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

(CAS No. 75-65-0)

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

September 2014

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ABBREVIATIONS

α2u-g	alpha2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
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
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
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
PCNA	proliferating cell nuclear antigen
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 of America

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PREFACE

This Toxicological Review critically reviews the publicly available studies on *tert*-butyl alcohol (*tert*-butanol) in order to identify its adverse health effects and to characterize exposure-response relationships. It was prepared under the auspices of EPA's Integrated Risk Information System (IRIS) Program. The assessment covers an oral RfD, an inhalation RfC, and a cancer weight of evidence descriptor.

The Toxicological Reviews for ethyl tert-butyl ether (ETBE) and *tert*-butanol are being developed simultaneously because they have a number of overlapping scientific issues:

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

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to IRIS Toxicological Reviews. This is the first IRIS assessment for this compound. The findings of this assessment and related documents produced during its development are available on the IRIS Web site (http://www.epa.gov/iris).

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 are available on the docket at http://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2013-0111).

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

In response to the NRC's 2011 recommendations, the IRIS Program has made changes to streamline the assessment development process, improve transparency, and create efficiencies in

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

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

In the draft *tert*-butanol assessment, the IRIS Program has attempted to transparently and uniformly identify strengths and limitations that would affect interpretation of results. All animal studies of *tert*-butanol that were considered to be of acceptable quality, whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to *tert*-butanol. These studies were evaluated for aspects of design, conduct, and reporting that could affect the interpretation of results and the overall contribution to the synthesis of evidence for determination of human hazard potential using the study quality considerations outlined in the Preamble. A brief summary of the evaluation is included in the section on methods for study selection and evaluation. Information on study features related to this evaluation is reported in evidence tables and documented in the synthesis of evidence. Discussion of study strengths and limitations (that ultimately supported preferences for the studies and data relied upon) were included in the text where relevant.

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

weight-of-evidence approach to identify the potential human hazards associated with chemical exposure.

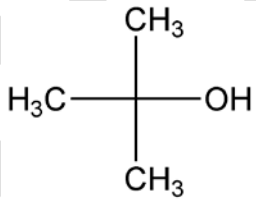
The IRIS *tert*-butanol assessment provides a streamlined presentation of information, integrated hazard identification of all toxic effects, and derivation of organ/system-specific reference values. Additionally, consistent with the goal that assessments should provide a scientifically sound and transparent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

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

Chemical Properties

tert-Butanol is a white crystalline solid or colorless liquid (above 77°F) with a camphor-like odor that is highly flammable (NIOSH, 2005; IPCS, 1987a). *tert*-Butanol contains a hydroxyl chemical functional group and is miscible with alcohol, ether, and other organic solvents and soluble in water (IPCS, 1987a). Selected chemical and physical properties of *tert*-butanol are presented in Table P-1.

Table P-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; *tert*iary 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 x 10^6 mg/L	HSDB (2007)
Octanol/Water Partition Coefficient (Log K_{OW})	0.35	HSDB (2007)
Henry's Law Constant	9.05 x 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)
Chemical structure		HSDB (2007)

Uses

tert-Butanol is primarily an anthropogenic substance that is produced in large quantities (HSDB, 2007) from a number of precursors, including 1-butene, isobutylene, acetyl chloride and dimethylzinc, and *tert*-butyl hydroperoxide. The domestic production volume of *tert*-butanol, including imports, was approximately four 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 U.S. prior to 2007 at levels of more than 2 billion gallons annually. Current use levels of MTBE and ETBE in the U.S. 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 is also 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 is also used to create artificial musk, fruit essences, and perfume (HSDB, 2007). It is also used in coatings on metal and paperboard food containers (Cal/EPA, 1999), industrial cleaning compounds, and can be used for chemical extractions in pharmaceutical application (HSDB, 2007).

Fate and Transport

Soil

The mobility of *tert*-butanol in soil is expected to be high due 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 *tert*-butanol's vapor pressure, volatilization from soil surfaces is expected to be an important dissipation process (HSDB, 2007). *tert*-Butanol is a tertiary alcohol and this class of alcohols generally degrades 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 on the order of months (approximately 200 days). Microbial degradation rates are increased in soils supplemented with nitrate and sulfate nutrients (HSDB, 2007).

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 is on the magnitude of 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 exists primarily 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 over one million pounds of *tert*-butanol has been released into the soil from landfills, land treatment,

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

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 wells throughout the United States (HSDB, 2007). California's Geotracker Database[2] lists 3496 detections of *tert*-butanol in groundwater associated with contaminated sites in that state since 2011. *tert*-Butanol has also been detected in drinking water wells in the vicinity of landfills (U.S. EPA, 2012c). Additionally, *tert*-Butanol leaking from underground storage tanks may 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 and in the environment.

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

General Population Exposure

tert-Butanol exposure can occur in many different settings. Contamination resulting from leaking underground storage tanks could potentially result in exposure to a large number of 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; therefore, exposure through ingestion of contaminated drinking water is likely occurring (HSDB, 2007).

Contaminated food can also contribute to *tert*-butanol ingestion through its use as a coating in metallic and paperboard food containers (Cal/EPA, 1999). *tert*-Butanol has been detected in food, namely beer and chickpeas, and identified in mother's milk (HSDB, 2007). Indirect exposure to *tert*-butanol may also occur as a result of ingestion of MTBE or ETBE, as *tert*-butanol is a metabolite of these compounds (NSF International, 2003).

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

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

Assessments by Other Federal, State and International Health Agencies

Toxicity information on *tert*-butanol has been evaluated by the American Conference of Governmental Industrial Hygienists (ACGIH, 2012), the National Institute for Occupational Safety and Health (NIOSH, 2007), the Occupational Safety and Health Administration (OSHA, 2006), and Food and Drug Administration. The results of these assessments are presented in Appendix A of the Supplemental Information. The California EPA carried out an expedited risk assessment for *tert*-butanol in drinking water and calculated a cancer slope factor based on rat kidney tumors observed in the NTP bioassays. It is important to recognize that these earlier assessments were prepared for different purposes using different methods and could consider only the studies that were available at the time.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

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

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

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

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

2. Process for developing and peer-reviewing IRIS assessments

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

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

Step 1. Development of a draft Toxicological Review. The draft assessment considers all pertinent publicly available studies and applies consistent criteria to evaluate study quality, identify health effects, identify mechanistic events and pathways,

integrate the evidence of causation for each effect, and derive toxicity values. A public meeting prior to the integration of evidence and derivation of toxicity values promotes public discussion of the literature search, evidence, and key issues.

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

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

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

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

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

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

The remainder of this Preamble addresses step 1, the development of a draft Toxicological Review. IRIS assessments follow standard practices of evidence evaluation and peer review, many of which are discussed in EPA guidelines (U.S. EPA, 2005a, b, 2000b, 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 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

Occurrence and Health Effects

tert-Butanol does not occur naturally, but it is produced by humans for multiple purposes, such as a solvent for paints, a denaturant for ethanol and several other alcohols, an octane booster in gasoline, a dehydrating agent, and the manufacture of flotation agents, fruit essences, and perfumes. *tert*-Butanol is also 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, as well as consuming contaminated water (or breast milk) or foods. Exposure may 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. No chronic inhalation exposure studies have been conducted. There is suggestive evidence that *tert*-butanol is carcinogenic to humans based on predominantly benign renal tumors in male rats and benign thyroid tumors in female mice.

Effects Other Than Cancer Observed Following Oral Exposure

EPA identified kidney effects as a hazard of *tert*-butanol exposure, with kidney toxicity observed after oral exposure in two strains of rats, one strain of mice, and in both sexes. In mice, the only kidney effect observed was an increase in kidney weight (absolute and/or relative) in both sexes of mice in the 13-week study, but no treatment-related histopathological lesions were reported in the kidneys of mice at 13 weeks or 2 years. Absolute and relative kidney weights were increased in both male and female rats after 13 weeks and 15 months of treatment. Histopathological examination also indicated kidney toxicity in both male and female rats, with increased incidence of nephropathy after 13 weeks of oral exposure and transitional epithelium

hyperplasia observed after 2 years of oral exposure. Additionally, increased suppurative inflammation was noted in females after 2 years of oral exposure. Mode of action analysis determined that male rat kidney effects were not mediated by α_{2u}-globulin, and these effects are concluded to be relevant for human health hazard assessment.

Oral Reference Dose (RfD) for Effects Other Than Cancer

Kidney toxicity, represented by kidney transitional epithelial hyperplasia, was chosen as the basis for the proposed overall oral reference dose (RfD) (see Table ES-1), as it was the only noncancer endpoint for which there is credible evidence of an association with *tert*-butanol exposure. 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 due to its consistency in both sexes, its specificity and its sensitivity as an indicator of kidney toxicity, and the observed dose-response relationship of effects across dose groups. Benchmark dose (BMD) modeling was utilized to derive the $BMDL_{10\%}$ of 16 mg/kg-day. The BMDL was converted to a human equivalent dose using body weight$^{3/4}$ scaling, and this value of 3.84 mg/kg-day was used as the point of departure (POD) for RfD derivation (U.S. EPA, 2011).

The proposed overall RfD was calculated by dividing the POD for 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. Summary of reference dose (RfD) derivation

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

Effects Other Than Cancer Observed Following Inhalation Exposure

EPA identified kidney effects as a human hazard of *tert*-butanol exposure. Both absolute and relative kidney weights were increased in male and female rats. There was an increase in nephropathy severity in male rats, which supported the increase in kidney weights. No available human studies evaluated the effects of inhalation exposure. Mode of action analysis determined that male rat kidney effects were not mediated by α_{2u}-globulin, and these effects are concluded to be relevant for human health hazard assessment.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

Kidney toxicity, represented by transitional epithelial hyperplasia, was chosen as the basis for the proposed inhalation reference concentration (RfC) (see Table ES-2), as it was the only noncancer endpoint for which there is credible evidence of an association with *tert*-butanol exposure. The chronic oral exposure study in rats (NTP, 1995) was used to derive the overall RfC. A PBPK model for *tert*-butanol in rats was developed internally, and route-to-route extrapolation was used to derive equivalent inhalation PODs. The POD adjusted for the human equivalent concentration (HEC) was 26.1 mg/m^3 and 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. Summary of reference concentration (RfC) derivation

Effect	Basis	RfC (mg/m^3)	Exposure description	Confidence
Kidney toxicity	Increased incidence of kidney transitional epithelial hyperplasia	9×10^{-1}	Chronic	HIGH
Proposed overall RfC	**Increased incidence of kidney transitional epithelial hyperplasia**	**9×10^{-1}**	**Chronic**	**HIGH**

Evidence for Human Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for *tert*-butanol provides "suggestive evidence of carcinogenic potential." Human data are not available to assess the carcinogenic potential of *tert*-butanol. In 2-year studies in F344 rats and $B6C3F_1$ mice, male rats exhibited dose-related increases in renal tubule adenoma and combined renal tubule adenoma or carcinoma. Although data support α_{2u}-globulin deposition in the kidney of male rats, there is insufficient evidence to support this as the only or primary mechanism for renal tumor development in male rats. Therefore, the renal tumors are considered relevant to humans. However, the observed renal tumors were predominantly benign, only occurred in a single sex/species combination, and were not observed in studies that exposed the same strain of rat to ETBE, which is rapidly metabolized to *tert*-butanol. In addition, a statistically significant increase in the incidence of thyroid follicular cell adenoma was observed in a 2-year drinking water study in female mice (NTP, 1995). These tumors were all benign and only a single sex/species combination was affected. There are no studies examining the carcinogenic potential of *tert*-butanol after inhalation exposure in animals. However, internal tumors developed after oral exposure and may occur regardless of exposure route, as blood concentrations were found to be similar after oral or

inhalation exposures. Genotoxicity data for *tert*-butanol are inconclusive. *tert*-Butanol was negative in a variety of genotoxicity assays in different cell systems including gene mutations, sister chromatid exchanges, micronucleus formation and chromosomal aberrations. However, DNA adducts in male Kunming mice and DNA damage in human HL-60 leukemia cells have been observed. Overall, the cancer descriptor "suggestive evidence of carcinogenic potential" is plausible, as some concern is raised by the positive evidence of predominantly benign renal tumors in male rats and benign thyroid tumors in female mice.

Quantitative Estimate of Carcinogenic Risk from Oral Exposure

Lifetime oral exposure to *tert*-butanol 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. The NTP (1995) study in rats and mice was the only available study for dose-response analysis. 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," EPA concluded that the main study was well-conducted and quantitative analysis may be useful for providing a sense of the magnitude of potential carcinogenic risk. For renal tumors, two slope factors were derived for this endpoint from the NTP (1995) bioassay: one based on the original reported incidences and one based on the Hard et al. (2011) reanalysis. The two estimates differed by less than 20%, and rounded to the same number at one significant figure. However, the Hard et al. (2011) reanalysis is considered preferable, as it is based on a Pathology Working Group (PWG) analysis. A slope factor was also derived for thyroid tumors in female mice. The modeled *tert*-butanol PODs were 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 more sensitive endpoint of renal tumors was used because there is no data to support neither renal nor thyroid tumors most relevant to humans. The oral slope factor of **1×10^{-2} per mg/kg-day**, based on the renal tubule tumor response in male F344 rats.

