Exposure-response array of developmental effects following oral exposure to *tert*-butanol.

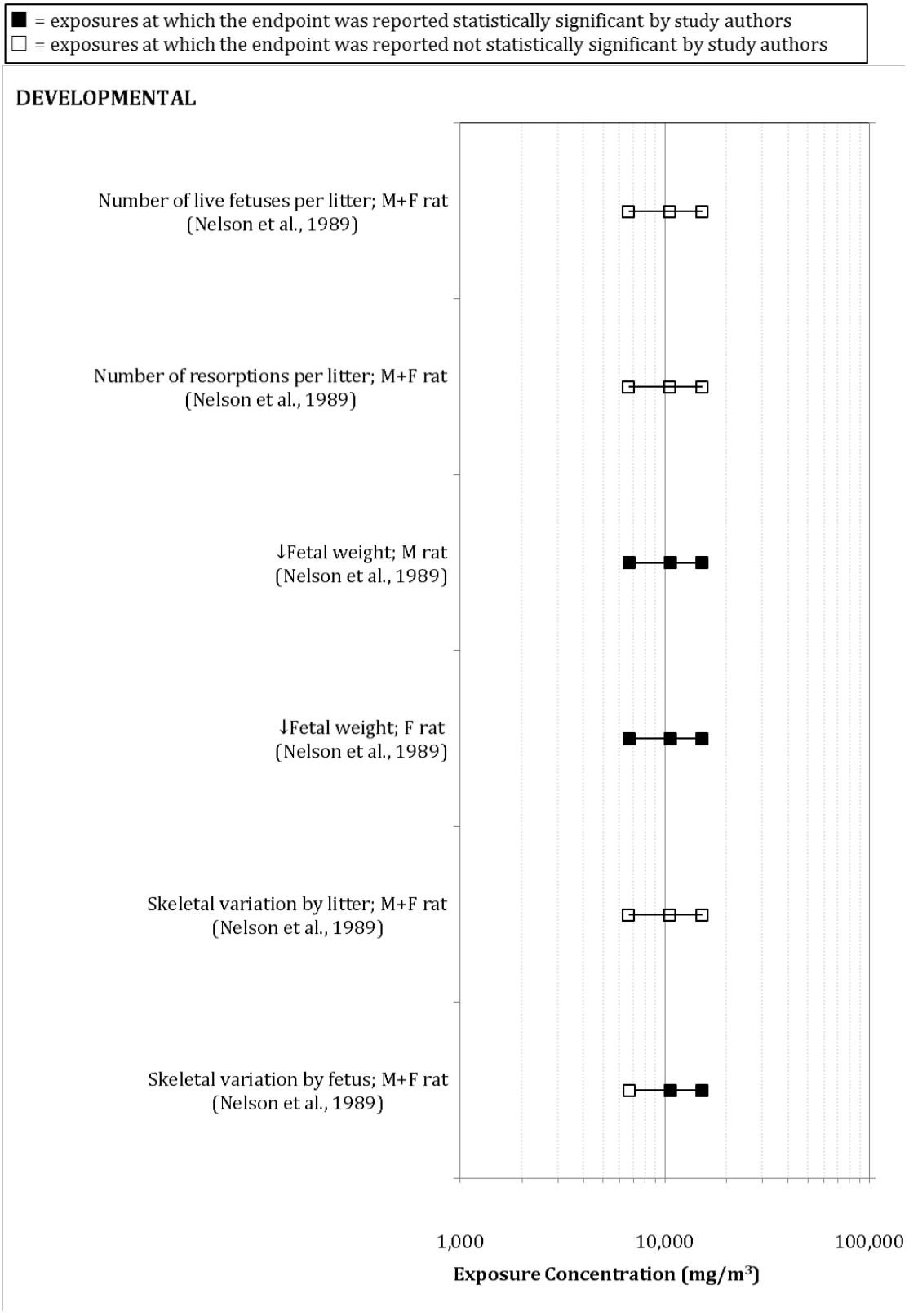


Figure 1-12. Exposure-response array of developmental effects following inhalation exposure to *tert*-butanol.

Integration of developmental effects

There is suggestive evidence of developmental effects associated with *tert*-butanol exposure. Exposure to *tert*-butanol during gestation resulted in increased fetal loss, decreased fetal body weight, and increases in skeletal variations in exposed offspring. Dams had body weight losses or gains (or both), decreased food consumption, and clinical signs of intoxication at the same doses of *tert*-butanol causing fetal effects. Therefore, determining whether *tert*-butanol exposure results in specific developmental toxicity or whether the fetal effects are due to maternal toxicity is difficult. The observed maternal effects are minimal, however, and thus, the developmental effects observed in the fetuses are not discounted as being secondary to maternal toxicity (U.S. EPA, 1991b) and the evidence is considered suggestive of developmental toxicity.

1.2.4. Neurodevelopmental Effects

Synthesis of effects related to neurodevelopment

Three studies evaluated neurodevelopmental effects (Nelson et al., 1991; Daniel and Evans, 1982)[one in male rats; one in female rats] following *tert*-butanol exposure via liquid diet (i.e., maltose/dextrin) or inhalation. No epidemiology studies on neurodevelopment are available. The animal studies evaluating neurodevelopmental effects of *tert*-butanol contain study design limitations. Daniel and Evans (1982) had a small number of animals per treatment group, lacked comparison of treatment-related effects to controls for all endpoints investigated, and did not use long-term neurodevelopmental testing. The two studies by Nelson et al. (1991) evaluated neurodevelopmental effects after either paternal or maternal exposure but did not run the exposures concurrently or provide exposure methods to indicate the studies were conducted similarly. The studies are arranged in the evidence tables by species and sex.

Various neurodevelopmental effects have been observed in the available studies. These include changes in rotarod performance following oral or inhalation exposures and decreases in open field behavior and cliff avoidance following oral exposure, and reduced time hanging on wire after inhalation exposure during gestation (Table 1-8).

Rotarod performance

Inconsistent results were observed across studies. Although Daniel and Evans (1982) found decreased rotarod performance in mouse pups of dams orally exposed during gestation, Nelson et al. (1991) observed an increase in rotarod performance in rat pups of dams exposed via inhalation during gestation.

Neurochemical measurements

Biochemical or physiological changes in the brain of offspring exposed during gestation or early in the postnatal period were examined in one study. In this study, Nelson et al. (1991) reported statistically significant changes in neurochemical measurements in the brain in offspring

of both dams exposed via inhalation during gestation and treated adult males mated with untreated dams. The strength of these results is compromised, however, because the two concentrations tested (in both experiments) were not run concurrently, and only data on statistically significant effects were reported. Therefore, comparison across doses or trend analysis for the effects is not feasible.

Physiological and psychomotor development

Daniel and Evans (1982) cross-fostered half the mouse pups born to treated mothers with untreated surrogate females to test the effects of maternal nutrition and behavioral factors on the pups' physiological and psychomotor development. Results indicated that pups fostered to control dams performed significantly better than those maintained with treated dams (Table 1-8)(Daniel and Evans, 1982). Data suggest that neurodevelopmental effects were not solely due to in utero exposure to *tert*-butanol (Daniel and Evans, 1982). Interpretation of these results is limited, however, as the neurodevelopmental data were presented only in figures and could not be compared with controls.

Table 1-8. Evidence pertaining to neurodevelopmental effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Daniel and Evans (1982) Swiss Webster (Cox) mouse; 15 pregnant dams/treatment Liquid diet (0, 0.5, 0.75, or 1.0%, w/v); GD6–20; after birth half the pups were nursed with their treated dams and the other half were fostered by untreated dams who recently gave birth 0 (isocaloric amounts of maltose/dextrin), 3,324, 4,879, or 6,677 mg/kg-d	• a dose-dependent increase in righting reflex time, with more time needed in animals maintained with maternal dams • a dose-dependent decrease in open field behavior, with less activity in pups maintained with maternal dams • a dose-dependent decrease in rotarod performance with the pups from maternal dams having lower performances • a dose-dependent decrease in the amount of time the pups were able to avoid a cliff, with animals maintained with their maternal dams having less avoidance time
Nelson et al. (1991) Sprague-Dawley rat; 15 pregnant dams/treatment Inhalation analytical concentration: 0, 6,000, or 12,000 mg/m^3; (dynamic whole body chamber) 7 hr/d GD 1–19	Data were not presented specifically by dose nor were any tables or figures of the data provided Maternal toxicity was noted by decreased food consumption and body weight gains Results in offspring • increase in rotarod performance in high-dose group (16 versus 26 revolutions/min for controls and 12,000 mg/m^3 animals, respectively) • decreased time held on wire in the performance ascent test in the low-dose group (16 sec versus 10 sec for controls and 1,750 mg/m^3 animals, respectively)

Reference and study design	Results
	The following differences in neurochemical measurements in the brain between control and treated offspring were observed, • 53% decrease in norepinephrine in the cerebellum at 12,000 mg/m^3 • 57% decrease in met-enkephalin in the cerebrum at 12,000 mg/m^3 and 83% decrease at 6,000 mg/m^3 • 61% decrease in β-endorphin in the cerebellum at 12,000 mg/m^3 • 67% decrease in serotonin in the midbrain at 6,000 mg/m^3
Nelson et al. (1991) Adult male Sprague-Dawley rats (18/treatment) mated to untreated females Inhalation analytical concentration: 0, 6,000, or 12,000 mg/m^3; (dynamic whole body chamber) 7 hr/d for 6 wk	Data were not presented specifically by dose nor were any tables or figures of the data provided Results (generally only specified as paternally treated versus controls) in offspring indicate • increase in rotarod performance (16 versus 20 revolutions/min for controls and 12,000 mg/m^3 animals, respectively) • decreased time in open field (less time to reach the outer circle of the field, 210 sec versus 115 seconds for controls and 12,000 mg/m^3 animals, respectively) The following differences in neurochemical measurements in the brain between control and treated offspring were observed • 39% decrease in norepinephrine in the cerebellum at 12,000 mg/m^3 • 40% decrease in met-enkephalin in the cerebrum at 12,000 mg/m^3 and 75% decrease at 6,000 mg/m^3 • 71% decrease in β-endorphin in the cerebellum at 12,000 mg/m^3 • 47% decrease in serotonin in the midbrain at 6,000 mg/m^3

* Statistically significant $p \leq 0.05$ as determined by study authors.