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

No chronic inhalation exposure studies to *tert*-butanol are available. However, through the oral route of exposure, lifetime 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. The NTP (1995) study in rats and mice was the only available study for dose-response analysis. 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," EPA concluded that the main study was well-conducted and quantitative analysis may be useful for providing a sense of the magnitude of potential carcinogenic risk. Since the available evidence for *tert*-butanol carcinogenicity is from a 2 year oral exposure, route-to-route extrapolation of the oral BMDL was performed to derive an inhalation equivalent BMCL. The BMCL was then converted to a human equivalent concentration (HEC) according to the RfC guidelines (U.S. EPA, 1994) by multiplying the BMCL by the blood:gas partition coefficient ratio. Using linear extrapolation from the resulting $BMCL_{10\text{-}HEC}$, a human equivalent inhalation unit risk was derived (inhalation unit risk = $0.1/BMCL_{10\text{-}HEC}$). Extrapolation from the oral study results for renal tubule adenoma or carcinoma in male F44 rats gives a unit risk of **2×10^{-3} per mg/m^3**, associated with lifetime inhalation exposure to *tert*-butanol.

Susceptible Populations and Lifestages for Cancer and Noncancer

No data were identified to indicate susceptible populations or lifestages.

Key Issues Addressed in Assessment

Due to the observation of kidney tumors and noncancer toxicity following chronic exposure to *tert*-butanol, an evaluation of whether *tert*-butanol caused α_{2u}-globulin nephropathy was performed. The presence of α_{2u}-globulin in the hyaline droplets was confirmed in male rats by α_{2u} immunohistochemical staining. Linear mineralization and tubular hyperplasia were reported in male rats, though only in the chronic study. Other subsequent steps in the pathological sequence, including necrosis, exfoliation, and granular casts, were either absent or not consistently observed across subchronic or chronic studies. None of the observed effects occurred in female rats or in either sex of mice. Because the available data supports the occurrence of at least two of the subsequent steps in the pathological sequence, these data are sufficient to conclude that α_{2u}-globulin nephropathy is occurring in the kidney of male rats following *tert*-butanol exposure. Thus, the noncancer lesions associated with α_{2u}-globulin nephropathy are not considered relevant to humans.

However, tumors develop at doses lower than some precursors of α_{2u}-globulin nephropathy, such as granular casts and tubular hyperplasia (Hard et al., 2011; NTP, 1995). Therefore, there is insufficient evidence to support a conclusion that α_{2u}-globulin nephropathy is the sole or primary contributor to renal tumor development. Because carcinogenic processes other than α_{2u}-globulin nephropathy cannot be ruled out, the renal tumors are considered relevant to humans.

In addition, some of the observed renal lesions in rats following exposure to *tert*-butanol are effects commonly associated with chronic progressive nephropathy (CPN), an age-related renal disease of laboratory rodents that occurs spontaneously. While it has been argued that CPN in rats

is not relevant to humans, it is acknowledged that the mechanism regulating CPN in rats is not understood. Moreover, no key events for the exacerbation of CPN have been identified, so no mode of action analysis can be performed. Therefore, kidney effects from *tert*-butanol exposure associated with CPN are considered relevant to humans.

Sufficient data were available to develop a PBPK model in rats for both oral and inhalation exposure in order to perform route-to-route extrapolation, so rat studies from both routes of exposure were considered for dose-response analysis. The only endpoint available from the subchronic inhalation study (NTP, 1997) was increased kidney weights, which is a less-specific endpoint compared to other endpoints available for analysis from the oral study (NTP, 1995). In regards to the carcinogenic effects, the 2-year oral study (NTP, 1995) was the only study to evaluate lifetime carcinogenic effects and was selected for route-to-route extrapolation.

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 in order to identify all potentially pertinent studies. In subsequent steps, references were screened to exclude papers not pertinent to an assessment of the health effects of *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, Toxline, Web of Science, and TSCATS through April 2014, using the keywords and limits described in Table LS-1. The overall literature search approach is shown graphically in Figure LS-1. An additional 7 citations were obtained using additional search strategies described in Table LS-2. After electronically eliminating duplicates from the citations retrieved through these databases, 2,532 unique citations were identified.

The resulting 2,532 citations were screened into categories as presented in Figure LS-1 using the title, abstract, and/or full text for pertinence to examine the health effects of *tert*-butanol exposure.

- 12 references were identified as potential "Sources of Health Effects Data" and were considered for data extraction to evidence tables and exposure-response arrays.
- 196 references were identified as "Supporting Studies;" these included 39 studies describing physiologically-based pharmacokinetic (PBPK) models and other toxicokinetic information, 70 studies providing genotoxicity and other mechanistic information, 1 human case report, 73 not relevant exposure paradigms (including acute, dermal, eye irritation, and injection studies), 6 preliminary toxicity studies, and 7 physical dependency studies. While still considered sources of health effects information, studies investigating the effects of acute and direct chemical exposures are generally less pertinent for characterizing health hazards associated with chronic oral and inhalation exposure. Therefore, information from these studies was not considered for extraction into evidence tables. Nevertheless, these studies were still evaluated as possible sources of supporting health effects information.
- 63 references were identified as secondary sources of health effects information (e.g., reviews and other agency assessments); these references were kept as additional resources for development of the Toxicological Review.
- 2,261 references were identified as not being pertinent to an evaluation of the health effects of *tert*-butanol and were excluded from further consideration (see Figure LS-1 for exclusion categories).

The complete list of references and the sorting of these materials can be found on the HERO website at http://hero.epa.gov.

Selection of Critical Studies for Inclusion in Evidence Tables

Each study retained after the literature search and screen was evaluated for aspects of its design or conduct that could affect the interpretation of results and the overall contribution to the evidence for determination of hazard potential. Some general questions that were considered in evaluating experimental animal studies are presented in Table LS-3. Much of the key information for conducting this evaluation can generally be found in the study's methods section and in how the study results are reported. Importantly, the evaluation at this stage does not consider the direction or magnitude of any reported effects.

To facilitate this evaluation, evidence tables were constructed that systematically summarize the important information from each study in a standardized tabular format as recommended by the NRC (2011). Twelve studies identified as "Sources of Health Effects Data" were considered for extraction into evidence tables for hazard identification in Chapter 1. Initial review of studies found two studies to be publications of the NTP (1995) data prior to the release of the final NTP report (Cirvello et al., 1995; Lindamood et al., 1992). One publication in the "Supporting Studies" category also was based on data from the NTP report (Takahashi et al., 1993). There were differences between the published reports and the final NTP report; therefore, the finalized NTP (1995) report was included in evidence tables. Data from the remaining 10 studies in the "Sources of Health Effects Data" category were extracted into evidence tables.

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

Database Evaluation

The database for *tert*-butanol is comprised of animal toxicity studies containing one 2-year bioassay that employs oral exposures in rats and mice; two oral subchronic studies in rats and one in mice; one inhalation subchronic study in rats and mice; a re-evaluation of the NTP (1995) rat data; two oral developmental studies; two inhalation developmental studies; and one one-generation reproductive study that also evaluates other systemic effects. Several acute and short term studies (including an 18-day inhalation study and a 14-day study by NTP) using oral and inhalation exposures were performed mostly in rats, but were grouped as supporting studies since the database of chronic and subchronic rat studies was considered sufficient. No cohort studies, case-control studies, or ecological studies exist in the published literature. There was one case report available. Health effect studies of gasoline and *tert*-butanol mixtures were not considered pertinent to the assessment since the separate effects of the gasoline components could not be determined; thus, these studies were excluded during the manual screen.

The "Sources of Health Effects Data" were comprised entirely of studies performed in rats and mice with drinking water, oral gavage, and inhalation exposures to *tert*-butanol. These 12 sources were conducted according to OECD Good Laboratory Practice (GLP) guidelines, presented extensive histopathological data, and/or clearly presented their methodology; thus, these are considered high quality. Preliminary, acute, and short-term studies contained information that supported and did not differ qualitatively from the results of the ≥30 day exposure studies; thus, these studies are not included in the evidence tables. Some of these shorter duration studies are presented in the text of the Toxicological Review and are used in sections such as "Mechanistic Evidence" to augment the discussion. A more detailed discussion of methodological concerns that were identified will precede each endpoint evaluated in the hazard identification section.

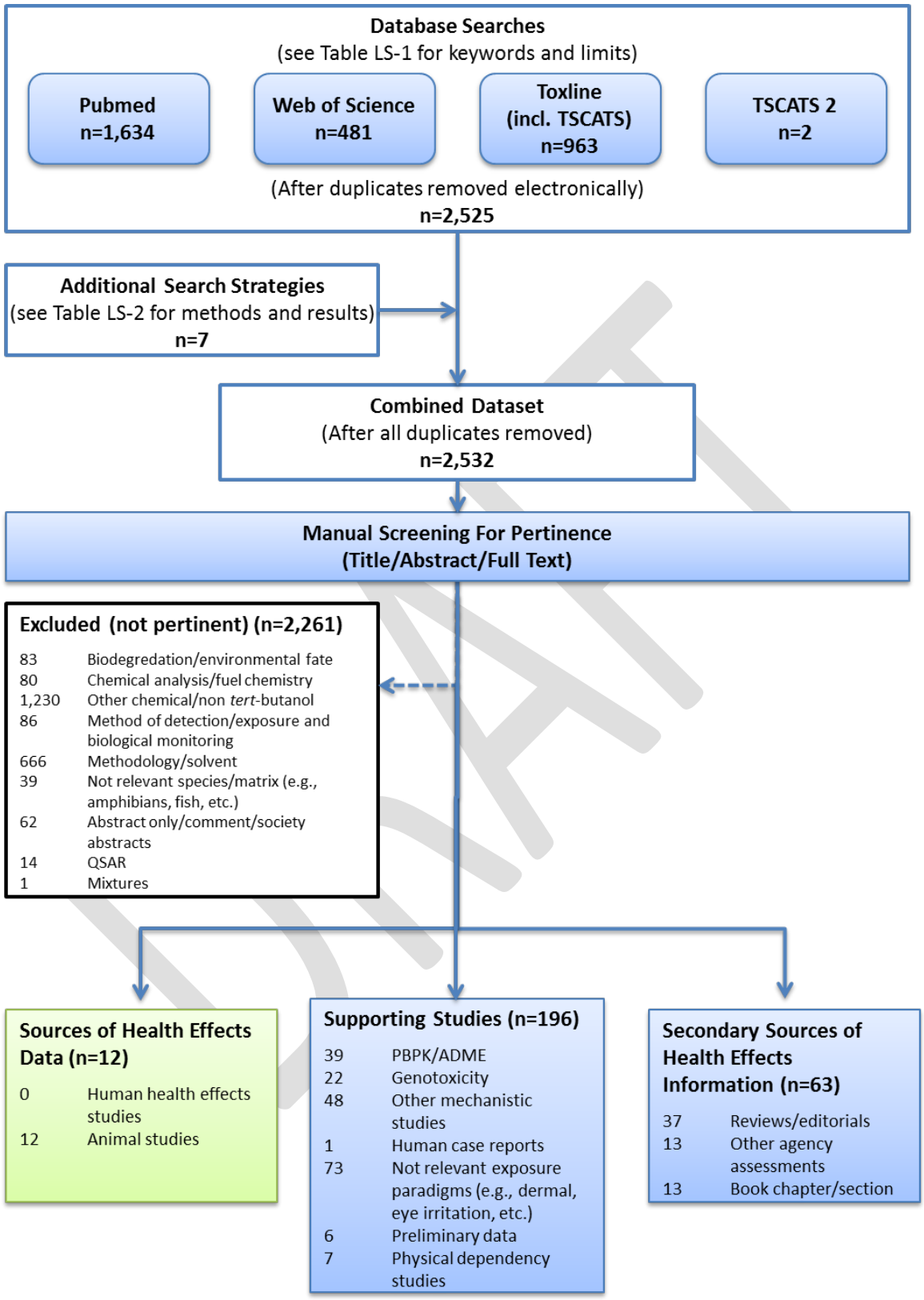


Figure LS-1. Study selection strategy.

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

Database (Search Date)	Keywords	Limits
PubMed (12/20/2012) (4/17/2014)	*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)	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 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)	*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)	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 by other international and federal agencies	IPCS (1987a). Butanols: Four isomers: 1-butanol, 2-butanol, tert-butanol, isobutanol [WHO EHC]. Geneva, Switzerland: World Health Organization.	1/2013	None
	OSHA (1992). Occupational safety and health guideline for *tert*-butyl alcohol. Cincinnati, OH: National Institute for Occupational Safety and Health.	1/2013	None

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

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

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

1. HAZARD IDENTIFICATION

1.1. PRESENTATION AND SYNTHESIS OF EVIDENCE BY ORGAN/SYSTEM

1.1.1. Kidney Effects

Synthesis of Effects in Kidney

This section reviews the studies that investigated whether exposure to *tert*-butanol can cause kidney effects in humans or animals. The database examining kidney effects following *tert*-butanol exposure contains no human data, six studies performed in rats or mice, and one re-evaluation of the rat data from NTP (1995). Studies employing short-term and acute exposures that examined kidney effects are not included in the evidence tables; however, they are discussed in the text if they provide data to support mode of action or hazard identification. No methodological concerns were identified that would lead one or more studies to be considered less informative for assessing human health hazard. A pathology working group (Hard et al., 2011) re-examined kidney histopathology from the NTP (1995) 13-week and 2-year studies in rats to evaluate questions involving MOAs for renal tubule development. All slides were analyzed in a blinded manner. Hard et al. (2011) did report different incidences of adenomas or carcinomas compared with the original NTP (1995) study; thus, these data were presented separately. Histopathological results from both Hard and NTP will be considered for hazard identification.

tert-Butanol exposure resulted in a number of kidney effects after both oral (drinking water) and inhalation exposure in both sexes of rats and mice. Kidney effects observed after oral exposure (Table 1-1;Table 1-2; Figure 1-1) include increased kidney weight in female rats and in male and female mice (13-week exposure), and kidney inflammation, kidney transitional epithelial hyperplasia, and increased incidence and/or severity of kidney nephropathy in female rats (2-year exposure) (NTP, 1995). In a 2-year oral exposure study in male rats, increased kidney weight, increased hyaline droplets, kidney transitional epithelial hyperplasia, kidney mineralization, renal tubule hyperplasia, and increased incidence and/or severity of kidney nephropathy were observed, with some of these effects seen at earlier time periods (NTP, 1995). Other kidney effects in male rats were observed in a 10-week oral exposure study (Acharya et al., 1997; Acharya et al., 1995). No changes in clinical chemistry that would typically be indicative of kidney damage have been observed with *tert*-butanol exposure. Although there were some changes in urinalysis parameters (e.g., decreased urine volume and increased specific gravity), this was accompanied by reduced water consumption and may not be related to an effect of kidney function.

The kidney is also the target organ for cancer effects (Table 1-3; Figure 1-1). Male F344 rats had an increased incidence of renal tubule adenomas and combined renal tubule adenoma or

carcinoma in a 2-year oral bioassay (Hard et al., 2011; NTP, 1995). The highest exposure group had an increase in mortality, which may in part explain the apparent non-monotonicity in the observed dose-response, in which the highest exposure group had a lower incidence of tumors than the middle exposure group.

An Independent Pathology Working Group (PWG), sponsored by Lyondell Chemical Company, re-evaluated the kidney changes in the NTP 2-year study (Hard et al., 2011). The PWG consisted of senior pathologists with experience in chemically-induced nephrotoxicity and renal neoplasia. In all cases, PWG members were blinded to treatment groups to preclude any possible bias, and used guidelines published by the Society of Toxicologic Pathology. The PWG confirmed the NTP findings of atypical tubule hyperplasia and renal tubule tumors in male rats at 2-years. In particular, they reported very similar overall tumor incidences in the exposed groups. However, the PWG evaluation of the control groups reported fewer renal tubule adenomas and carcinomas than the original NTP study. As a result, based on the PWG evaluation, all treated groups had statistically significant increases in renal tubule adenomas and carcinomas (combined) as compared to controls. Additionally, the PWG considered fewer of the tumors to be carcinomas as compared to the original NTP study.

No chronic (2-year) inhalation exposure study is available, but minimal kidney effects were observed in rats (mainly the males) after *tert*-butanol exposure by inhalation for 13 weeks at concentrations ranging from 406–6,368 mg/m^3 (NTP, 1997) (Table 1-1; Table 1-2; Figure 1-2). Absolute kidney weights were elevated (9.8–11%) in male rats exposed at ≥3,274 mg/m^3 (not dose-dependent); relative kidney weights were statistically elevated (~9%) in males at ≥3,274 mg/m^3 and 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). Although the kidney effects were less severe after inhalation exposure, a direct comparison can only be made on the basis of internal dose. ARCO (1983) found that blood levels of *tert*-butanol and its metabolites are equivalent after a single oral dose of 350 mg/kg compared to a single 6-hour inhalation exposure to 6,164 mg/m^3. That would indicate, based on bolus exposures, that the inhalation exposures used in the NTP (1997) study were in the range of the lower doses used in the NTP (1995) oral study. On the other hand, based on PBPK modeling, chronic exposure in the range of the NTP (1995) bioassay doses of 90–420 mg/kg-day lead to the same average blood concentration of *tert*-butanol as 6-hour/day, 5 day/week inhalation exposures to 860–4500 mg/m^3, suggesting that the oral and inhalation exposures in NTP (1995) and NTP (1997), respectively, overlap on the basis of internal dose. Finally, the lack of either mortality or changes in body weight (both observed with oral exposure) observed after the inhalation exposure suggests that a direct comparison cannot be made.

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

Reference and study design	Results
Kidney weight (percent change as compared to control)	
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d Males: 9 weeks beginning 4 weeks prior to mating Females: 4 weeks prior to mating through PND21	**Males** Dose (mg/kg-d) / Left absolute weight / Left relative weight / Right absolute weight / Right relative weight 0 / 0 / 0 / 0 / 0 64 / +6 / +8 / +6 / +8 160 / +9 / +14* / +6 / +11* 400 / +12* / +14* / +14* / +17* 1,000 / +18* / +28* / +20* / +31* **Females** Dose (mg/kg-d) / Left absolute weight / Left relative weight / Right absolute weight / Right relative weight 0 / 0 / 0 / 0 / 0 64 / −1 / −2 / +2 / 0 160 / 0 / 0 / +1 / 0 400 / +3 / +2 / +4 / +2 1,000 / +4 / 0 / +7 / +2
NTP (1995) F344/N rat; 10/sex/treatment Drinking water 0, 2.5, 5, 10, 20, 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	**Males** Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 230 / +12* / +19* 490 / +17* / +26* 840 / +16* / +32* 1,520 / +26* / +54* 3,610 / All dead / All dead **Females** Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 290 / +19* / +17* 590 / +16* / +15* 850 / +29* / +28* 1,560 / +39* / +40* 3,620 / +36* / +81*

Table 1-1. Changes in kidney weight in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results					
NTP (1995) $B6C3F_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	**Males**			**Females**		
	Dose (mg/kg-d)	Absolute weight	Relative weight	Dose (mg/kg-d)	Absolute weight	Relative weight
	0	0	0	0	0	0
	350	+1	+1	500	0	−3
	640	+3	+2	820	−3	−1
	1,590	+2	+8	1,660	+1	0
	3,940	+6	+22*	6,430	+6	+15*
	8,210	0	+48*	11,620	+12*	+35*
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	**Males**			**Females**		
	Dose (mg/kg-d)	Absolute weight	Relative weight	Dose (mg/kg-d)	Absolute weight	Relative weight
	0	0	0	0	0	0
	90	+4	+8	180	+8*	+14*
	200	+11	+15*	330	+18*	+21*
	420	+7	+20*	650	+22*	+42*
	Only animals sacrificed at 15 months were evaluated for organ weights. Organs were not weighed in the 2-year mouse study					
NTP (1997) F344/N rat; 10/sex/treatment 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	**Males**			**Females**		
	Concentration (mg/m^3)	Absolute weight	Relative weight	Absolute weight	Relative weight	
	0	0	0	0	0	
	406	+1	+1	−4	−1	
	824	−2	−1	0	+1	
	1,643	+3	+2	+4	+4	
	3,273	+11*	+8*	+2	+2	
	6,368	+9.8*	+9*	+4	+9*	

Table 1-1. Changes in kidney weight in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results				
NTP (1997) $B6C3F_1$ mouse; 10/sex/treatment 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		Males		Females	
	Concentration (mg/m^3)	Absolute weight	Relative weight	Absolute weight	Relative weight
	0	0	0	0	0
	406	−6	−4	+1	−3
	824	−1	+3	+5	+9
	1,643	+4	+3	+1	−2
	3,273	−10	−3	0	+7
	6,368	+3	+6	+3	+15*

[a] The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by the study authors.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.

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

Reference and study design	Results
Acharya et al. (1997; 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%)*
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	There were no changes in kidney histopathology observed.

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

Reference and study design	Results					
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			**Females**		
	Dose (mg/kg-d)	Mineralization	Nephropathy	Dose (mg/kg-d)	Mineralization	Nephropathy
	0	0/10	7/10 (1.0)	0	10/10 (1.7)	2/10 (1.0)
	230	0/10	10/10 (1.6*)	290	10/10 (2.0)	3/10 (1.0)
	490	2/10 (1.5)	10/10 (2.6*)	590	10/10 (2.0)	5/10 (1.0)
	840	8/10*(1.4)	10/10 (2.7*)	850	10/10 (2.0)	7/10* (1.0)
	1,520	4/10*(1.0)	10/10 (2.6*)	1,560	10/10 (2.0)	8/10* (1.0)
	3,610	4/10*(1.0)	7/10 (1.1)	3,620	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/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Histopathology data for the 13-week study were not provided, but the kidney was evaluated indicating that no changes in kidney histopathology were observed in the 13-week study.					

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

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, 10 mg/mL) M: 0, 90, 200, 420[a] mg/kg-d F: 0, 180, 330, 650[a] mg/kg-d 2 years	Incidence (severity): **Males** Dose (mg/kg-d) / Mineralization (interim) / Mineralization (terminal) / Linear mineralization (terminal) 0 / 1/10 (1.0) / 26/50 (1.0) / 0/50 90 / 2/10 (1.0) / 28/50 (1.1) / 5/50* (1.0) 200 / 5/10 (1.8) / 35/50 (1.3) / 24/50* (1.2) 420 / 9/10* (2.3) / 48/50* (2.2) / 46/50* (1.7) Dose (mg/kg-d) / Renal tubule hyperplasia (extended evaluation) / Transitional epithelium hyperplasia / Nephropathy severity 0 / 12/50 (2.3) / 25/50 (1.7) / 3.0 90 / 16/50 (2.3) / 32/50 (1.7) / 3.1 200 / 14/50 (2.2) / 36/50* (2.0) / 3.1 420 / 23/50* (2.8) / 40/50* (2.1) / 3.3* **Females** Dose (mg/kg-d) / Mineralization[b] Interim / Mineralization[b] Terminal / Inflammation (suppurative) incidence 0 / 10/10 (2.8) / 49/50 (2.6) / 2/50 180 / 10/10 (2.9) / 50/50 (2.6) / 3/50 330 / 10/10 (2.9) / 50/50 (2.7) / 13/50* 650 / 10/10 (2.8) / 50/50 (2.9) / 17/50* Dose (mg/kg-d) / Renal tubule hyperplasia / Transitional epithelium hyperplasia / Nephropathy severity 0 / 0/50 / 0/50 / 1.6 180 / 0/50 / 0/50 / 1.9* 330 / 0/50 / 3/50 (1.0) / 2.3* 650 / 1/50 (1.0) / 17/50*(1.4) / 2.9*

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

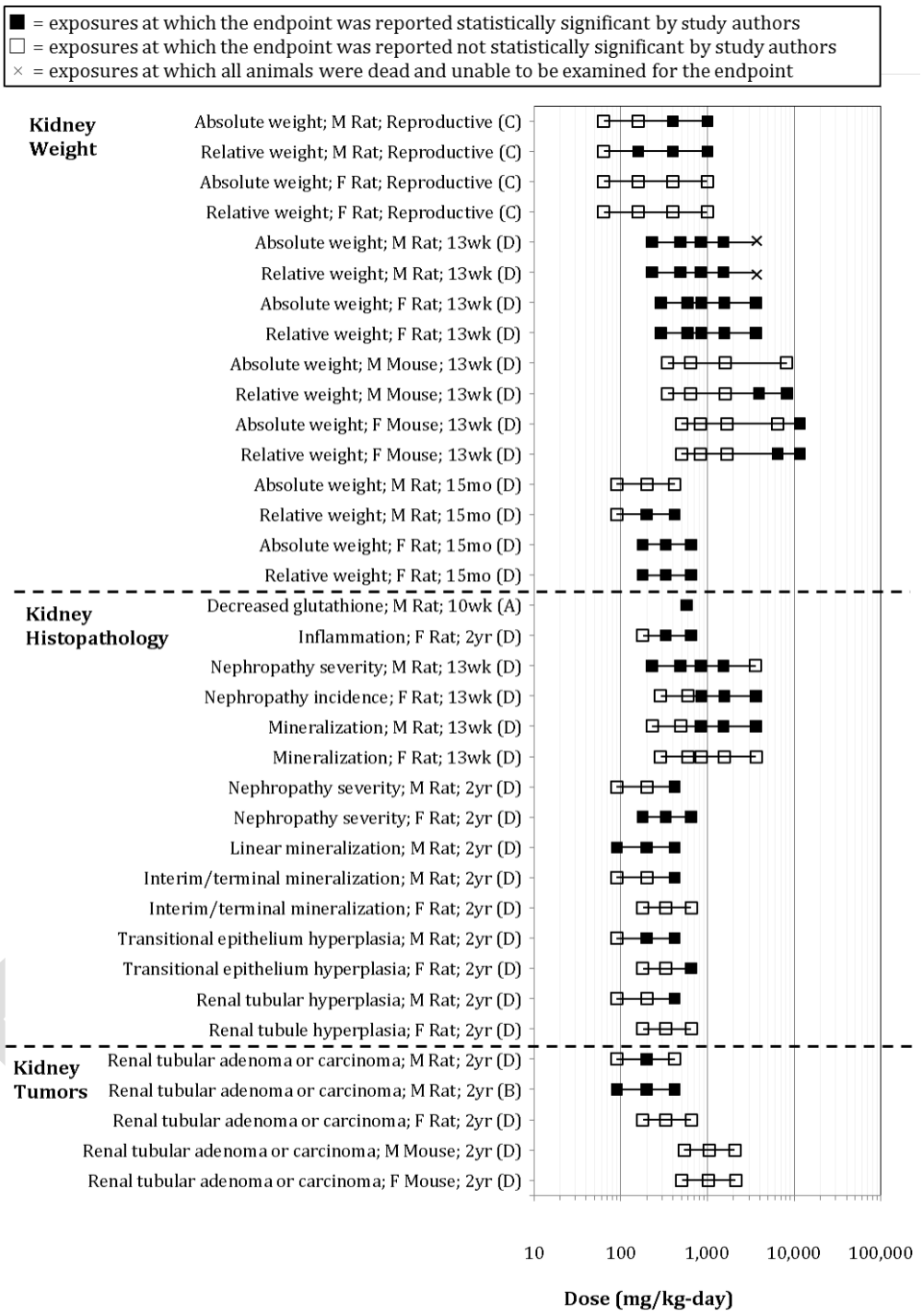
Reference and study design	Results
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	No changes in kidney related histopathology observed.[c]
NTP (1997) F344/N rat; 10/sex/treatment Analytical 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	**Male** Concentration (mg/m^3) / Average severity of chronic nephropathy 0 / 1.0 406 / 1.4 824 / 1.4 1,643 / 1.6 3,273 / 1.9 6,368 / 2.0 Severity categories: 1= minimal, 2= mild. No results from statistical tests reported
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment 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	There were no kidney effects observed.