Note: Conversions from diet concentrations to mg/kg-d performed by study authors.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

Mechanistic Evidence

No mechanistic evidence is available for reproductive, developmental, or neurodevelopmental effects.

Integration of neurodevelopmental effects

Neurodevelopmental effects, including decreased brain weight, changes in brain biochemistry, and changes in behavioral performances, have been observed. Each study evaluating neurodevelopmental effects, however, had limitations in study design, reporting, or both. In addition, results were not always consistent between studies or across dose. At this time, there is inadequate information to draw conclusions regarding neurodevelopmental toxicity.

1.2.5. Reproductive Effects

Synthesis of effects related to reproduction

Several studies evaluated reproductive effects [a one-generation, oral reproductive study (Lyondell Chemical Co., 2004) and subchronic evaluations in rats and mice following oral and inhalation exposure (NTP, 1997, 1995)] in animals exposed to *tert*-butanol via oral gavage, drinking water, or inhalation for ≥63 days. The studies are arranged in the evidence tables by sex, route of exposure, duration of exposure, and species. The collection of studies evaluating reproductive effects of *tert*-butanol is limited by the absence of two-generation reproductive oral or inhalation studies and by having no human studies on reproduction. The design, conduct, and reporting of each study were reviewed, and each study was considered adequate to provide information pertinent to this assessment.

Reproductive endpoints, such as reproductive organ weights, estrous cycle length, and sperm effects were examined following either oral or inhalation exposure (Lyondell Chemical Co., 2004; NTP, 1997, 1995) (Table 1-9;Figure 1-13; Figure 1-14). In males, the only significant effect observed was a slight decrease in sperm motility for F0 males treated with 1000 mg/kg-day of *tert*-butanol (Lyondell Chemical Co., 2004). No significant changes in sperm motility were reported following oral exposure in other rat studies or via inhalation exposure in mice or rats. In addition, the reduced motility in treated animals falls within the range of historical control data and, therefore, its biological significance is uncertain. In female $B6C3F_1$ mice, estrous cycle length was increased 28% following oral exposure to 11,620 mg/kg-day (NTP, 1995). No significant changes in estrous cycle length were observed following oral exposure in rats, or inhalation exposure in mice or rats.

Table 1-9. Evidence pertaining to reproductive effects in animals following exposure to *tert*-butanol

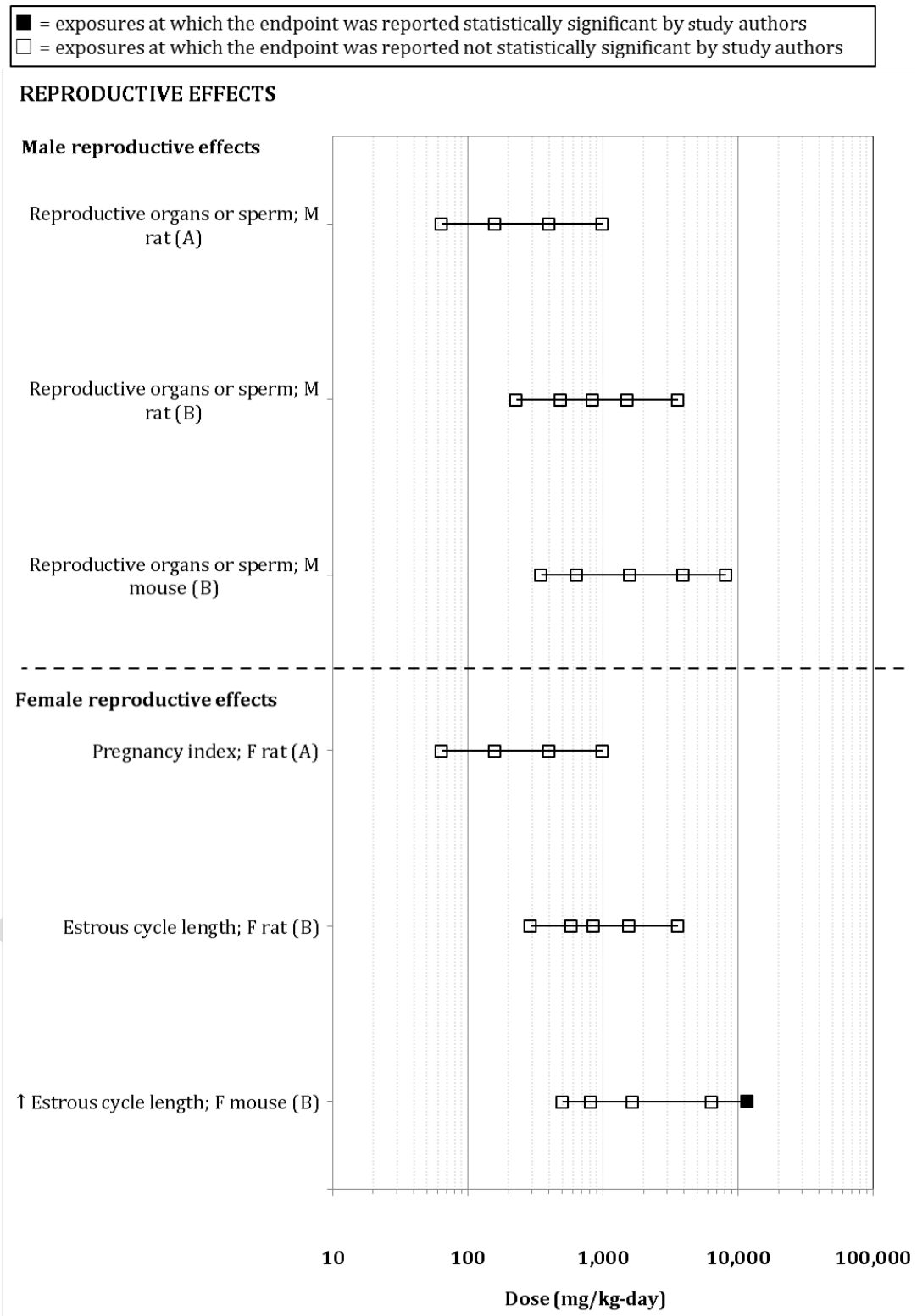
Reference and study design	Results
Male reproductive effects	
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d F0 males: 9 weeks beginning 4 weeks prior to mating PND21	F0 reproductive effects Sperm motility (only control and high-dose groups examined) 0: 94% 1000: 91%* No other significant effect on weights of male reproductive organs or sperm observed
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d 13 weeks	No significant effect on weights of male reproductive organs or sperm observed

Reference and study design	Results
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d 13 weeks	No significant effect on weights of male reproductive organs or sperm observed
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole body chamber) 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on weights of male reproductive organs or sperm observed Evaluations were performed only for concentrations ≥542 ppm (1,643 mg/m^3)
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole body chamber) 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on weights of male reproductive organs or sperm observed Evaluations were performed only for concentrations ≥542 ppm (1,643 mg/m^3)
Female reproductive effects	
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d F0 females: 4 weeks prior to mating through PND21	Pregnancy index 91.7% 91.7% 100% 100% 91.7%
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	No significant effect on female estrous cycle (0, −2, −4, 0, +8 % change relative to control)
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	↑ length of estrous cycle *Response relative to control*: 0, +5, +5, +5, +6, +28*%

Reference and study design	Results
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole body chamber) 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on female estrous cycle (0, −4, +2, +4 % change relative to control) Evaluations were performed only for concentrations ≥542 ppm (1,643 mg/m^3)
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole body chamber) 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on female estrous cycle (0, −3, −9, −5 % change relative to control) Evaluations were only performed for concentrations ≥542 ppm (1,643 mg/m^3)

1 * Statistically significant $p \leq 0.05$ as determined by the study authors.

2 Notes: Conversions from drinking water concentrations to mg/kg-d performed by study authors.
3 Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
4 Percentage change compared to control = (treated value – control value) ÷ control value × 100



Sources: (A) Lyondell Chemical Co. (2004); (B) NTP (1995).

Figure 1-13. Exposure-response array of reproductive effects following oral exposure to *tert*-butanol.