[a] The high-dose group had an increase in mortality.
[b] Linear mineralization not observed in female rats.
[c] Organs were not weighed in mice during the 2-year study. Relative organ weights refer to relative to body weight
* Statistically significant $p \leq 0.05$ as determined by the study authors.
Conversions from drinking water concentrations to mg/kg-d 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

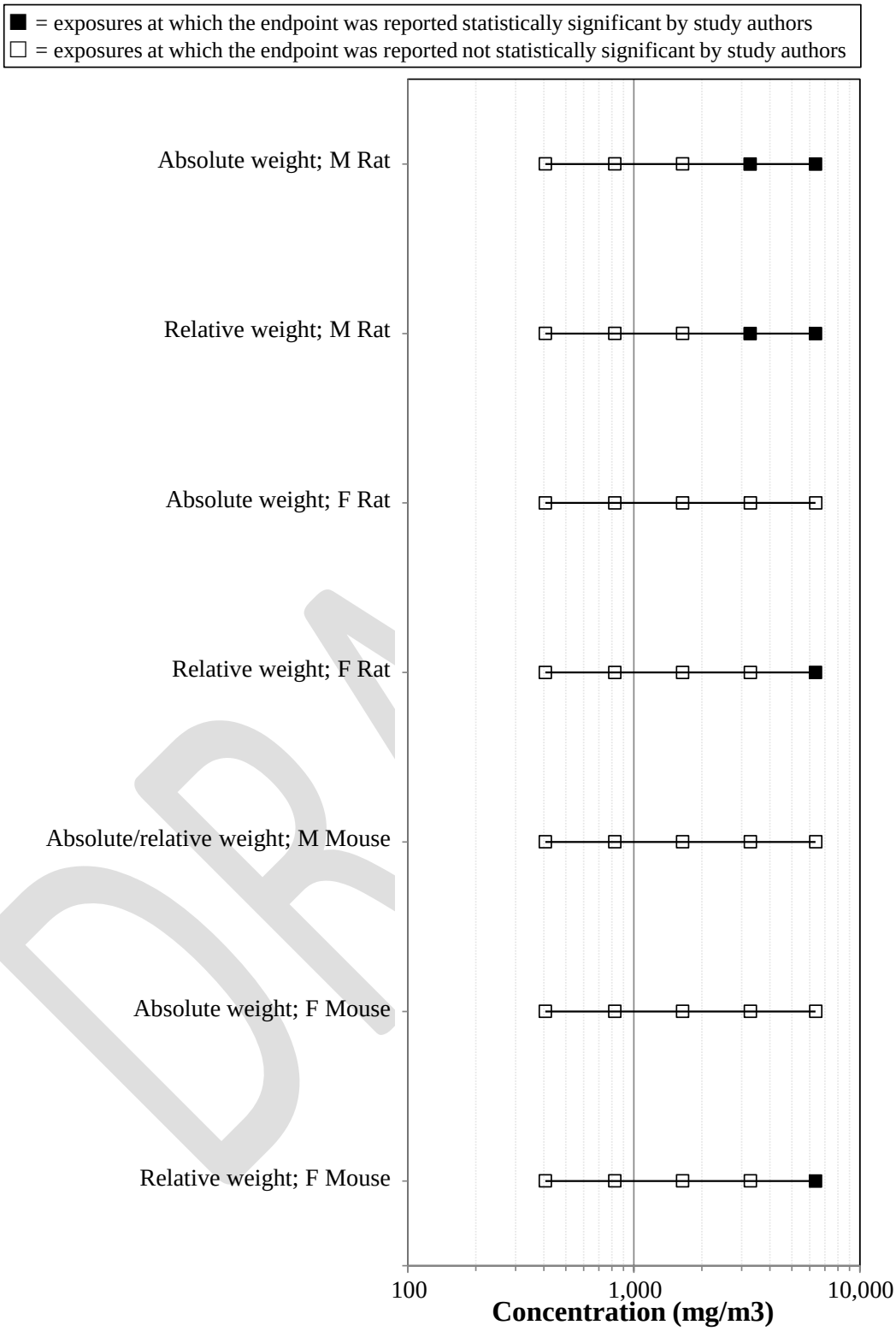
Reference and study design	Results
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	**Male** Dose (mg/kg-d) / Renal tubule adenoma (single) / Renal tubule adenoma (multiple) / Renal tubule carcinoma / Renal tubule adenoma (single or multiple) or carcinoma 0 / 7/50 / 1/50 / 0/50 / 8/50 90 / 7/50 / 4/50 / 2/50 / 13/50 200 / 10/50 / 9/50* / 1/50 / 19/50* 420 / 10/50 / 3/50 / 1/50 / 13/50 **Female** Dose (mg/kg-d) / Renal tubule adenoma (single) / Renal tubule adenoma (multiple) / Renal tubule carcinoma / Renal tubule adenoma (single or multiple) or carcinoma 0 / 0/50 / 0/50 / 0/50 / 0/50 180 / 0/50 / 0/50 / 0/50 / 0/50 330 / 0/50 / 0/50 / 0/50 / 0/50 650 / 0/50 / 0/50 / 0/50 / 0/50 Results do not include the animals sacrificed at 15 months.
Hard et al. (2011) reanalysis of the slides from male rats 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 changes in kidney-related tumors

[a] The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by the study authors.
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 1-1. Exposure response array for kidney effects following oral exposure to *tert*-butanol.



Source: NTP (1997)

Figure 1-2. Exposure-response array of kidney effects following subchronic inhalation exposure to *tert*-butanol (no chronic studies available).

Mode of Action Analysis—Kidney Effects

Mode of Action Analysis for α_{2u}-globulin-associated nephropathy

Description of the hypothesized MOA

Several studies were identified that evaluated the role of α_{2u}-globulin in *tert*-butanol-induced renal tumor development (Borghoff et al., 2001; Williams and Borghoff, 2001; Takahashi et al., 1993). α_{2u}-Globulin is a member of a large superfamily of low-molecular-weight proteins and 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 the male rat.

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

U.S. EPA (1991a) states that two questions must be addressed to determine the extent to which α_{2u}-globulin mediated processes induce renal tumors and nephropathy. First, it must be determined whether the α_{2u}-globulin process is occurring in male rats and therefore could be a factor in renal effects. U.S. EPA (1991a) states that the criteria for answering this question in the affirmative are as follows:

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

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

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

    (a) single-cell necrosis,

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

(c) granular casts,

(d) linear mineralization, and

(e) tubule hyperplasia.

The available data relevant to this question in male rats are summarized in Table 1-5 and Figure 1-3 and Figure 1-4, and will be evaluated below in accordance with the mode of action (MOA) framework from the EPA cancer guidelines (U.S. EPA, 2005a).

If the α_{2u}-globulin process is operative, then U.S. EPA (1991a) states that a second question must be answered as to whether the renal effects are solely due to the α_{2u}-globulin process, are a combination of the α_{2u}-globulin process and other carcinogenic processes, or are due primarily to other processes. U.S. EPA (1991a) states that the following types of data may be useful for answering this question:

1) Hypothesis-testing data
2) Biochemical information
3) Sustained cell division in the proximal tubule of the male rat
4) Structure-activity relationships
5) Covalent binding to macromolecules
6) Genotoxicity
7) Nephrotoxicity
8) Animal bioassay data in other species-, sex-combinations
9) Other information not specifically listed

The available data relevant to this question are summarized in Table 1-6, and will be evaluated below in accordance with the MOA framework from the EPA cancer guidelines (U.S. EPA, 2005a).

From these two questions, U.S. EPA (1991a) states that one of three possible conclusions can be made:

- If renal tumors in male rats are attributable solely to the α_{2u}-globulin process, then U.S. EPA (1991a) states that such tumors will not be used for human cancer hazard identification or for dose-response extrapolations.

- If renal tumors in male rats are not linked to the α_{2u}-globulin process, then U.S. EPA (1991a) states that such tumors are an appropriate endpoint for human hazard identification and are considered, along with other appropriate endpoints, for quantitative risk estimation.

- If some renal tumors in male rats are attributable to the α_{2u}-globulin process and some attributable to other carcinogenic processes, then U.S. EPA (1991a) states that 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.

Additionally, U.S. EPA (1991a) states that if the α_{2u}-globulin process is occurring in male rats, then the associated nephropathy in male rats (described above) would not be an appropriate endpoint to determine noncancer effects occurring in humans. In such a case, the characterization of human health hazard for renal toxicity would need to rely on other types of nephrotoxic effect data in male rats and/or on nephrotoxic effect data in female rats or other species.

Table 1-4. Additional kidney effects potentially relevant to mode of action in animals following exposure to *tert*-butanol

Reference and study design	Results
Williams and Borghoff (2001) F344 rats; 4/sex Single gavage dose: 500 mg/kg	Males: ↑ binding of *tert*-butanol to α_{2u}-globulin compared to females* Females: no change in binding 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 F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Accumulation of hyaline droplets: **Male** Dose (mg/kg-d) — Hyaline droplet accumulation 0 — 0/10 230 — +[e] 490 — ++ 840 — ++ 1,520 — ++ 3,610 — 0/10 No information provided on females. No results from statistical tests reported.
Hard et al. (2011) Reanalysis of the slides in the NTP (1995) study	Males: Confirmed accumulation of hyaline droplets increased with increasing dose-levels in 13 week study above. No incidence data available. Females: not evaluated

Table 1-4. Additional kidney effects potentially relevant to mode of action in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results
Borghoff et al. (2001) F344 rat; 5/sex/treatment Analytical concentration:0, 250, 450, 1,750 ppm (0,771, 1,387 or 5,395mg/m^3) 6hr/d 10 days	Males: positive trend for accumulation of protein droplets ($p < 0.05$), significant increase in accumulation of α_{2u}-globulin at 5,395 mg/m^3 as compared to controls (no incidence data provided) Females: No positive staining for α_{2u}-globulin was observed in exposed female rats.

[a] The high-dose group had an increase in mortality.
[b] Linear mineralization not observed in female rats.
[c] Organs were not weighed in mice during the 2-year study.
[d] Standard & extended evaluation combined.
[e] + or ++ indicated an increased accumulation relative to controls, as reported by the authors; no additional incidence data and no results from statistical tests available.
* Statistically significant $p \leq 0.05$ as determined by the study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.

Table 1-5. Summary of data on the α_{2u}-globulin process in male rats exposed to *tert*-butanol

Criterion	Duration	Results	Reference
(1) hyaline droplets are increased in size and number	10 d	+	Borghoff et al. (2001)
	13 wk	(+)	NTP (1995)
	13 wk	–	NTP (1997)
	13 wk	(+)[a]	Hard et al. (2011)
(2) the protein in the hyaline droplets is α_{2u}-globulin	12 hr	+	Williams and Borghoff (2001)
	10 d	+	Borghoff et al. (2001)
(3) Several (but not necessarily all) additional steps in the pathological sequence are present in male rats, such as:			
(a) Single-cell necrosis	13 wk	–	NTP (1995)
	2 yr	–	NTP (1995)
	2 yr	–	Hard et al. (2011)
(b) exfoliation of epithelial cells into the tubular lumen	13 wk	–	NTP (1995)
	2 yr	–	NTP (1995)
	2 yr	–	Hard et al. (2011)
(c) granular casts	13 wk	–	NTP (1995)
	13 wk	(+)[a,c]	Hard et al. (2011)
	13 wk	–	NTP (1997)
	2 yr	–[b]	NTP (1995)
(d) linear mineralization	13 wk	–	NTP (1995)
	13 wk	–	NTP (1997)
	2 yr	+	NTP (1995)
	2 yr	(+)[a]	Hard et al. (2011)
(e) tubule hyperplasia	2 yr	+	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] Re-analysis of one control and one treated group from NTP (1995)
[b] Protein casts reported, not granular casts
[c] Precursors to granular casts reported

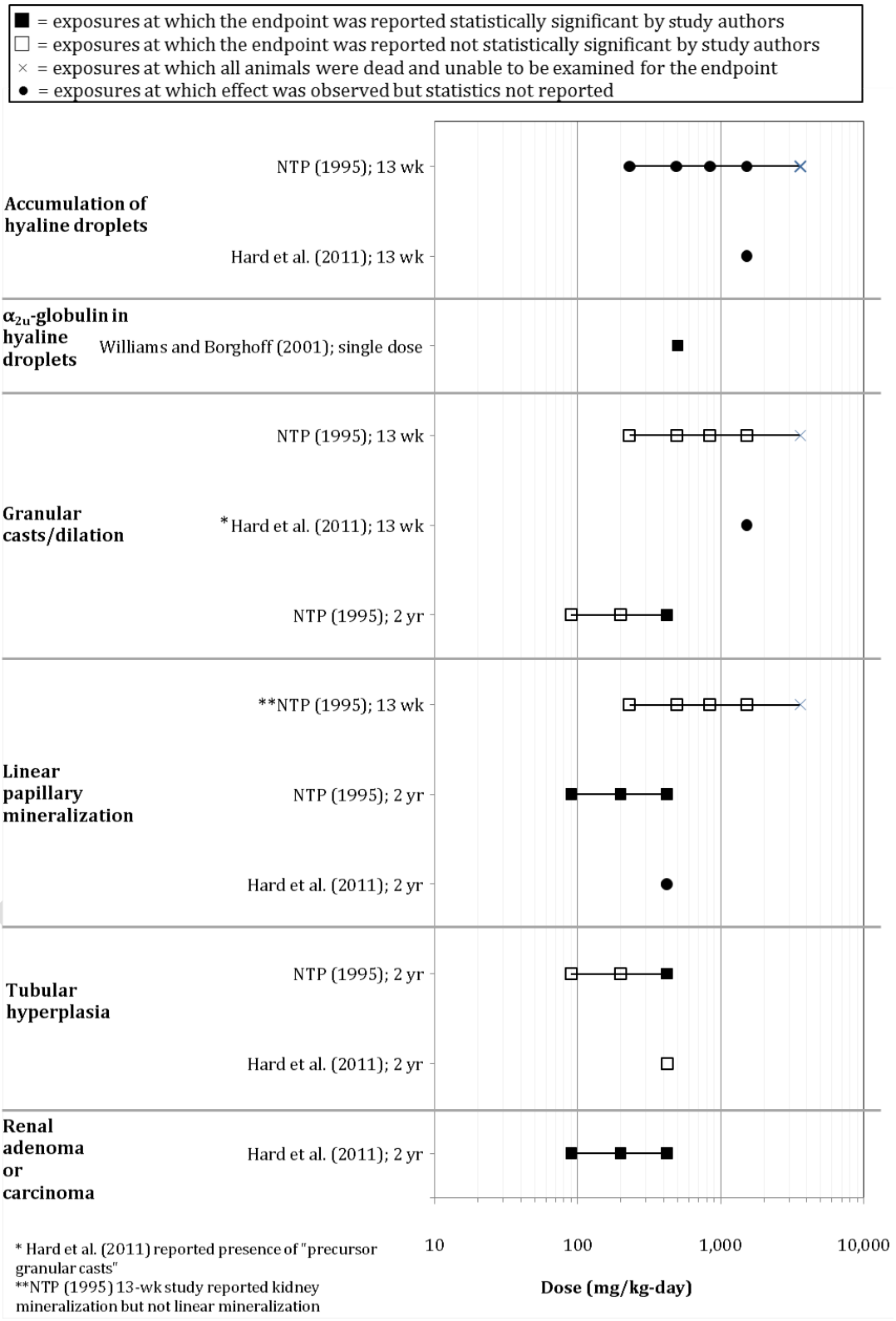


Figure 1-3. Exposure-response array for components of α_{2u}-globulin nephropathy and renal tumors in male rats after oral exposure to *tert*-butanol.

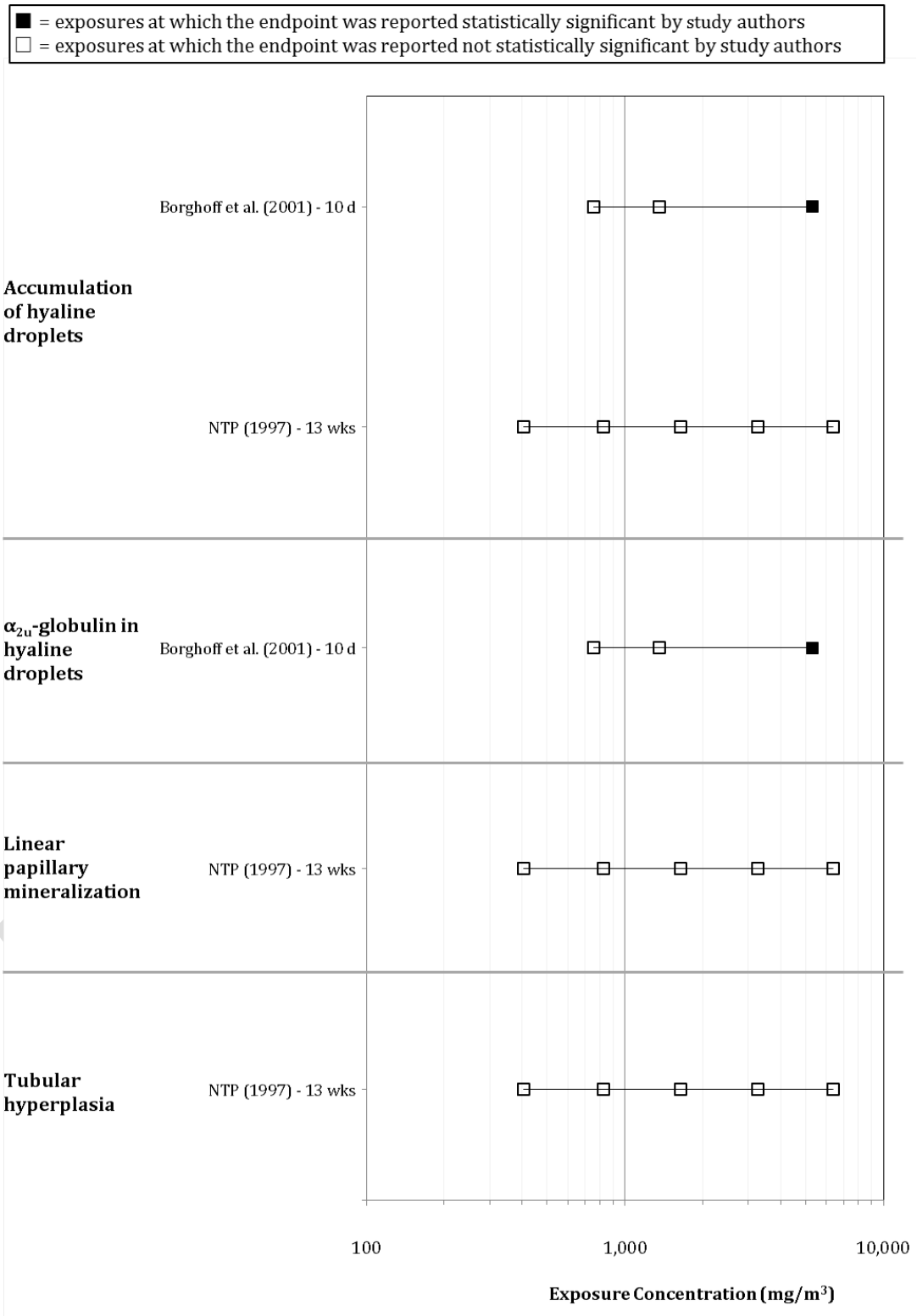


Figure 1-4. Exposure-response array for components of α_{2u}-globulin nephropathy and renal tumors in male rats after inhalation exposure to *tert*-butanol.

Table 1-6. Summary of additional data informing the contribution of the α_{2u}-globulin process on the renal tumor development in male rats exposed to *tert*-butanol

Type of data Description	Reference
(1) Hypothesis-testing data	
No data	
(2) Biochemical information	
Reversible, non-covalent binding of *tert*-butanol to α_{2u}-globulin.	Williams and Borghoff (2001)
(3) Sustained cell division in the proximal tubule of the male rat	
Hyperplasia at 2 yr reported in both male and female rats, attributed to CPN.	Hard et al. (2011)
No effect on proliferation at 13 wk	NTP (1997)
Increased proliferation at 13 wk based on PCNA assay	NTP (1995)
Increased proliferation of the P2 segment at 10 days based on BrdU labeling	Borghoff et al. (2001)
(4) Structure-activity relationships	
No data	
(5) Covalent binding to macromolecules	
No data	
(6) Genotoxicity	
Limited database to conclude *tert*-butanol is genotoxic or non-genotoxic	See Appendix B.3.
(7) Nephrotoxicity	
Increased tubular regeneration and intratubule protein cast formation at 2 yr in males and females, with effects in females occurring at lower dose.	NTP (1995)
Increased severity of CPN in male rats after 13 wk inhalation exposure	NTP (1997)
Increased CPN in male and female F344/N rats following drinking water exposure for 13 wk.	NTP (1995)
Increased CPN in male and female F344/N rats following drinking water exposure for 2 yr.	NTP (1995)
(8) Animal bioassay data in other species-, sex-combinations	
Two renal tubular adenocarcinomas (not statistically significant as compared to concurrent controls) reported in male mice following drinking water exposure for 2 yr. These tumors are very rare in mice.	NTP (1995)
(9) Other data	
Dose-response and temporal concordance (see Figures 1-3 and 1-4).	

Strength, consistency, specificity of association

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

The first criterion considered is whether hyaline droplets are increased in size and number in male rats. Protein droplet accumulation was statistically significantly increased in the kidneys of male rats exposed to 5,305 mg/m^3 *tert*-butanol for 6 hr/day for 10 days (Borghoff et al., 2001). Data from drinking water studies (NTP, 1995; Takahashi et al., 1993; Lindamood et al., 1992) demonstrated a statistically significant increase, except at the highest dose, in hyaline droplet formation and severity in the proximal tubule of male rats following oral exposure to *tert*-butanol for 13 weeks. Treated males had large hyaline droplets with crystal accumulation, but the controls had small droplets without crystals. NTP (1997) 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, and there was no difference between the controls and treatment groups.

The second criterion considered is whether the protein in the hyaline droplets in male rats is α_{2u}-globulin. Two studies measured α_{2u}-globulin immunoreactivity in the hyaline droplets of the renal proximal tubular epithelium (Borghoff et al., 2001; Williams and Borghoff, 2001). Borghoff et al. (2001) observed α_{2u}-globulin immunoreactivity present in the hyaline droplets in the male rats. No α_{2u}-globulin immunostaining was observed in the kidneys of the female rats. Williams and Borghoff (2001) found the content of α_{2u}-globulin statistically significantly elevated in 72% of the kidneys of male rats treated with *tert*-butanol compared with controls treated with corn oil.

The third criterion considered is whether several (but not necessarily all) additional steps in the pathological sequence are present in male rats. Several, but not all, of the subsequent histopathological lesions were observed in the available subchronic or chronic *tert*-butanol exposure studies. Linear mineralization was the lesion most consistently observed in male rats and was found to be statistically significantly increased in male rats after 2 years of oral exposure (NTP, 1995) (see Table 1-2). The 13-week study in rats (NTP, 1995) reported mineralization, but it was not characterized as linear mineralization. Additionally, although the inhalation study by NTP (1997) did not report linear mineralization at 13 weeks, this may be due to the lower internal dose as compared to the oral studies. Atypical tubule hyperplasia was statistically significantly increased at the highest dose following 2 years of oral exposure (NTP, 1995). Granular casts were increased at the 13 week time point (NTP, 1995), though statistical significance was not determined. The reanalysis of the 13-week data by Hard et al. (2011) concluded that the lesions were precursors to granular casts and not the casts themselves. Other studies did not observe granular casts at 13 weeks or 2 years (NTP, 1997, 1995). The formation of protein casts were observed, but these may be part of the pathology of CPN and are not thought to be related to α_{2u}-globulin nephropathy (NTP, 1995). Neither necrosis nor epithelial exfoliation was reported in any study.

In summary, the evidence supports the conclusion that *tert*-butanol causes increases in the size and number of hyaline droplets, and the accumulating protein in the hyaline droplets is α_{2u}-globulin. Additionally, several, but not all, of the additional steps in the pathological sequence

were observed, and not always consistently across studies. Therefore, the overall strength, consistency, and specificity of the association between *tert*-butanol and the hypothesized key events is moderate.

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

As summarized in Table 1-6, there are many potential sources of additional data that may inform the contribution of the α_{2u}-globulin process on renal tumor development. No hypothesis-testing data, structure-activity relationships, or covalent binding data were located, so these types of data are not discussed. Additional data related to dose-response concordance and temporal relationships are discussed in subsequent sections.

In terms of biochemical information, Williams and Borghoff (2001) report that *tert*-butanol reversibly and non-covalently binds to α_{2u}-globulin. This provides additional support to the evidence that the α_{2u}-process is occurring, but it does not inform the relative contribution to renal tumor development.

Sustained cell division in the proximal tubule of the male rat is consistent with, though not specific to, the α_{2u}-process. Proliferation of the proximal tubule was significantly increased in male rats after 10 days of inhalation exposure to *tert*-butanol at concentrations of 771–5,395 mg/m^3 (Borghoff et al., 2001) and after 13 weeks of oral exposure to 1520 mg/kg-day (NTP, 1995; Takahashi et al., 1993; Lindamood et al., 1992), but not after 13 week inhalation exposure up to 6368 mg/m^3 (NTP, 1997). Therefore, it is unclear the extent to which increased cell division is sustained. While hyperplasia was reported in chronic studies (Hard et al., 2011), it was observed in both male and female rats, and attributed to CPN.