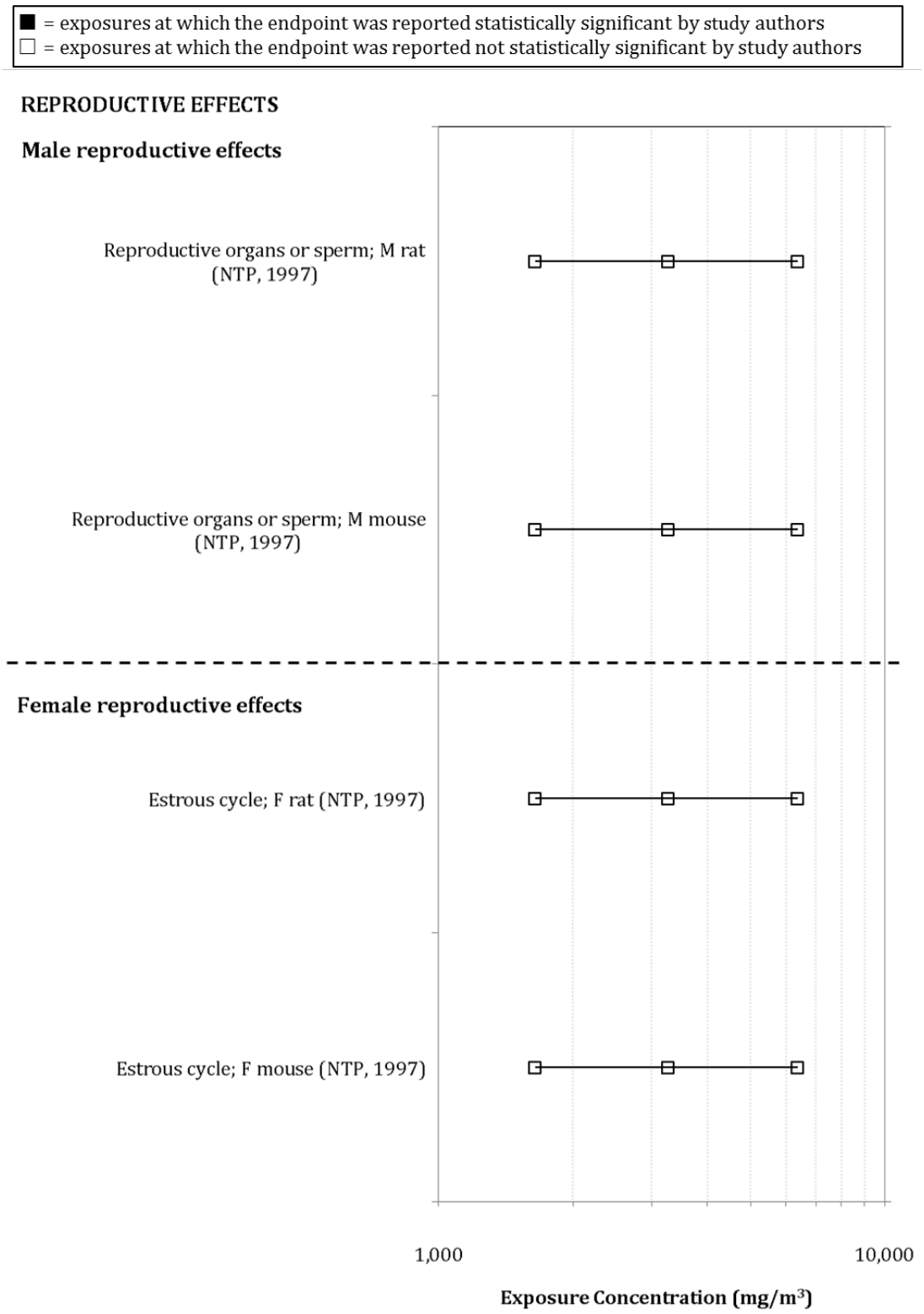


Figure 1-14. Exposure-response array of reproductive effects following inhalation exposure to *tert*-butanol.

Integration of reproductive effects

At this time, no conclusions are drawn in regard to reproductive toxicity. The database is limited to a one-generation study (Lyondell Chemical Co., 2004; NTP, 1995). No two-generation reproductive studies are available that evaluate oral or inhalation exposure. In males, the only observed effect was a slight decrease in sperm motility for F0 males in the highest dose group of rats treated with *tert*-butanol. This effect was not observed, however, in other studies with orally treated rats and mice or in rats exposed via inhalation. In females, NTP (1995) reported an increased length of the estrous cycle in the highest dose group of orally exposed mice. This effect was not observed in similarly treated rats or in mice and rats exposed via inhalation.

1.2.6. Other Toxicological Effects

Effects other than those related to kidney, thyroid, reproductive, developmental, and neurodevelopmental toxicity were observed in some of the available rodent studies; these include liver and urinary bladder effects. Due to a lack of consistency in the liver effects and minimal to mild effects with a lack of progression in urinary bladder, however, inadequate information is available to draw conclusions regarding liver or urinary bladder toxicity at this time.

Additionally, central nervous system (CNS) effects similar to those caused by ethanol (animals appearing intoxicated and having withdrawal symptoms after cessation of oral or inhalation exposure) were observed. Due to study quality concerns (e.g., lack of data reporting, small number of animals per treatment group), however, adequate information to assess CNS toxicity is unavailable at this time. For more information on these other toxicological effects, 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 *tert*-butanol exposure based on several endpoints, including suppurative inflammation in female rats, transitional epithelial hyperplasia in male and female rats, severity of nephropathy in male and female rats, incidences of nephropathy in female rats, mineralization in male rats, and increased kidney weights in both male and female rats. These effects are similar to the kidney effects observed with ETBE exposure (e.g., CPN and urothelial hyperplasia) and MTBE (e.g., CPN and mineralization) (ATSDR, 1996).

Several effects were observed in the kidneys of rats. Based on mechanistic evidence indicating that an α_{2u}-globulin-related process is operating in male rats (Hard et al., 2011; Cirvello et al., 1995; NTP, 1995; Lindamood et al., 1992), any kidney effects associated with α_{2u}-globulin nephropathy are not considered relevant for human hazard identification. In addition, CPN played a role in the renal tubule nephropathy observed following *tert*-butanol exposure, and effects associated with such nephropathy are not considered relevant for human hazard identification. Although increases in severity (males and females) or incidence (females) of nephropathy were

related to *tert*-butanol exposure and could have arisen from chemical-specific processes independent from CPN, the association of these effects with CPN makes this measure less suitable for dose-response analysis, and therefore these effects were not considered for the derivation of reference values. Furthermore, some uncertainty exists regarding whether mineralization is also associated with CPN in male rats; due to this uncertainty, and because other kidney effects were identified as being associated with *tert*-butanol exposure and yet independent from CPN, mineralization in male rats was not considered for dose-response analysis. The remaining effects (suppurative inflammation, transitional epithelial hyperplasia, and increased kidney weights) are considered the result of *tert*-butanol exposure and relevant to human hazard characterization. These effects therefore are suitable for consideration for dose-response analysis and derivation of reference values, in Section 2.

There is suggestive evidence of developmental effects associated with *tert*-butanol exposure. Increased fetal loss, decreased fetal body weight, and increases in skeletal variations in exposed offspring were observed following exposure to relatively high doses of *tert*-butanol during gestation.. These effects are similar to the developmental effects observed with MTBE exposure (e.g., decreased fetal body weight and increases in skeletal variations) (ATSDR, 1996).

No mechanistic evidence is available for developmental effects of *tert*-butanol. Although the evidence is suggestive of developmental toxicity, due to the uncertainty as to whether fetal effects were due to direct effects of *tert*-butanol or indirect effects of maternal toxicity and the lack of consistency across some endpoints, developmental effects were not considered for dose-response analysis and derivation of reference values in Section 2. Furthermore, no adverse effects were reported in one- and two-generation reproductive/developmental studies on ETBE (Gaoua, 2004a, b), providing further support for the lack of evidence supporting reproductive or developmental effects as possible human hazards following *tert*-butanol exposure.

At this time, there is inadequate information to draw conclusions regarding neurodevelopmental effects as a human hazard of *tert*-butanol exposure. Although neurodevelopmental effects have been observed, the studies had limitations in design or reporting, or both, and results were inconsistent between studies and across dose groups. No mechanistic evidence is available to inform the MOA for neurodevelopmental effects of *tert*-butanol. These effects were not considered further for dose-response analysis and derivation of reference values.

At this time, no conclusions are drawn regarding reproductive effects as a human hazard of *tert*-butanol exposure. The only reproductive effect observed due to *tert*-butanol exposure was increased length of estrous cycle (NTP, 1995) in the highest dose group of orally exposed mice, and this effect was not observed in orally exposed rats or in mice and rats exposed via inhalation. Further, the database was limited and contained only two oral exposure studies and one subchronic inhalation study. No mechanistic or MOA information is available for reproductive effects of *tert*-butanol. These effects were not considered further for dose-response analysis and derivation of reference values.

At this time, there is inadequate information to draw conclusions regarding liver or urinary bladder toxicity due to lack of consistency of effects and minimal/mild effects showing a lack of progression, respectively. No mechanistic evidence is available for these effects. The liver and urinary bladder effects were not considered further for dose-response analysis and the derivation of reference values.

1.3.2. Carcinogenicity

Summary of evidence

In F344/N rats, administration of *tert*-butanol in drinking water increased the incidence of renal tubule tumors, mostly adenomas, in males; no renal tumors in females were reported (Hard et al., 2011; NTP, 1995). As discussed in Section 1.2.1, some of these tumors might be associated with α_{2u}-globulin nephropathy, an MOA considered specific to the male rat (U.S. EPA, 1991a). Evidence in support of this hypothesized MOA includes the accumulation of hyaline droplets in renal tubule cells, the presence of α_{2u}-globulin in the hyaline droplets, and additional aspects associated with α_{2u}-globulin nephropathy, including linear papillary mineralization and foci of tubular hyperplasia. Other evidence, however, is not supportive: The accumulation of hyaline droplets was minimal; concentrations of α_{2u}-globulin were low at doses that induced tumors; and no significant necrosis or cytotoxicity was associated with compensatory regenerative proliferation or induction of granular casts observed within a timeframe consistent with α_{2u}-globulin-mediated nephropathy. Renal tumors also could be associated with chronic progressive nephropathy, but the data on CPN are not coherent: Dose-response relationships for CPN, renal tubule hyperplasia, and renal tubule tumors were different; in addition, CPN was nearly as severe in female rats as in male rats, yet no female rats developed renal tumors. Thus, some renal tumors may be attributable to α_{2u}-globulin nephropathy and some to other, yet unspecified, processes. Taken together, and according to EPA's guidance on renal tumors in male rats (U.S. EPA, 1991a), renal tumors induced by *tert*-butanol are relevant for human hazard identification.

In B6C3F$_1$ mice, administration of *tert*-butanol in drinking water increased the incidence of thyroid follicular cell adenomas in females, and adenomas or carcinomas (only one carcinoma observed) in males (NTP, 1995), as discussed in Section 1.2.2. According to EPA's thyroid tumor guidance (U.S. EPA, 1998a), chemicals that produce thyroid tumors in rodents might pose a carcinogenic hazard to humans.

In addition, as mentioned in Section 1.1.4, *tert*-butanol is a primary metabolite of MTBE and of ETBE, two compounds tested in rats and mice that could provide supplementary information on the carcinogenicity of *tert*-butanol. For MTBE, the most recent cancer evaluation by a national or international health agency is from IARC (1999). IARC reported that oral gavage exposure in Sprague-Dawley rats resulted in testicular tumors in males and lymphomas and leukemias (combined) in females; inhalation exposure in male and female F344 rats resulted in renal tubule adenomas in males; and inhalation exposure in male and female CD-1 mice resulted in

hepatocellular adenomas in females (IARC, 1999). For ETBE, a draft IRIS assessment under development concurrently with this assessment reports that inhalation exposure in male and female F344 rats resulted in hepatocellular tumors, mostly adenomas, in males; no significant tumor increases were reported for 2-year studies by drinking water exposure in male and female F344 rats or by oral gavage in male and female Sprague-Dawley rats.

Integration of evidence

This evidence leads to consideration of two hazard descriptors under EPA's cancer guidelines (U.S. EPA, 2005a). The descriptor *likely to be carcinogenic to humans* is appropriate when the evidence is "adequate to demonstrate carcinogenic potential to humans" but does not support the descriptor *carcinogenic to humans*. One example from the cancer guidelines is "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans." *tert*-Butanol matches the conditions of this example, having increased tumor incidences in two species, in both sexes, and at two sites.

Alternatively, 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. The results for *tert*-butanol raise a concern for cancer but none of the effects is particularly strong. The kidney tumors resulted, in part, from an MOA that is specific to male rats, while no kidney tumors occurred in female rats. The thyroid tumors induced in male and female mice were almost entirely benign. In addition, while MTBE was also associated with male rat kidney tumorigenesis, there is little coherence of results between *tert*-butanol and ETBE associated tumorigenesis in rats. MTBE or ETBE effects following chronic oral exposure in mice have not been investigated, however, so no evidence exists to evaluate the coherence of the thyroid tumorigenesis observed following *tert*-butanol exposure in $B6C3F_1$ mice.

These considerations, interpreted in light of the cancer guidelines, support the conclusion that there is *suggestive evidence of carcinogenic potential* for *tert*-butanol. Although increased tumor incidences were reported for two species, two sexes, and two sites, none of the tumor responses was strong or coherent with the results for ETBE, and this was decisive in selecting a hazard descriptor.

The descriptor *suggestive evidence of carcinogenic potential* applies to all routes of human exposure. Oral administration of *tert*-butanol to rats and mice induced tumors at sites beyond the point of initial contact, and inhalation exposure for 13 weeks resulted in absorption and distribution of *tert*-butanol into the systemic circulation, as discussed in Section 1.2.1. According to the cancer guidelines, this information provides sufficient basis to apply the cancer descriptor developed from oral studies to other exposure routes.

Biological considerations for dose–response analysis

Regarding hazards to bring forward to Section 2 for dose-response analysis, EPA's guidance on renal tumors in male rats (U.S. EPA, 1991a) advises that unless the relative contribution of

α_{2u}-globulin nephropathy and other process can be determined, dose-response analysis should not be performed. As discussed in Section 1.2.1, the available data do not allow such determination, and so an analysis of kidney tumors does not appear in Section 2.

EPA's guidance on thyroid tumors and EPA's cancer guidelines (U.S. EPA, 1998a) advises that for thyroid tumors resulting from thyroid–pituitary disruption, dose–response analysis should use nonlinear extrapolation, in the absence of MOA information to indicate otherwise. As discussed in Section 1.2.2, increases in thyroid follicular cell hyperplasia in male and female mice provide partial support for thyroid-pituitary disruption. Other necessary data on *tert*-butanol, however, are not adequate or are not supportive. There is little correlation among thyroid, pituitary, and liver effects in female mice, and no data are available to evaluate the potential for antithyroid effects in male mice. Data are not adequate to conclude that thyroid hormone changes exceed the range of homeostatic regulation or to evaluate effects on extrahepatic sites involved in thyroid-pituitary disruption. Also, no data are available to evaluate reversibility of effects upon cessation of exposure. Thus, according to EPA's thyroid tumor guidance, concluding that the thyroid tumors result from thyroid-pituitary disruption is premature, and dose-response analysis should use linear extrapolation. The data are well suited to dose-response analysis, coming from an NTP study that tested multiple dose levels.

1.3.3. Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

No chemical-specific data that would allow for the identification of populations with increased susceptibility to *tert*-butanol exposure exist. In vitro studies have implicated the liver microsomal mixed function oxidase (MFO) system, namely CYP450 (Cederbaum et al., 1983; Cederbaum and Cohen, 1980), as playing a role in the metabolism of *tert*-butanol. No studies, however, have identified the specific CYPs responsible for the biotransformation of *tert*-butanol. Pharmacokinetic differences among the fetus, newborns, children, and the aged might alter responses to chemicals compared to adults, resulting in differences in health effects. In the presence of environmental chemicals, metabolic homeostasis is maintained by the liver's ability to detoxify and eliminate xenobiotics. This process is accomplished, in part, by the expression of xenobiotic metabolizing enzymes and transporters (XMETs), which metabolize and transport xenobiotics and determine whether exposure will result in altered responses. The expression of XMETs, including various CYPs, has been found to be underexpressed in the mouse fetus and neonate (Lee et al., 2011) and decreased in older mice (Lee et al., 2011) and rats (Lee et al., 2008). Decreased ability to detoxify and transport *tert*-butanol out of the body could result in increased susceptibility to *tert*-butanol in the young and old.

In regard to cancer, although children are more sensitive than adults to thyroid carcinogenesis resulting from ionizing radiation, relative differences in lifestage sensitivity to chemically induced thyroid carcinogenesis are unknown (U.S. EPA, 1998a). In addition, the data on *tert*-butanol mutagenicity are inconclusive.

Collectively, there is little evidence on *tert*-butanol itself to identify susceptible populations or lifestages.

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. The RfD can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

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

EPA identified kidney effects as a potential human hazard of *tert*-butanol exposure (see Section 1.2.1). Studies within this effect category were evaluated using general study quality characteristics [as discussed in Section 6 of the Preamble; see also U.S. EPA (2002)] to help inform the selection of studies from which to derive toxicity values. No other hazards were identified for further for consideration in the derivation of reference 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 *tert*-butanol, 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 to provide information about the shape of the dose-response curve, and (3) power to detect effects at low exposure levels. The database for *tert*-butanol includes both chronic and subchronic studies showing effects in the kidney that are suitable for deriving reference values.

Kidney Toxicity

EPA identified kidney effects as a potential human hazard of *tert*-butanol-induced toxicity based on findings in male and female rats (summarized in Section 1.3.1). Kidney toxicity was observed across multiple chronic, subchronic, and short-term studies following oral and inhalation exposure. Kidney effects observed after chronic exposure, such as suppurative inflammation and transitional epithelial hyperplasia, may impact the ability of the kidney to filter waste. Observed changes in kidney weight could also indicate toxic effects in the kidney. For the oral *tert*-butanol database, there are several studies available that evaluated these kidney effects. Lyondell Chemical Co. (2004) conducted a reproductive study in Sprague-Dawley rats that was of shorter duration, and reported changes in kidney weight but did not examine changes in histopathology. NTP

conducted a 2-year drinking water study (NTP, 1995) in F344 rats that evaluated multiple doses in both males and females, and reported on all three endpoints highlighted above. NTP (1995) was identified as most suitable for dose-response assessment considering the study duration, comprehensive reporting of outcomes, and multiple doses tested.

In the NTP (1995) 2-year drinking water study, male F344 rats were exposed to approximate doses of 0, 90, 200, or 420 mg/kg-day; female F344 rats were exposed to approximate doses of 0, 180, 330, or 650 mg/kg-day. Reduced body weights and survival were observed and reflected in some of the effects. Kidney effects, including changes in organ weight, histopathology, or both, were observed in both sexes of rats after 13 weeks, 15 months, and 2 years of treatment (NTP, 1995). Specific endpoints chosen for dose-response analysis were absolute kidney weight (observed in males and females), kidney suppurative inflammation (observed in females), and kidney transitional epithelial hyperplasia (observed in males and females). For absolute kidney weight, data from 15 months was selected as described in Section 1.2.1; for the other endpoints, data at the longest duration of 2 years were selected.

2.1.2. Methods of Analysis

No biologically based dose-response models are available for *tert*-butanol. In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. The models in EPA's Benchmark Dose Software (BMDS) were applied. Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b), 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 the level of change that is considered biologically significant, a BMR of 1 standard deviation from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data is used to estimate the BMD and BMDL, and also to facilitate a consistent basis of comparison across endpoints, studies, and assessments. Endpoint-specific BMRs, where feasible, are described further below. When modeling was feasible, the estimated BMDLs were used as points of departure (PODs); the PODs are summarized in Table 2-1. Further details including the modeling output and graphical results for the model selected for each endpoint can be found in Appendix C of the Supplemental Information to this Toxicological Review.

Kidney weights were analyzed as absolute weights rather than relative to body weight. In general, absolute and relative kidney weight data can both be considered appropriate endpoints for analysis (Bailey et al., 2004). In the NTP (1995) 2-year drinking water study, body weight in exposed animals noticeably decreased relative to controls at the 15-month interim sacrifice (see Table 1-1), but this decrease in body weight impacted the measure of relative kidney weight resulting in an exaggeration of the kidney weight change. There was greater confidence in the absolute kidney weight measure; thus, it was considered more appropriate for dose-response analysis, and changes in relative kidney weights were not analyzed. A 10% relative change from

control was used as a BMR for absolute kidney weight by analogy with a 10% change in body weight as an indicator of toxicity. A BMR of 10% extra risk was considered appropriate for the quantal data on incidences of kidney suppurative inflammation and kidney transitional epithelial hyperplasia.

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 toxicokinetic modeling (PBPK). Other approaches include using chemical-specific information in the absence of a complete PBPK model. As discussed in Appendix B of the Supplemental Information, human PBPK models for inhalation of ETBE or inhalation and dermal exposure to MTBE have been published, which include *tert*-butanol submodels. A validated human PBPK model for *tert*-butanol, however, is not available for extrapolating doses from animals to humans. In lieu of either chemical-specific models or data to inform the derivation of human equivalent oral exposures, body weight scaling to the ¾ power (i.e., $BW^{3/4}$) is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans for the purpose of deriving an oral RfD.

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

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

where

BW_a = animal body weight

BW_h = human body weight

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

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

Table 2-1 summarizes all PODs and the sequence of calculations leading to the derivation of a human-equivalent POD for each endpoint discussed above.

Table 2-1. Summary of derivations 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 absolute kidney weight at 15 months NTP (1995)	Rat/M	Linear (constant variance)	10%	657	296	296	71
Increased absolute kidney weight at 15 months NTP (1995)	Rat/F	Exponential (M4) (constant variance)	10%	164	91	91	22
Kidney inflammation (suppurative) NTP (1995)	Rat/F	Log-probit	10%	254	200	200	48
Kidney transitional epithelial hyperplasia NTP (1995)	Rat/M	Log-logistic	10%	30	16	16	3.84
Kidney transitional epithelial hyperplasia NTP (1995)	Rat/F	Multistage, 3-degree	10%	412	339	339	81.4

[a]For modeling details, see Appendix C in Supplemental Information.
[b]For studies in which animals were not dosed daily, EPA would adjust administered doses to calculate the TWA daily doses prior to BMD modeling. However, this adjustment was not required for the NTP (1995) study.
[c]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011).