There are a limited number of studies available to assess the genotoxic potential of *tert*-butanol (see Appendix B.3 in Supplemental Information for further details). *tert*-Butanol was generally negative in a variety of genotoxicity assays and cell systems including *Salmonella typhimurium*, *Escherichia coli* and *Neurospora crassa* (Mcgregor et al., 2005; Zeiger et al., 1987; Dickey et al., 1949). Studies also demonstrate negative results for gene mutations, sister chromatid exchanges, micronucleus formation, and chromosomal aberrations (NTP, 1995; McGregor et al., 1988). However, DNA adducts were found in male Kunming mice (Yuan et al., 2007), and DNA damage was observed in human HL-60 leukemia cells (Tang et al., 1997). In another study by Sgambato et al. (2009), an initial increase in DNA damage was observed as measured by nuclear fragmentation, but the damage declined drastically following 4 hours of exposure and disappeared entirely after 12 hours of exposure to *tert*-butanol.

In terms of nephrotoxicity, a number of renal effects have been reported in female rats and/or in mice. Kidney transitional epithelial hyperplasia and inflammation were significantly increased in both male and female F344 rats exposed for 2 years via oral exposure. F344 rats exposed to *tert*-butanol at dose ranges of 230–1520 mg/kg-day and 850–3,620 mg/kg-day in males and females, respectively, exhibited a statistically significant increase in the incidence of nephropathy compared with controls after 13 weeks of exposure (NTP, 1995). Nephropathy

severity was also significantly increased at 420 mg/kg-day in males and 180-650 mg/kg-day in females after 2 years of exposure (NTP, 1995). Average severity of chronic nephropathy was minimal to mild in males after a 13-week inhalation exposure (NTP, 1997). Female rats also had lesions associated with nephropathy (NTP, 1995), but none of the lesions were similar to those observed in the male rat that are associated with α_{2u}-globulin nephropathy.

With respect to renal tumors, no statistically significant increases in renal tumors were reported in *tert*-butanol-exposed female rats or mice compared with concurrent controls. Two renal tubular adenocarcinomas were reported in male mice following drinking water exposure for 2 years (NTP, 1995) (one each in the low and high dose groups). Although such tumors are very rare in mice, with historical control incidences of 2/1351 (0.15%) in feeding studies and 4/1093 (0.37%) in chamber studies (Haseman et al., 1998), these data are not sufficient to indicate that the kidney tumors observed in mice exposed to *tert*-butanol are treatment-related.

Overall, the strength, consistency, and specificity of the data supporting a *tert*-butanol-induced α_{2u}-globulin process as the sole actor for renal effects in male rats is weak to moderate.

Dose-response concordance

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

As shown in Figure 1-3 and Figure 1-4, the dose-response concordance among hypothesized key events is mixed.

Borghoff et al. (2001) exposed male and female F344 rats to *tert*-butanol at concentrations of 758, 1,364, or 5,305 mg/m^3 for 6 hr/day for 10 days to assess the role of α_{2u}-globulin nephropathy and renal cell proliferation. Significant tubular proliferation in males was observed at all exposure levels, but accumulation of α_{2u}-globulin-positive hyaline droplets was increased only at the highest dose (Borghoff et al., 2001). These data suggest that cell proliferation may be related to the α_{2u}-globulin process only at the highest exposure concentration.

The dose-response relationships observed after 13 weeks and 2-years were also only moderately concordant. Data from a drinking water study (NTP, 1995; Takahashi et al., 1993; Lindamood et al., 1992) demonstrated hyaline droplet formation in the proximal tubule of male rats at all tested doses (except at the highest dose where all rats died during weeks 5-12) following oral exposure to *tert*-butanol for 13 weeks. PWG reevaluation by Hard et al. (2011) reported observing precursors to granular casts at the only dose level evaluated (1,520 mg/kg-day). Spontaneous mineralization was observed, but the linear mineralization characteristic of this MOA was not observed at any dose. At the 2-year timepoint, linear mineralization was observed at all exposure levels from 90-420 mg/kg-day, and renal tubular hyperplasia was observed at the highest dose of 420 mg/kg-day (NTP, 1995), consistent with the expected dose-response relationship. Notably, however, granular casts were not observed.

Overall, the dose-response concordance of the association between *tert*-butanol and the hypothesized key events is moderate.

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

Dose-response concordance between the hypothesized key events and the occurrence of renal tumors can inform whether carcinogenesis is solely due to the α_{2u}-globulin process. Male F344 rats exhibited an increased incidence of renal tubule adenomas and combined renal tubule adenoma or carcinoma in a 2-year oral bioassay (Hard et al., 2011; NTP, 1995). Increased tumors were observed at 90, 200, and 420 mg/kg-day. Although some effects related to α_{2u}-globulin nephropathy, including hyaline droplets and linear mineralization, were observed at all doses, tubule hyperplasia was not observed at doses lower than 420 mg/kg-day in any study and only precursor granular casts were observed at a much higher dose of 1,520 mg/kg-day. Moreover, the middle dose of *tert*-butanol induced the greatest incidence of tumors, so increasing the dose from 200 to 420 mg/kg-day led to additional markers of α_{2u}-globulin nephropathy in the form of tubule hyperplasia, but without any increase in tumor burden. Thus, *tert*-butanol induced tumors at lower doses than for other precursor effects such as hyperplasia and granular casts, suggesting a weak dose response concordance with the incidence of tumors.

Therefore, on the basis of weak dose-response concordance, the data suggest that the observed tumors are not solely due to α_{2u}-globulin and that other processes are primarily responsible for tumors.

Temporal relationship

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

As shown in Table 1-2 and Table 1-4, hyaline droplets and α_{2u}-globulin accumulation were observed after a single dose or 10-day exposure; precursors to granular casts were observed at 13 weeks; and tubular hyperplasia was observed at 2 years. The observations are consistent with the expected temporal relationship (Hard et al., 2011; Borghoff et al., 2001; Williams and Borghoff, 2001; NTP, 1995). However, the absence of other key events such as necrosis, exfoliation, and granular casts in most other studies at the anticipated time points weaken the case for α_{2u}-globulin MOA. Additionally, the NTP's 13-week study in rat reported kidney mineralization, but it was not characterized as linear mineralization.

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

This question cannot be answered from data on temporality.

Biological plausibility and coherence

Both U.S. EPA and IARC have accepted the general biological plausibility and coherence of role of α_{2u}-globulin-mediated nephropathy in renal tumor induction (Swenberg and Lehman-McKeeman, 1999; U.S. EPA, 1991a), and those rationales will not be repeated here.

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

2007). For instance, dose-response concordance was not observed for several endpoints such as linear mineralization, tubular hyperplasia, granular casts, and hyaline droplets following exposure to α_{2u}-globulin-inducing chemicals such as d-limonene, decalin, propylene glycol mono-t-butyl ether, and Stoddard solvent IIC (SS IIC). Although some of these chemicals induced histopathological lesions that exhibited a dose response, all of them failed to induce a dose-response trend for at least one of the endpoints in the sequence. Furthermore, no endpoint in the pathological sequence was predictive for tumor incidence. Tumor incidence did not exhibit a dose response following either d-limonene or decalin exposure. Finally, tumor incidence was not predicted by the severity of a particular effect in the α_{2u}-globulin sequence as demonstrated by SS IIC which induced some of the most severe nephropathy precursors relative to the other chemicals but did not significantly increase kidney tumors (Doi et al., 2007). Thus, these analyses suggest that another MOA may be operative for inducing tumors.

Moreover, renal tumors were not observed following exposure to ETBE, which is rapidly metabolized to *tert*-butanol. Specifically, Suzuki et al. (2012) and Saito et al. (2013) reported no increase in renal tumors in both sexes of Fischer 344 rats following 2-year oral or inhalation exposures to ETBE at doses that yield similar internal concentrations (based on PBPK modeling) of *tert*-butanol compared with the concentrations of the *tert*-butanol bioassays. After 13 weeks of exposure to *tert*-butanol or ETBE, hyaline droplets were increased in a dose-response manner. ETBE exposure increased hyaline droplets at lower internal concentrations of *tert*-butanol than by direct *tert*-butanol administration. Similar to hyaline droplets, linear mineralization was increased at an internal *tert*-butanol concentration approximately tenfold lower following ETBE exposure than a *tert*-butanol exposure. By contrast, tubule hyperplasia and renal tumors were both observed following a 2-year exposure to *tert*-butanol but not following ETBE exposure. Renal tumors and tubule hyperplasia were not observed following any ETBE exposure despite achieving similar blood concentrations of *tert*-butanol as the NTP (1995) study. The failure of internal *tert*-butanol concentrations to induce histopathological lesions early in the α_{2u}-globulin pathological sequence at blood levels that later induced hyperplasia and tumors suggests a lack of coherence across the two data sets.

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

Is the hypothesized MOA sufficiently supported in test animals?

This conclusion is divided into two sub-questions: whether the α_{2u}-globulin process is occurring in male rats, and whether it is the sole contributor to renal effects in male rats.

With respect to the first question, *tert*-butanol induced increases in α_{2u}-globulin deposition and hyaline droplet accumulation, and several of the subsequent steps in the pathological sequence were observed. These data provide sufficient evidence that the α_{2u}-globulin process can be operating given sufficient *tert*-butanol exposure.

With respect to the second question, male rats are more sensitive to the kidney effects of *tert*-butanol, and the available data indicate that male rats accumulate α_{2u}-globulin in the kidney, which is a specific MOA for male rats. Many of the steps in the pathological sequence of lesions related to α_{2u}-globulin-associated nephropathy were observed exclusively in male rats but not in female rats, or mice of either sex, and renal tumors occurred only in male rats. However, there is insufficient evidence to support a conclusion that α_{2u}-globulin nephropathy is the sole or primary contributor to renal tumor development. Given the inconsistencies and limitations of the genotoxicity database, the effect of *tert*-butanol with respect to genotoxicity cannot be ruled out. Additionally, *tert*-butanol induced tumors at lower doses than other precursor effects such as hyperplasia and granular casts, with no further increase in tumor incidence coinciding with the additional markers of α_{2u}-globulin nephropathy. Thus, renal tumors observed in male rats are unlikely to be confounded by the presence of α_{2u}-globulin nephropathy. Therefore, on the basis of a weak dose-response concordance, the data support a conclusion that processes other than α_{2u}-globulin nephropathy are likely responsible for renal tumor development.

Is the hypothesized MOA relevant to humans?

Based on the conclusion that processes other than α_{2u}-globulin nephropathy are likely responsible for renal tumor development induced by *tert*-butanol, U.S. EPA (1991a) states that the following conclusion will be made:

- If renal tumors in male rats are not linked to the α_{2u}-globulin process, then (U.S. EPA, 1991a) states that such tumors are an appropriate endpoint for human hazard identification and are considered, along with other appropriate endpoints, for quantitative risk estimation.

Therefore, kidney tumors are relevant to humans for purposes of hazard identification and dose-response assessment. Because female rats and both sexes of mice do not have α_{2u}-globulin present, kidney effects in these animals are considered relevant to humans for both hazard identification and dose-response.

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

This question is not applicable.

Alternative MOA hypotheses with inadequate data for analysis

Other nephrotoxic responses, such as exacerbation of CPN, inflammation, transitional epithelial hyperplasia, and increased kidney weight, are observed in rats and/or mice, suggesting other possible processes are operative. It has been proposed that enhanced chronic progressive nephropathy (CPN) is a mode of action for chemically-induced kidney tumors in male rats and that

renal tubule tumors induced by chemicals that concomitantly exacerbate CPN are not relevant to humans (Hard and Khan, 2004).

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

Increased severity of CPN occurred in both male and female rats as a result of *tert*-butanol exposure. Some of the observed renal lesions in male rats following exposure to *tert*-butanol are effects commonly associated with CPN. Hard et al. (2011) concluded that the observation of transitional epithelial hyperplasia in the 2-year drinking study conducted by NTP (1995) was associated with CPN, and not a direct effect of *tert*-butanol exposure. However, there was a strong, statistically-significant, treatment-related, dose-response relationship between chronic *tert*-butanol exposure and increased incidence of transitional epithelial hyperplasia in both male and female rats in the NTP (1995) study. The severity of CPN also increased with *tert*-butanol exposure, although the dose-response relationship in males was very weak (only a 10% increase in mean severity at the highest dose). The very different dose-response relationships argue against a close association. Moreover, even if transitional epithelial hyperplasia were associated with CPN, there is no evidence to support that the effect is independent of *tert*-butanol treatment, given the robust dose-response relationships. Therefore, the data are insufficient to dismiss transitional epithelial hyperplasia as causally related to *tert*-butanol exposure.

Additionally, there have been a few research groups who have discussed the role of CPN and α_{2u}-globulin accumulation on the renal tumors observed in male rats exposed to *tert*-butanol. Cruzan et al. (2007) concluded that α_{2u}-globulin, exacerbation of CPN, or a combination of both were the MOAs for the kidney tumors in males. Hard et al. (2011) also concluded that both α_{2u}-globulin-induced nephropathy and exacerbated CPN were MOAs for the kidney tumors observed in the male rats in the 2-year drinking study conducted by NTP (1995). However, the underlying mechanisms regulating CPN and its exacerbation are not well understood, and to date, there is no scientific consensus on the relevance of CPN in rats to human health hazard (Melnick et al., 2012; Hard et al., 2009). Moreover, no key events for the exacerbation of CPN have been identified, so no MOA analysis can be performed under the EPA Cancer Guidelines MOA framework (U.S. EPA, 2005a). Therefore, kidney effects from *tert*-butanol exposure associated with CPN are considered relevant to humans.