NA= not applicable

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], also described in the Preamble, five possible areas of uncertainty and variability were considered when determining the application of UFs 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 *tert*-butanol (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 because $BW^{3/4}$ scaling was used to extrapolate oral doses from laboratory animals to humans.

Although $BW^{3/4}$ scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes, some residual uncertainty in the extrapolation remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's $BW^{3/4}$ guidance (U.S. EPA, 2011) recommends use of an uncertainty factor of 3.

A subchronic to chronic uncertainty factor, UFS, of 1 was applied to all PODs because the endpoints were all observed following chronic exposure.

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% relative change in absolute kidney weight, a 10% extra risk of kidney suppurative inflammation, and a 10% extra risk of transitional cell hyperplasia were selected assuming they represent minimal biologically significant response levels.

A database uncertainty factor, UF, of 1 was applied to all PODs. The *tert*-butanol oral toxicity database includes chronic and subchronic toxicity studies in rats and mice (Acharya et al., 1997; Acharya et al., 1995; NTP, 1995) and developmental toxicity studies in rats and mice (Lyondell Chemical Co., 2004; Faulkner et al., 1989; Daniel and Evans, 1982). In the developmental studies, no effects were observed at exposure levels below 1000 mg/kg-day, and effects observed at ≥1000 mg/kg-day were accompanied by evidence of maternal toxicity. These exposure levels are much higher than the PODs for kidney effects, suggesting developmental toxicity is not as sensitive an endpoint as kidney effects. No immunotoxicity or multigenerational reproductive studies are available for *tert*-butanol. Studies on ETBE, which is rapidly metabolized to systemically available *tert*-butanol, are informative for consideration of the gaps in the *tert*-butanol oral database. The database for ETBE does not indicate immunotoxicity (Banton et al., 2011; Li et al., 2011), suggesting immune system effects would not be a sensitive target for *tert*-butanol. No adverse effects were reported in one- and two-generation reproductive/developmental studies on ETBE (Gaoua, 2004a, b), indicating that reproductive/developmental effects would not be a sensitive target for *tert*-butanol. Additionally, a one-generation, reproductive toxicity study in rats from a Toxic Substances Control Act submission (Lyondell Chemical Co., 2004) is available for *tert*-butanol. This study did not observe reproductive effects. Although the oral toxicity database for *tert*-butanol has some gaps, the available data on *tert*-butanol, informed by the data on ETBE, do not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD. Therefore, a database UF_D of 1 was applied.

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

Table 2-2 is a continuation of Table 2-1 and summarizes the application of UFs to each POD to derive a candidate value for each data set, preliminary to the derivation of the organ/system-specific RfDs. These candidate values are considered individually in the selection of a representative oral reference value for a specific hazard and subsequent overall RfD for *tert*-butanol. Figure 2-1 presents graphically the candidate values, UFs, and POD_{HED} values, with each

bar corresponding to one data set described in Tables 2-1 and 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 absolute kidney weight; male rat at 15 months NTP (1995)	71	$BMDL_{10\%}$	3	10	1	1	1	30	2×10^{0}
Increased absolute kidney weight; female rat at 15 months NTP (1995)	22	$BMDL_{10\%}$	3	10	1	1	1	30	7×10^{-1}
Kidney inflammation (suppurative); female rat NTP (1995)	48	$BMDL_{10\%}$	3	10	1	1	1	30	2×10^{0}
Kidney transitional epithelial hyperplasia; male rat NTP (1995)	3.8	$BMDL_{10\%}$	3	10	1	1	1	30	1×10^{-1}
Kidney transitional epithelial hyperplasia; female rat NTP (1995)	81	$BMDL_{10\%}$	3	10	1	1	1	30	3×10^{0}

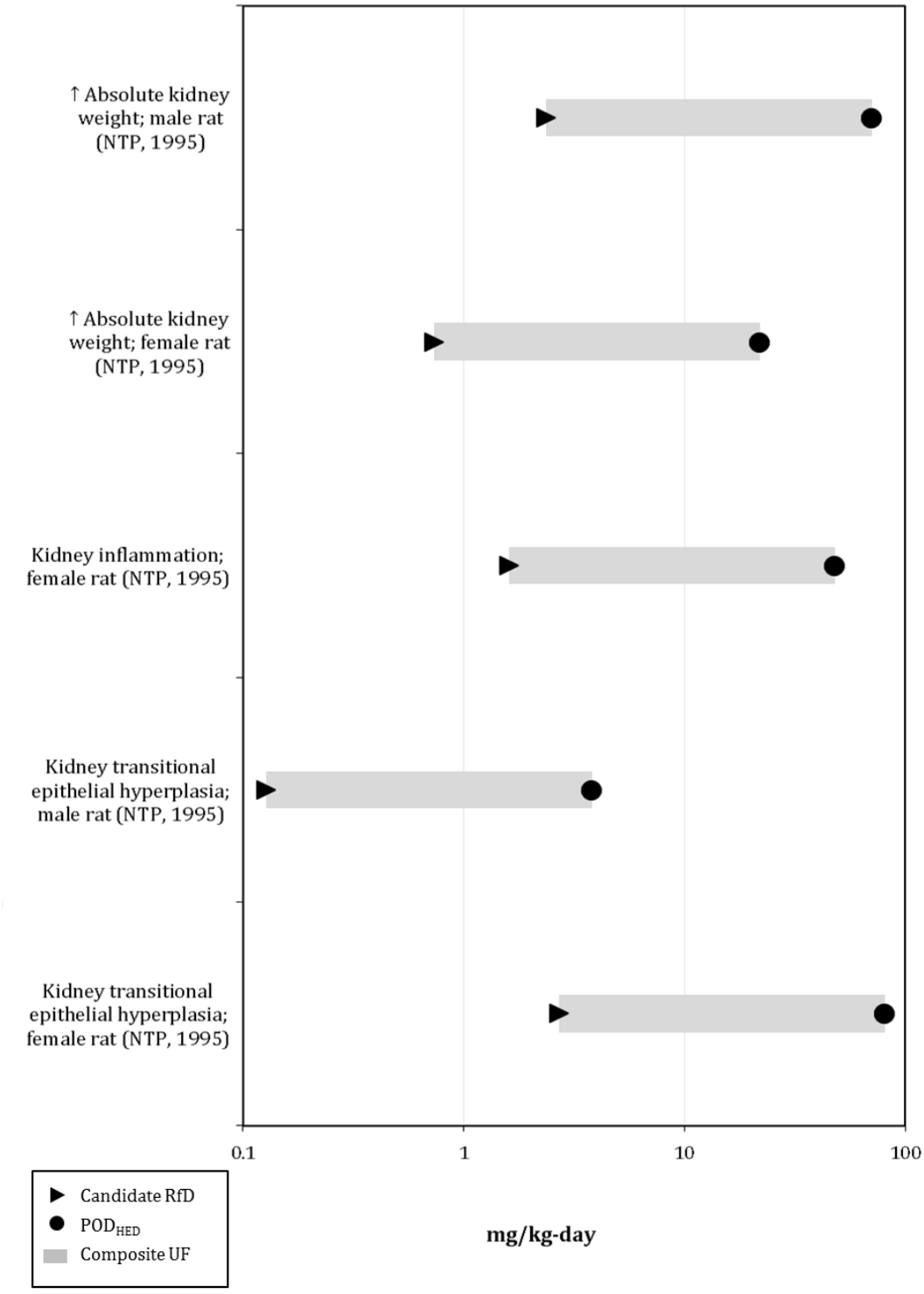


Figure 2-1. Candidate values with corresponding POD and composite UF. Each bar corresponds to one data set described in Table 2-1 and Table 2-2.

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 *tert*-butanol, candidate values were for several different kidney effects in both sexes, spanning a range from 1×10^{-1} to 3×10^{0} mg/kg-day, for an overall 30-fold range. To estimate an exposure level below which kidney toxicity from *tert*-butanol exposure is not expected to occur, the RfD for increased incidence of transitional epithelial hyperplasia in male rats (**1×10^{-1} mg/kg-day**) was selected as the kidney-specific reference dose for *tert*-butanol. Unlike kidney suppurative inflammation, this effect was observed in both sexes, with males appearing to be more sensitive than females. Additionally, this indicator of kidney toxicity is more specific and more sensitive than the relatively non-specific endpoint of absolute kidney weight changes. Confidence in this kidney-specific RfD is high. The PODs are based on benchmark dose modeling, and the candidate values are derived from a well-conducted long-term study, involving a sufficient number of animals per group, including both sexes, and assessing a wide range of kidney endpoints.

Table 2-3. Organ/system-specific RfDs and overall RfD for *tert*-butanol

Effect	Basis	RfD (mg/kg-day)	Study exposure description	Confidence
Kidney	Incidence of transitional epithelial hyperplasia (NTP (1995))	1×10^{-1}	Chronic	High
Overall RfD	**Kidney**	**1×10^{-1}**	**Chronic**	**High**

2.1.5. Selection of the Overall Reference Dose

For *tert*-butanol, only kidney effects were identified as a 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^{-1} mg/kg-day**) is the overall RfD for *tert*-butanol. This value is based on increased incidence of transitional epithelial hyperplasia in male rats exposed to *tert*-butanol.

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 *tert*-butanol, there is potential for early lifestage susceptibility to *tert*-butanol exposure 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 (NTP, 1995) is high. This study was well conducted, complied with Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations, involved a sufficient number of animals per dose group (including both sexes), and assessed a wide range of tissues and endpoints. Although the toxicity database for *tert*-butanol has some gaps, they are informed by the data on ETBE, a parent compound of *tert*-butanol. Therefore, the confidence in the database is high. 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 *tert*-butanol 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 UFs generally applied to reflect limitations of the data used.

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

As for oral exposure, EPA identified kidney effects as a potential human hazard of *tert*-butanol inhalation exposure (summarized in Section 1.3.1). No chronic inhalation study for *tert*-butanol is available; there is only one 13-week study in rats and mice (NTP, 1997). Sufficient data were available to modify and utilize a PBPK model in rats for both oral and inhalation exposure in order to perform a route-to-route extrapolation, so rat studies from both routes of exposure were considered for dose-response analysis.

The database for *tert*-butanol includes oral and inhalation studies and data sets that are potentially suitable for use in deriving inhalation reference values. Specifically, effects associated with *tert*-butanol 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

EPA identified kidney effects as a potential human hazard of *tert*-butanol exposure based on findings of organ weight changes and histopathology primarily in male rats. These findings were observed across multiple chronic, subchronic, and short-term studies following oral and inhalation exposure. The subchronic NTP (1997) inhalation study is the only route-specific study available, and was carried forward for further analysis. For oral studies considered for route-to-route extrapolation, see Section 2.1.1 for a summary of considerations for selecting oral studies for dose-response analysis. Overall, the NTP 2-year drinking water study NTP (1995) was identified as the study most suitable for dose-response assessment, given the study duration, comprehensive reporting of outcomes, use of multiple species tested, multiple doses tested, and availability of a PBPK model for route-to-route extrapolation. This study was discussed previously in Section 2.1.1 as part of the derivation of the oral reference dose, so is not reviewed here again. The NTP (1997) subchronic inhalation study shares many strengths with the 2-year drinking water study (NTP, 1995), and is described in more detail below.

NTP (1997) was a well-designed subchronic study that evaluated the effect of *tert*-butanol exposure on multiple species at multiple inhalation doses. Absolute kidney weights were elevated (10–11%) in male rats exposed at ≥3,273 mg/m^3; relative kidney weights were elevated (~9%) in males at ≥3,273 mg/m^3 and in females at 6,368 mg/m^3. Male rats exhibited an increase in the severity of chronic nephropathy (characterized as number of foci of regenerative tubules). Few endpoints were available for consideration in the subchronic inhalation study, but changes in kidney weights also were observed in the oral studies, such as the NTP (1995) 2-year drinking water study.

2.2.2. Methods of Analysis

No biologically based dose-response models are available for *tert*-butanol. In this situation, EPA evaluates a range of dose-response models considered consistent with underlying biological processes 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* (U.S. EPA, 2012b), the benchmark dose or concentration (BMD/C) and the 95% lower confidence limit on the BMD/C (BMD/CL) were estimated using a BMR of 10% change from the control mean for absolute kidney weight changes (as described in Section 2.1.2). As noted in Section 2.1.2., a BMR of 10% extra risk was considered appropriate for the quantal data on incidences of kidney suppurative inflammation and kidney transitional epithelial hyperplasia. The estimated BMD/CLs were used as PODs. Where dose-response modeling was not feasible, NOAELs or LOAELs were identified and summarized in Table 2-4. Further details, including the modeling output and graphical results for the best-fit model for each endpoint, can be found in Appendix C of the Supplemental Information.

PODs from Inhalation Studies

Because the RfC 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 (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 inhalation study (NTP, 1997) was adjusted to reflect a continuous exposure by multiplying it by (6 hours per day) ÷ (24 hours per day) and (5 days per week) ÷ (7 days per 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 *tert*-butanol in the laboratory animal (rat or mouse) and humans. According to the RfC guidelines (U.S. EPA, 1994), *tert*-butanol is a Category 3 gas because extra-respiratory effects were observed. Kaneko et al. (2000) measured a blood:gas partition coefficient [$(H_{b/g})_A$] of 531 ± 102 for *tert*-butanol in the male Wistar rat, while Borghoff et al. (1996) measured a value of 481 ± 29 in male F344 rats. A blood:gas partition coefficient [$(H_{b/g})_H$] of 462 was reported for *tert*-butanol in humans (Nihlén et al., 1995). The calculation $(H_{b/g})_A \div (H_{b/g})_H$ was used to calculate a blood:gas partition coefficient ratio to apply to the delivered concentration. Because F344 rats were used in the study, the blood:gas partition coefficient for F344 rats was used. Thus, the calculation was 481 ÷ 462 = 1.04. Therefore, a ratio of 1.04 was used to calculate the HEC. This allowed a $BMCL_{HEC}$ to be derived as follows:

$$BMCL_{HEC} = BMCL_{ADJ}\ (mg/m^3) \times (\text{interspecies conversion})$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (481 \div 462)$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (1.04)$$

Table 2-4 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD 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[b] (mg/m^3)	BMCL[b] (mg/m^3)	POD_{ADJ}[b] (mg/m^3)	POD_{HEC}[c] (mg/m^3)
Kidney							
Increased absolute kidney weight NTP (1997)	Male F344 rats	Hill	10%	1931	1705	304	304
Increased absolute kidney weight NTP (1997)	Female F344 rats	No model selected[d]	10%	--	--	1137	1137

[a]For modeling details, see Appendix C in Supplemental Information.
[b]BMCs, BMCLs, and PODs were adjusted for continuous daily exposure by multiplying by (hours exposed per day / 24 hr) × (days exposed per week / 7 days).
[c]POD_{HEC} calculated by adjusting the POD_{ADJ} by the DAF (=1.0, rounded from 1.04) for a Category 3 gas (U.S. EPA, 1994).
[d]BMD modeling failed to calculate a BMD value successfully (see Appendix C); POD calculated from no-observed adverse effect level (NOAEL) of 6368 mg/m^3.

PODs from oral studies – use of PBPK model for route-to-route extrapolation

A PBPK model for *tert*-butanol in rats has been modified, as described in Appendix B of the Supplemental Information. Using this model, route-to-route extrapolation of the oral BMDLs to derive inhalation PODs was performed as follows. First, the internal dose in the rat at each oral BMDL (assuming continuous exposure) was estimated using the PBPK model, to derive an "internal dose BMDL." Then, the inhalation air concentration (again, assuming continuous exposure) that led to the same internal dose in the rat was estimated using the PBPK model. The resulting BMCL was then converted to a human equivalent concentration POD (POD_{HEC}) using the methodology previously described in "PODs from inhalation studies":

$$BMCL_{HEC} = BMCL_{ADJ}\ (mg/m^3) \times (\text{interspecies conversion})$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (481 \div 462)$$
$$= BMCL_{ADJ}\ (mg/m^3) \times (1.04)$$

A critical decision in the route-to-route extrapolation is selection of the internal dose metric that establishes "equivalent" oral and inhalation exposures. For *tert*-butanol-induced kidney effects, the two options are the concentration of *tert*-butanol in blood and rate of *tert*-butanol metabolism. Note that using the kidney concentration of *tert*-butanol will lead to the same route-to-route extrapolation relationship as *tert*-butanol in blood because the distribution from blood to kidney is independent of route. Data are not available that suggest that metabolites of *tert*-butanol mediate its renal toxicity. Without evidence that suggests otherwise, *tert*-butanol is assumed the active

toxicological agent. Therefore, the concentration of *tert*-butanol in blood was selected as the dose metric.

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

Table 2-5. Summary of derivation of inhalation points of departure derived from route-to-route extrapolation from oral exposures

Endpoint and reference	Species/sex	BMR	BMDL (mg/kg-d)	Internal dose[a] (mg/L)	Equivalent POD_{HEC}[b] (mg/m^3)
Kidney					
Mean absolute kidney weight at 15 months NTP (1995)	Rat/M	10%	296	22.4	551
Mean absolute kidney weight at 15 months NTP (1995)	Rat/F	10%	91	4.76	155
Kidney inflammation (suppurative) NTP (1995)	Rat/F	10%	200	12.6	359
Kidney transitional epithelial hyperplasia NTP (1995)	Rat/M	10%	16	0.745	26.1
Kidney transitional epithelial hyperplasia NTP (1995)	Rat/F	10%	339	27.9	638

[a] Average blood concentration of *tert*-butanol under continuous oral exposure at the BMDL.
[b] Continuous inhalation human equivalent concentration that leads to the same average blood concentration of *tert*-butanol as continuous oral exposure at the BMDL.

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. Several PODs for the candidate inhalation values were derived using a route-to-route extrapolation from the PODs estimated from the chronic oral toxicity study in rats (NTP, 1995) in the derivation of the oral RfD (Section 2.1). With the exception of the subchronic inhalation (NTP, 1997) study, the uncertainty factors (UFs) selected and applied to PODs derived from the chronic oral (NTP, 1995) study for route-to-route extrapolation are the same as those for the RfD for *tert*-butanol (see Section 2.1.3). The model used to perform this route-to-route extrapolation is a well-characterized model considered appropriate for the purposes of this assessment. One source of uncertainty regarding the route-to-route extrapolation is the assumption of that 100% of inhaled *tert*-butanol reaches the gas-exchange region, that is, 100% of the inhaled

tert-butanol could be absorbed and distributed to the rest of the body in rats. If not all of the compound is bioavailable for the rat, a lower blood concentration would be expected compared to the current estimate, and thus, a higher RfC would be calculated.

For the PODs derived from the subchronic inhalation (NTP, 1997) study, a UF_S of 10 was applied to account for extrapolation from subchronic to chronic duration.

Table 2-6 is a continuation of Table 2-4 and Table 2-5, and summarizes the application of UFs to each POD to derive a candidate value for each data set. The candidate values presented in the table below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative inhalation reference value for a specific hazard and subsequent overall RfC for *tert*-butanol.

Figure 2-2 presents graphically the candidate values, UFs, and POD_{HEC} values, with each bar corresponding to one data set described in Tables 2-4, 2-5, and 2-6.

Table 2-6. Effects and corresponding derivation of candidate values

Endpoint (sex and species) and reference	POD_{HEC}[a] (mg/m^3)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/m^3)
Kidney									
Increased absolute kidney weight at 13 weeks; male rat NTP (1997)	304	$BMCL_{10\%}$	3	10	1	10	1	300	1×10^0
Increased absolute kidney weight at 13 weeks; female rat NTP (1997)	1137	NOAEL	3	10	1	10	1	300	4×10^0
Increased absolute kidney weight at 15 months; male rat NTP (1995)	551	$BMCL_{10\%}$	3	10	1	1	1	30	2×10^1 *
Increased absolute kidney weight at 15 months; female rat NTP (1995)	155	$BMCL_{10\%}$	3	10	1	1	1	30	5×10^0 *
Kidney inflammation (suppurative); female rat NTP (1995)	359	$BMCL_{10\%}$	3	10	1	1	1	30	1×10^1 *
Kidney transitional epithelial hyperplasia; male rat NTP (1995)	26.1	$BMCL_{10\%}$	3	10	1	1	1	30	9×10^{-1} *
Kidney transitional epithelial hyperplasia; female rat NTP (1995)	638	$BMCL_{10\%}$	3	10	1	1	1	30	2×10^1 *

*These candidate values are derived using route-to-route extrapolated PODs based on NTP's chronic drinking water study.