Summary of kidney toxicity

Kidney toxicity was consistently observed after oral exposure in two strains of rats and in one strain of mice and in both sexes. Absolute and relative kidney weights also were increased in male and female rats in both the 13-week and 2-year studies. In male and female rats, histopathological examination of the kidneys indicated kidney lesions exhibiting a dose-response trend, increased incidence of nephropathy after 13 weeks and 2 years, and increased transitional epithelium hyperplasia and suppurative inflammation (females only) after 2 years. In mice, the only kidney effect observed was an increase in kidney weight (absolute and/or relative) in both sexes of mice in the 13-week study. Organs were not weighed in the 2-year mouse study, so no determination can be made. Furthermore, there were no treatment-related histopathological lesions in the kidneys of mice at 13 weeks or 2 years.

Male rats are more sensitive to the kidney effects of *tert*-butanol, and the available data indicate that male rats accumulate α_{2u}-globulin in the kidney, which is a specific MOA for male rats. MOA analysis determined that the renal tumors observed in male rats are mediated by other processes besides α_{2u}-globulin. Therefore, in the absence of a known MOA, EPA considers the male and female kidney effects observed in experimental animals to be relevant to assessing human health hazard.

EPA identified kidney effects as a human hazard of *tert*-butanol exposure. Data on kidney tumors associated with *tert*-butanol exposure are discussed as part of the overall weight of evidence for carcinogenicity in Section 1.2.2.

1.1.2. Thyroid Effects

Synthesis of Effects in Thyroid

This section reviews the studies that investigated whether exposure to *tert*-butanol can cause thyroid effects in humans or animals. The database examining thyroid effects following *tert*-butanol exposure contains no human data and two chronic studies (one in rats and one in mice). Studies employing short term and acute exposures that examined thyroid effects are not included in the evidence tables; however, they are discussed in the text if they provide data to support mode of action or hazard identification. No methodological concerns were identified that would lead one or more studies to be considered less informative for assessing human health hazard.

A 2-year inhalation study is not available. Thyroid effects were not observed in studies in rats (NTP, 1995). Thyroid toxicity was observed in mice of both sexes after 2 years of oral exposure via drinking water (NTP, 1995). Follicular cell hyperplasia, as well as follicular cell adenomas, was present in both male and female mice. The evidence was stronger in females due to the dose-related increase in follicular cell hyperplasia reaching statistical significance in the highest two doses, and the presence of a statistically significant increase in follicular cell adenomas in the high-dose group. There was also a statistically significant increase in follicular cell hyperplasia at all

doses in male mice, but only a marginal increase in follicular cell adenomas in the mid-dose group. One high-dose male mouse developed a follicular cell carcinoma. The lower tumor incidence in males may be due to the increased mortality seen in the high-dose group. NTP (1995) noted that thyroid follicular cell tumorigenesis follows a progression from hyperplasia to adenoma and carcinoma, suggesting that hyperplasia is a preneoplastic lesion in the thyroid.

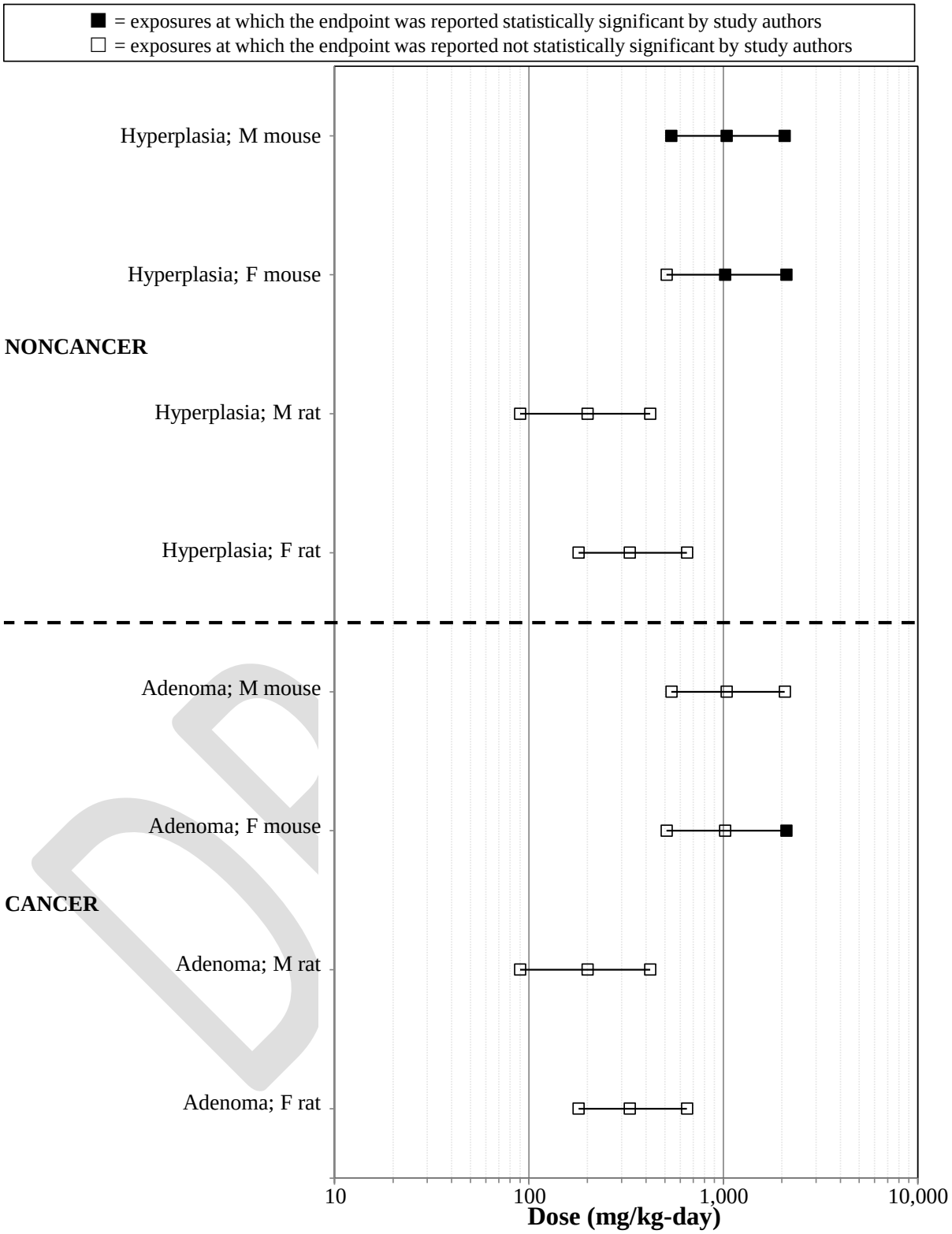
Table 1-7. Evidence pertaining to thyroid effects in animals following oral exposure to *tert*-butanol

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	0/50	650	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	18/57* (2.1)	2,110	47/59* (2.2)
Tumors				
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]			
	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	0/50	0/50	
	Female			
	0	1/50	1/50	
	180	0/50	0/50	
	330	1/50	1/50	
	650	0/50	0/50	

Table 1-7. Evidence pertaining to thyroid effects in animals following oral exposure to *tert*-butanol (*continued*)

Reference and study design	Results					
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	Mortality-adjusted rate (%)	Follicular cell carcinoma or adenoma	Mortality-adjusted rate (%)	Follicular cell carcinoma[c]
	Male					
	0	1/60	3.6	1/60	3.6	0/60
	540	0/59	0.0	0/59	0.0	0/59
	1,040	4/59	10.1	4/59	10.1	0/59
	2,070	1/57	5.9	2/57	8.7	1/57
	Female					
	0	2/58	5.6	2/58	5.6	0/58
	510	3/60	8.6	3/60	8.6	0/60
	1,020	2/59	4.9	2/59	4.9	0/59
	2,110	9/59*	19.6	9/59*	19.6	0/59

[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.
* Statistically significant $p \leq 0.05$ as determined by the study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.



Source: NTP (1995)

Figure 1-5. Exposure-response array of thyroid follicular cell effects following chronic oral exposure to *tert*-butanol.

Mode of Action Analysis—Thyroid Effects

There are inadequate data to determine the MOA for *tert*-butanol-induced thyroid follicular cell lesions. The mechanism of formation of these lesions resulting from *tert*-butanol exposure has not been specifically studied; however, Blanck et al. (2010) conducted a short-term study examining the hepatic and thyroid effects of *tert*-butanol exposure to provide additional data on the thyroid tumors observed in the chronic NTP (1995) study. *tert*-Butanol did not have any effect on liver weight when compared to the control group, but the livers were visibly enlarged, in some cases accompanied by centrilobular hepatocellular hypertrophy, in some treated groups. There were no treatment-related histological alterations in the thyroid in *tert*-butanol treated mice. Only a slight statistically nonsignificant increase in thyroid stimulating hormone (TSH) was observed after 3 days of exposure, but both thyroxine (T_4) and triiodothyronine (T_3) levels were decreased. Sustained increases in TSH, resulting in sustained thyroid follicular cell proliferation, may eventually result in progression of hyperplasia to adenoma and carcinoma (U.S. EPA, 1998a). Based on alterations in hepatic phase I and phase II enzyme activities and gene expression, the data from Blanck et al. (2010) suggest a possible role for increased thyroid hormone clearance in the liver in *tert*-butanol-induced thyroid tumors. The available support for this hypothesis, however, is weak. In particular, Blanck et al. (2010) did not find any significant changes in TSH levels, though the study duration was short (≤14 days), and there are no data regarding thyroid cell proliferation after exposure to *tert*-butanol.

Summary of thyroid toxicity

EPA identified thyroid effects as a potential human hazard of *tert*-butanol exposure. The thyroid endpoints reported following chronic exposure to *tert*-butanol include follicular cell hyperplasia, follicular cell adenoma, and follicular cell carcinoma. Together with the evidence of significantly increased incidence of thyroid follicular cell adenomas in high-dose females, these observations support the finding that the increased hyperplasia in male mice is a preneoplastic effect rather than an adaptive response. There is no conclusive MOA for the development of thyroid follicular cell adenomas, although there is some evidence supporting greater clearance of thyroid hormones by the liver causing continual secretion of TSH by the pituitary leading to follicular cell hyperplasia and tumors. Data on thyroid tumors associated with *tert*-butanol exposure are discussed further as part of the overall weight of evidence for carcinogenicity in Section 1.2.2.

1.1.3. Reproductive and Developmental Effects

Synthesis of reproductive and developmental toxicity

This section reviews the studies that investigated whether exposure to *tert*-butanol can cause reproductive and developmental effects in humans or animals. This section contains information on reproductive effects, systemic developmental effects, as well as neurodevelopmental effects following *tert*-butanol exposure. The database examining reproductive

or developmental effects following *tert*-butanol exposure contains no human data and 7 studies performed in rats and mice. Three studies evaluating reproductive effects included changes in reproductive organs (one one-generation reproductive study and two subchronic studies). In addition, there was one two-year oral study in rats and mice that evaluated reproductive histopathology and did not find any treatment-related effects. The studies selected for discussion below exposed animals via oral gavage, drinking water, and inhalation for ≥63 days. The collection of reproductive studies on *tert*-butanol is limited by the absence of any two-generation reproductive oral or inhalation studies. Four studies evaluated developmental effects (three developmental studies and one one-generation reproductive study). As mentioned in the Study Selection, the studies selected for discussion below exposed animals to *tert*-butanol via inhalation, gavage, and drinking water. No methodological concerns were identified that would lead one or more studies to be considered less informative for assessing human health hazard, but the Faulkner et al. (1989) study did not provide results in the dam that could be used to adequately determine if fetal effects occurred due to maternal toxicity. Three studies evaluated neurodevelopmental effects following *tert*-butanol exposure in rats and mice. These studies selected for discussion below exposed animals via liquid diet and inhalation. The collection of neurodevelopmental studies on *tert*-butanol is limited in that all studies were conducted prior to Developmental Toxicity Guidelines being available from the U.S. EPA (1991b) and OECD; as such, there are study design considerations for each of the studies. Daniel and Evans (1982) had a small number of animals per treatment group, presentation of results provided limited use of the data with no comparisons to controls, and there was no long term neurodevelopmental testing. Nelson et al. (1991) evaluated neurodevelopmental effects after either paternal or maternal exposure. Although the study authors used two different exposure concentrations, the exposures were not run concurrently nor was there information provided on exposure methods to indicate the studies were conducted similarly.

Reproductive effects

Reproductive endpoints, such as sex organ weights, estrous cycle length, and sperm effects were examined following either oral or inhalation exposure in three subchronic studies (Lyondell Chemical Co., 2004; NTP, 1997, 1995)(Table 1-8;Figure 1-6;Figure 1-7). The only reproductive effect noted was an increase in estrous cycle length. Estrous cycle length was increased (28% increase, $p < 0.01$) in female mice orally exposed to 11,620 mg/kg-day. No significant changes in estrous cycle length were observed following oral exposure in rats, or inhalation exposure in mice or rats.