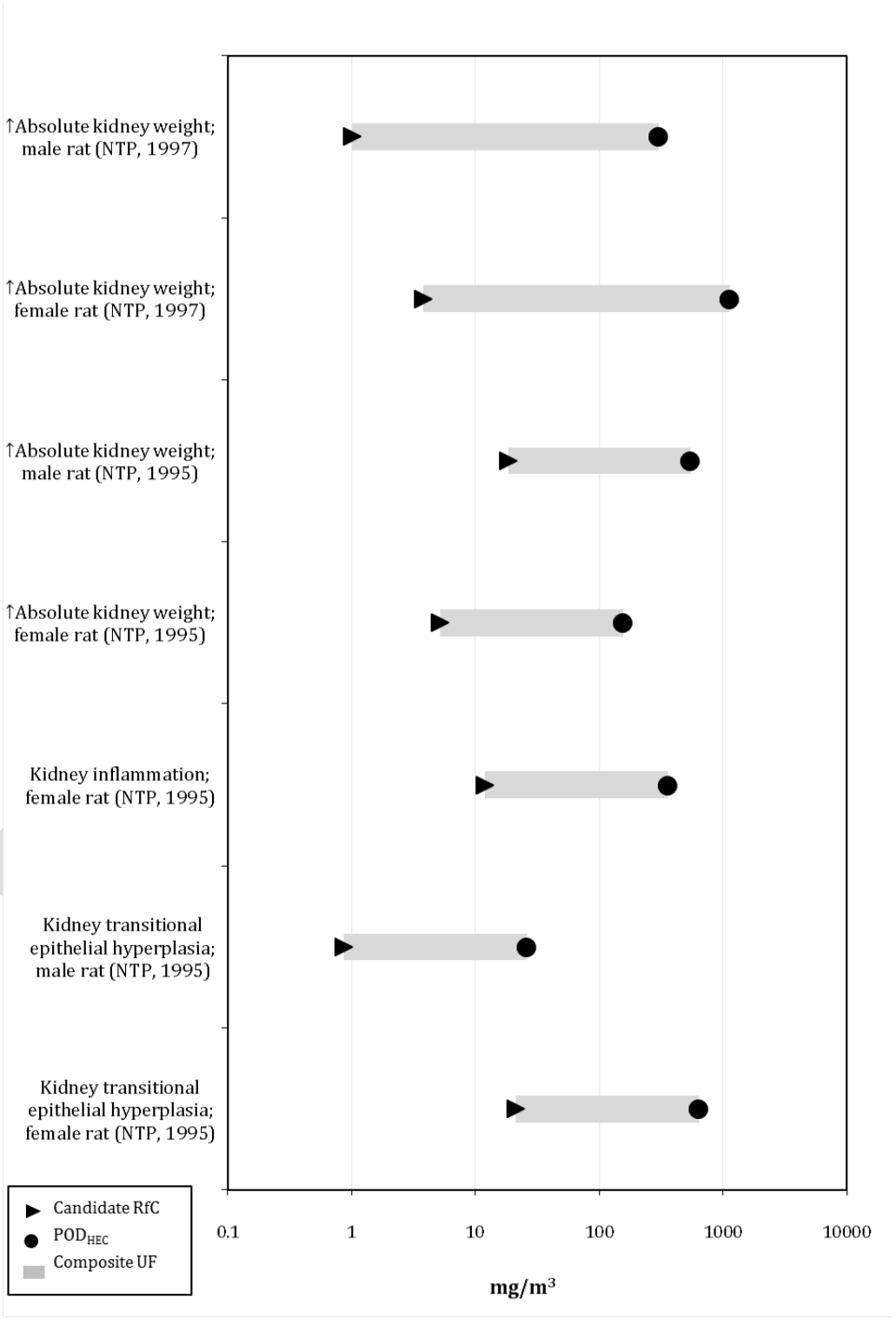


Figure 2-2. Candidate RfC values with corresponding POD and composite UF.

2.2.4. Derivation of Organ/System-Specific Reference Concentrations

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

Kidney Toxicity

For the derivation of candidate values, whether PODs from the subchronic inhalation study of NTP (1997) would provide a better basis than the route-to-route extrapolated PODs based on the chronic oral study of NTP (1995) must be considered. Candidate values were derived for increased kidney weight observed in the subchronic inhalation study (NTP, 1997) and several kidney effects observed in the chronic oral study (NTP, 1995) in both sexes of rat, spanning a range from 9×10^{-1} to 2×10^{1} mg/m^3, for an overall 20-fold range. To estimate an exposure level below which kidney toxicity from *tert*-butanol exposure is not expected to occur, the RfC for increased incidence of transitional epithelial hyperplasia in male rats (**9×10^{-1} mg/m^3**) was selected as the kidney-specific RfC for *tert*-butanol, consistent with the selection of the kidney-specific RfD (see Section 2.1.4). As discussed in Section 2.1.4, unlike kidney suppurative inflammation, this effect was observed in both sexes, with males appearing to be more sensitive than females. Additionally, it is based on a longer (chronic) duration and a more specific and sensitive indicator of kidney toxicity than the relatively non-specific endpoint of kidney weight change. Confidence in this kidney-specific RfC is high. The PODs are based on BMD modeling, and the candidate values are derived from a well-conducted study, involving a sufficient number of animals per group, including both sexes, assessing a wide range of kidney endpoints, and availability of a PBPK model for route-to-route extrapolation.

Table 2-7. Organ/system-specific RfCs and overall RfC for *tert*-butanol

Effect	Basis	RfC (mg/m^3)	Study exposure description	Confidence
Kidney	Incidence of transitional epithelial hyperplasia (NTP, 1995)	9×10^{-1}	Chronic	High
Overall RfC	**Kidney**	**9×10^{-1}**	**Chronic**	**High**

2.2.5. Selection of the Overall Reference Concentration

For *tert*-butanol, kidney effects were identified as the primary hazard; thus, a single organ-/system-specific RfC was derived. The kidney-specific RfC of **9×10^{-1} mg/m^3** is selected as the overall RfC, representing an estimated exposure level below which deleterious effects from *tert*-butanol exposure are not expected to occur.

The overall RfC 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 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 potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfC. In the case of *tert*-butanol, there is potential for early lifestage susceptibility to *tert*-butanol exposure 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). A PBPK model was utilized to perform a route-route extrapolation to determine a POD for the derivation of the RfC from the NTP (1995) oral study and corresponding critical effect. Confidence in the principal study (NTP, 1995) is high. This study was well-conducted, complied with FDA GLP regulations, involved a sufficient number of animals per group (including both sexes), and assessed a wide range of tissues and endpoints. Although there are some gaps in the toxicity database for *tert*-butanol, these areas are informed by the data on ETBE, a parent compound of *tert*-butanol. Therefore, the confidence in the database is high. Reflecting high confidence in the principal study, high confidence in the database, and minimal uncertainty surrounding the application of the modified PBPK model for the purposes of a route-to-route extrapolation, the overall confidence in the RfC for *tert*-butanol is high.

2.2.7. Previous IRIS Assessment

No previous inhalation assessment for *tert*-butanol 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 *tert*-butanol. To derive the RfD, the UF approach (U.S. EPA, 2000a, 1994) was applied to a POD based on kidney toxicity in rats treated chronically. UFs were applied to the POD to account for extrapolating from an animal bioassay to human exposure, and the likely existence of a diverse human population of varying susceptibilities. These extrapolations are carried out with default approaches given the lack of data to inform individual steps. To derive the RfC, this same approach was applied, but a PBPK model was used to extrapolate from oral to inhalation exposure.

The database for *tert*-butanol contains no human data on adverse health effects from subchronic or chronic exposure, and the PODs were calculated from data on the effects of *tert*-butanol reported by studies in rats. The database for *tert*-butanol exposure includes one lifetime bioassay, several reproductive/developmental studies, and several subchronic oral studies.

Although the database is adequate for reference value derivation, there is uncertainty associated with the lack of a comprehensive multigeneration reproductive toxicity study.

Additionally, only subchronic and short-term inhalation studies have been conducted, and no chronic inhalation studies are available. Developmental studies identified significant increases in fetal loss, decreases in fetal body weight, and possible increases in skeletal variations in exposed offspring or pups. However, effects were not always consistent across exposure routes, and maternal toxicity was present whenever developmental effects were observed.

The toxicokinetic and toxicodynamic differences for *tert*-butanol between the animal species in which the POD was derived and humans are unknown. The *tert*-butanol database lacks an adequate model that would inform potential interspecies differences (A limited data set exists for *tert*-butanol appearing as a metabolite from ETBE exposure in humans, but none for direct exposure to *tert*-butanol.) Generally, it was found that rats appear more susceptible than mice, and males appear more susceptible than females to *tert*-butanol toxicity. However, the underlying mechanistic basis of these apparent differences is not understood. Most importantly, it is unknown which animal species and/or sexes may be more comparable to humans.

Another uncertainty to consider relates to the MOA analysis conducted for the kidney effects. The assessment concluded that *tert*-butanol is a weak inducer of α_{2u}-globulin which is operative in male kidney tumors; therefore, noncancer effects related to α_{2u}-globulin were considered not relevant for hazard identification and, therefore, not suitable for dose response consideration. If this conclusion was incorrect and the noncancer effects characterized in this assessment as being related to α_{2u}-globulin were relevant to humans, then the RfD and RfC values could be underestimating toxicity. Similarly, the renal effects characterized as CPN and dismissed as not being treatment related, if considered relevant, would likewise contribute to the hazard potential and dose-response analysis for the kidney-specific RfD and RfC.

2.3. ORAL 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.

2.3.1. Analysis of Carcinogenicity Data

As noted in Section 1.3.2, that there is "suggestive evidence of carcinogenic potential" for *tert*-butanol. 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.

No human data relevant to an evaluation of the carcinogenicity of *tert*-butanol were available. The cancer descriptor was based on the 2-year drinking water study in rats and mice by

(NTP, 1995), which reported renal tumors in male rats and thyroid tumors in both male and female mice. This study was considered suitable for dose-response analysis. It was conducted in accordance with FDA GLP regulations, and all aspects were subjected to retrospective quality assurance audits. The study included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting of methods and results. Additionally, the renal tumors were reexamined by a Pathology Working Group (Hard et al., 2011).

Based on a mode of action analysis, it was concluded that the α_{2u}-globulin process was at least partially responsible for the male rat renal tumors, in addition to other, unknown, processes. Because the relative contribution of each process to tumor formation cannot be determined (U.S. EPA, 1991a), the male rat renal tumors are not considered suitable for quantitative analysis. Conversely, the mouse thyroid tumors are suitable for dose-response analysis and unit risk estimation, as described in Section 1.3.2.

2.3.2. Dose-Response Analysis—Adjustments and Extrapolations 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 *tert*-butanol, a standard model is used to curve-fit the data and 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 *tert*-butanol, the mode(s) of carcinogenic action for thyroid follicular cell tumors has not been established (see Section 1.3.2). Therefore, linear low-dose extrapolation was used to estimate human carcinogenic risk.

The dose-response modeling used administered dose because a PBPK model to characterize internal dosimetry in mice was not available. For the analysis of male mice thyroid tumors, the incidence data were adjusted to account for the increased mortality in high-dose male mice, relative to the other groups, that reduced the number of mice at risk for developing tumors. The Poly-3 method (Bailer and Portier, 1988) was used to estimate the number at risk of developing tumors, by weighting the length of time each animal was on study (details in Appendix C of the

Supplemental Information). This method was not applied to the female mice data because a difference in survival with increasing exposure was not appreciable and only one tumor, in the high-dose group, occurred before study termination.

The data modeled and other details of the modeling are provided in Appendix C. The BMDs and BMDLs recommended for each data set are summarized in Table 2-8. The modeled *tert*-butanol PODs were scaled to HEDs according to EPA guidance (U.S. EPA, 2011, 2005a). In particular, the BMDL was converted to an HED by assuming that doses in animals and humans are toxicologically equivalent when scaled by body weight raised to the $^3/_4$ power. Standard body weights of 0.025 kg for mice and 70 kg for humans were used (U.S. EPA, 1988). The following formula was used for the conversion of oral BMDL to oral HED for mouse endpoints:

$$\text{HED in mg/kgday-} = (\text{BMDL in mg/kgday-}) \times (\text{animal body weight}/70)^{1/4}$$
$$= (\text{BMDL in mg/kgday-}) \times 0.14$$

PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, corresponding to 10% extra risk in female mice and 5% extra risk in male mice.

2.3.3. Derivation of the Oral Slope Factor

The PODs estimated for each tumor data set are summarized in Table 2-8. The lifetime oral cancer slope factor for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (slope factor = $BMR/BMDL_{BMR} = 0.1/BMDL_{10}$). This slope represents a plausible upper bound on the true population average risk. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for male and female mice and are listed in Table 2-8.

The oral slope factor based on the incidence of thyroid follicular cell adenomas in female mice was 5×10^{-4} per mg/kg-day. Despite high mortality in high-dose male mice, estimating slope factors using the poly-3 method was feasible for addressing competing risks. Whether using the full data set (including the only thyroid follicular cell carcinoma observed at the highest dose) or omitting the high-dose group altogether (under the assumption that mortality in this group was too extensive to interpret the results), oral slope factors based on the incidence of thyroid follicular cell adenomas or carcinomas in male mice were similar when rounded to one significant digit—5×10^{-4} per mg/kg-day or 6×10^{-4} per mg/kg-day, respectively.

The recommended slope factor for lifetime oral exposure to *tert*-butanol is **5×10^{-4} per mg/kg-day**, based on the thyroid follicular cell adenoma or carcinoma response in male or female $B6C3F_1$ mice. This slope factor should not be used with exposures exceeding 1400 mg/kg-day, the highest POD from the two data sets, because above this level the cancer risk might not increase linearly with exposure. The slope of the linear extrapolation from the central estimate BMD_{10HED} derived from the female mouse data set is $0.1/[0.14 \times (2002 \text{ mg/kg-day})] = 4 \times 10^{-4}$ per mg/kg-day.

Table 2-8. Summary of the oral slope factor derivation

Tumor	Species/ sex	Selected model	BMR	BMD (mg/kg-d)	POD= BMDL (mg/kg-d)	$BMDL_{HED}$[a] (mg/kg-d)	Slope factor[b] $(mg/kg\text{-}day)^{-1}$
Thyroid follicular cell adenoma	$B6C3F_1$ mouse/Female	3° Multistage	10%	2002	1437	201	5×10^{-4}
Thyroid follicular cell adenoma or carcinoma	$B6C3F_1$ mouse/Male	All dose groups: 1° Multistage	5%[c]	1788	787	110	5×10^{-4}
		High dose omitted: 2° Multistage	5%[c]	1028	644	90	6×10^{-4}

[a]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011).
[b]Human equivalent slope factor = $0.1/BMDL_{10HED}$; see Appendix C of the Supplemental Information for details of modeling results.
[c]Because the observed responses were <10%, a BMR of 5% was used to represent the observed response range for low-dose extrapolation; human equivalent slope factor = $0.05/BMDL_{5HED}$.

2.3.4. Uncertainties in the Derivation of the Oral Slope Factor

There is uncertainty when extrapolating data from animals to estimate potential cancer risks to human populations from exposure to *tert*-butanol.

Table 2-9 summarizes several uncertainties that could affect the oral slope factor. There are no other chronic studies to replicate these findings or that examined other animal models, no data in humans to confirm a cancer response in general or the specific tumors observed in the NTP (1995) bioassay, and no other data (e.g., MOA) to support alternative approaches for deriving the oral slope factor.

Table 2-9. Summary of uncertainties in the derivation of the oral slope factor for *tert*-butanol

Consideration and impact on cancer risk value	Decision	Justification
Selection of tumor type and relevance to humans: Mouse thyroid tumors are the basis for estimating human cancer risk, as the fraction of rat kidney tumors not attributed to the male rat specific α2μ-globulin process could not be determined. Alternatively , quantifying rat kidney tumors could ↑ slope factor to 1 x 10^{-2} mg/kg-day (see Appendix C, Supplemental Information)	Thyroid tumors in female and male mice were selected U.S. EPA (1998a), U.S. EPA (1991a).	MOA data suggested that mouse thyroid tumors were relevant to humans. Quantitation of thyroid tumors in male mice was impacted only slightly by high mortality in the high-dose group, and supports the estimate based on female mice.
Selection of data set: No other studies are available.	NTP (1995), oral (drinking water) study, was selected to derive cancer risks for humans.	NTP (1995), the only chronic bioassay available, was a well-conducted study. Additional bioassays might add support to the findings, facilitate determination of what fraction of kidney tumors are not attributable to α2μ-globulin process, or provide results for different (possibly lower) doses, which would affect (possibly increase) the oral slope factor.
Selection of dose metric: Alternatives could ↓ or ↑ slope factor	Used administered dose.	For mice, PBPK-estimated internal doses could impact the OSF value for thyroid tumors if the carcinogenic moiety is not proportional to administered dose, but no PBPK model was available, and no information is available to suggest if any metabolites elicit carcinogenic effects.
Interspecies extrapolation of dosimetry and risk: Alternatives could ↓ or ↑ slope factor (e.g., 3.5-fold ↓ [scaling by body weight] or ↑ 2-fold [scaling by BW 2/3])	The default approach of body $weight^{3/4}$ was used.	No data to suggest an alternative approach for *tert*-butanol. Because the dose metric was not an area under the curve, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks. Although the true human correspondence is unknown, this overall approach is expected neither to over- or underestimate human equivalent risks.
Dose-response modeling: Alternatives could ↓ or ↑ slope factor	Used multistage dose-response model to derive a BMD and BMDL.	No biologically based models for *tert*-butanol were available. The multistage model has biological support and is the model most consistently used in EPA cancer assessments.

Consideration and impact on cancer risk value	Decision	Justification
Low-dose extrapolation: ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation	Linear extrapolation of risk in low-dose region used U.S. EPA (1998a).	Linear low-dose extrapolation for agents without a known MOA is supported (U.S. EPA, 2005a) and recommended for rodent thyroid tumors arising from an unknown MOA (U.S. EPA, 1998a).
Statistical uncertainty at POD: ↓ oral slope factor 1.4-fold if BMD used as the POD rather than BMDL	BMDL (preferred approach for calculating slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure at 10% extra risk of thyroid tumors.
Sensitive subpopulations: ↑ oral slope factor to unknown extent	No sensitive populations have been identified.	No chemical-specific data are available to determine the range of human toxicodynamic variability or sensitivity, including the susceptibility of children. Because determination of a mutagenic MOA has not been made, an age-specific adjustment factor is not applied.

2.3.5. Previous IRIS Assessment: Oral Slope Factor

No previous cancer assessment for *tert*-butanol is available in IRIS.

2.4. INHALATION UNIT RISK FOR CANCER

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

No chronic inhalation exposure studies to *tert*-butanol are available. Lifetime oral exposure has been associated with increased renal tubule adenomas and carcinoma in male F344 rats, increased thyroid follicular cell adenomas in female B6C3F$_1$ mice, and increased thyroid follicular cell adenomas and carcinomas in male B6C3F$_1$ mice. Because only a rat PBPK model exists, however, route-to-route extrapolation cannot be performed for thyroid tumors in mice at this time. The NTP (1995) drinking water study in rats and mice was the only chronic bioassay available for dose-response analysis. Still, the rat PBPK model and kidney tumors from the NTP (1995) drinking water study were not used for route-to-route extrapolation because enough information to determine the relative contribution of α_{2u}-globulin nephropathy and other processes to the overall renal tumor response (U.S. EPA, 1991a) is not available. Alternatively, if kidney tumors were considered acceptable for quantitation, then route-to-route extrapolation could be conducted to calculate an IUR (see Appendix C in Supplemental Information).

2.4.1. Previous IRIS Assessment: Inhalation Unit Risk

An inhalation cancer assessment for *tert*-butanol was not previously available on IRIS.

2.5. APPLICATION OF AGE-DEPENDENT ADJUSTMENT FACTORS

As discussed in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 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 *tert*-butanol carcinogenicity is not known (see Section 1.3.2), application of ADAFs is not recommended.

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