Table 1-8. 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 F0 females: 4 weeks prior to mating through PND21	F0 reproductive effects No 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 F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	No significant effect on weights of male reproductive organs or sperm observed
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 F: 0, 500, 820, 1,660, 6,430, 11,620[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 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 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)

Table 1-8. Evidence pertaining to reproductive effects in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results
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 males: 9 weeks beginning 4 weeks prior to mating 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) 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	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) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d 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*%
NTP (1997) F344/N rat; 10/sex/treatment 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 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)

* Statistically significant $p \leq 0.05$ as determined by the study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

Developmental effects

Data from three developmental studies (two oral, one inhalation) suggest that fetal effects are generally observed at doses that cause toxicity in the dams as measured by clinical signs (e.g., decreased body weight gain, and/or food consumption) (Table 1-9; Figure 1-6; Figure 1-7).

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 PND 2–10; however, data suggest that the effect may be due to maternal behavior or nutritional status. In addition, one study reported increased incidence of variations of the skull or sternebrae in two mouse strains, but the difference was not statistically significant (Faulkner et al., 1989). Similar developmental effects were also observed after whole-body inhalation exposure in Sprague-Dawley rats for 7 hours/day on GDs 1–19 (Nelson et al., 1989). Fetal effects included concentration-related reductions in body weight in male and female fetuses and higher incidence of skeletal variations when analyzed on the basis of individual fetuses (but not on a per litter basis). In contrast to the oral exposure studies in mice and rats, however, there was no effect on measures of fetal loss.

Table 1-9. Evidence pertaining to developmental 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, 1.0%, w/v) 0 (isocaloric amounts of maltose/dextrin), 3,324, 4,879, 6,677 mg/kg-d GD 6–20	No statistical analysis was conducted on any of these data **Maternal** Percent change compared to control: Dose (mg/kg-d) / Food consumption (mean g/animal/day) / Body weight gain / Number of litters (% pregnant dams) 0 / 0 / 0 / 11 (77%) 3,324 / +2 / −3 / 12 (80%) 4,879 / −3 / −19 / 8 (53%) 6,677 / −4 / −20 / 7 (47%) 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: Dose (mg/kg-d) / Number of neonates/litter / Fetal body weight on PND 2 0 / 0 / 0 3,324 / −1 / −7 4,879 / −29 / −19 6,677 / −49 / −38 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)

Table 1-9. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results
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	Maternal results not reported. **Fetal** Percent change compared to control: / Incidence: Dose (mg/kg-d) / Live fetuses/litter / Fetal weight / Sternebral variations / Skull variations 0 / 0 / 0 / 4/28 / 1/28 1,556 / −41* / −4 / 7/30 / 3/30 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) and resorptions per litter (+118%) increased ($p < 0.05$)
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	Maternal results not reported. **Fetal** Percent change compared to control: / Incidence: Dose (mg/kg-d) / Live fetuses/litter / Fetal weight / Sternal variations / Skull variations 0 / 0 / 0 / 5/21 / 1/21 1,556 / −58%* / −4 / 9/16 / 7/16 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) and resorptions per litter (+428%) increased ($p < 0.05$)

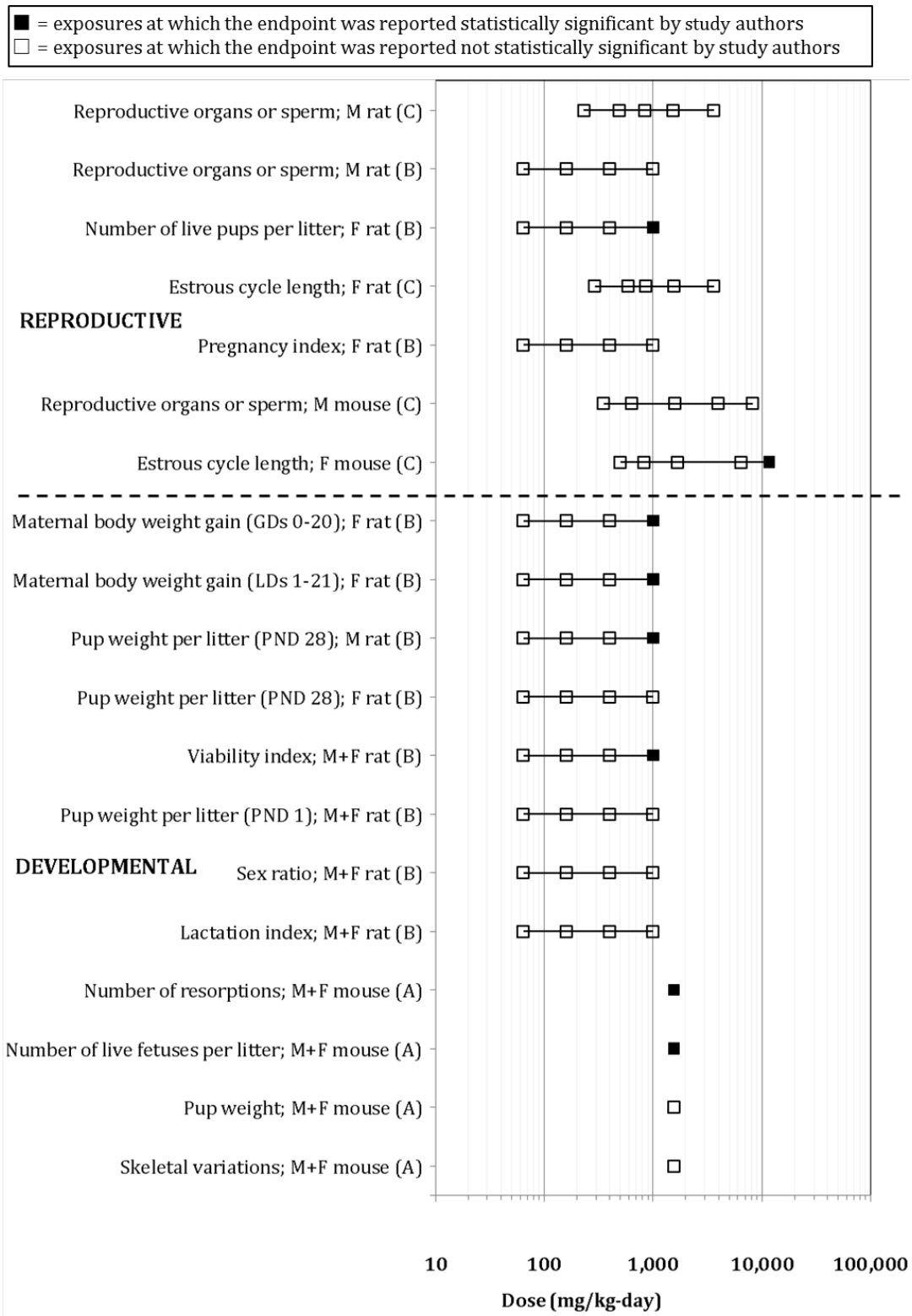
Table 1-9. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results
Lyondell Chemical Co. (2004) OECD guideline 421 study: 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 1000 or 400 mg/kg/d showed CNS effects (e.g., ataxia, lethargy) which became 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
Nelson et al. (1989) Sprague-Dawley rat; 15 pregnant dams/treatment	**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**

Table 1-9. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol (*continued*)

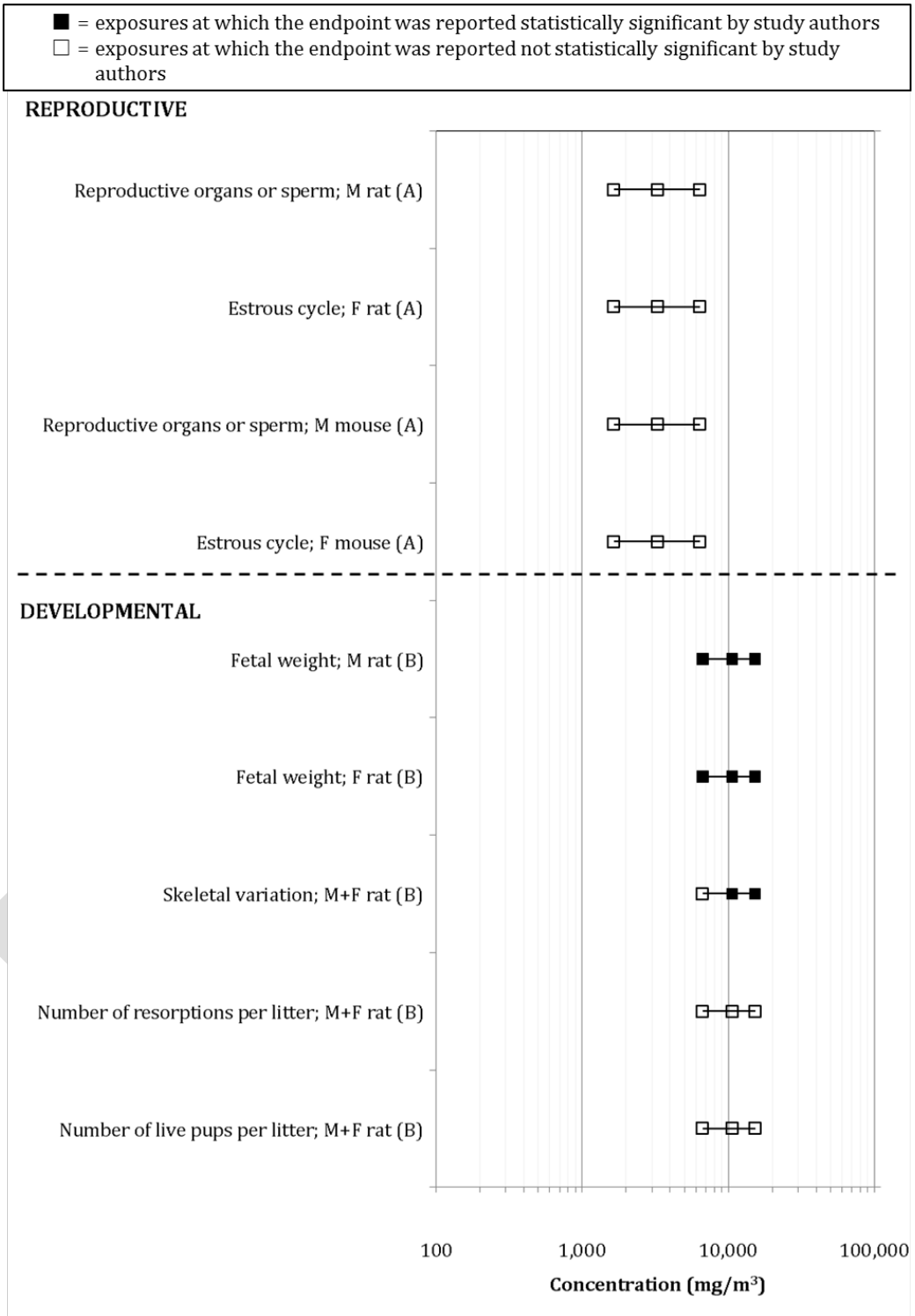
Reference and study design	Results
Analytical concentration: 0, 2,200, 3,510, 5,030 ppm (0, 6,669, 10,640, 15,248 mg/m^3), (dynamic whole body chamber) 7 hr/d GD 1–19 Generation method, analytical concentration and method were reported	Percent change compared to control: Dose (mg/m^3) / Number of live fetuses/litter / Resorptions per litter 0 / 0 / 0 6,669 / 0 / +9 10,640 / +15 / −18 15,248 / +8 / 0 Percent change compared to control: / Incidence: Dose (mg/m^3) / Fetal weight (males) / Fetal weight (females) / Skeletal variation by litter / Skeletal variation by fetus 0 / 0 / 0 / 10/15 / 18/96 6,669 / −9* / −9* / 14/17 / 35/104 10,640 / −12* / −13* / 14/14 / 53/103* 15,248 / −32* / −31* / 12/12 / 76/83* 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.

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



Sources: (A) Faulkner et al. (1989); (B) Lyondell Chemical Co. (2004); (C) NTP (1995)

Figure 1-6. Exposure-response array of reproductive and developmental effects following oral exposure to *tert*-butanol.



Sources: (A) NTP (1997); (B) Nelson et al. (1989)

Figure 1-7. Exposure-response array of reproductive and developmental effects following inhalation exposure to *tert*-butanol.

Neurodevelopmental Effects

In addition to the developmental effects noted above, neurodevelopmental effects also have been observed. This includes changes in rotarod performance following oral or inhalation exposures, as well as decreases in open field behavior and cliff avoidance following oral exposure, and reduced time hanging on wire after inhalation exposure during gestation (Table 1-10; Figure 1-6; Figure 1-7).

Rotarod performance

Looking across studies, not all the findings were consistent. While 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

In addition to behavioral effects, one study evaluated biochemical or physiological changes in the brain of offspring exposed during gestation or early in the postnatal period. Nelson et al. (1991) found statistically significant changes in neurochemical measurements in the brain in offspring of dams exposed via inhalation during gestation; however, the two concentrations tested were not run concurrently, and very little data were provided.

Physiological and psychomotor development

Data also suggest that neurodevelopmental effects were not solely due to in utero exposure (Daniel and Evans, 1982). Daniel and Evans (1982) cross-fostered half of the mouse pups born to treated mother 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 with control dams performed significantly better than those maintained with treated dams (Table 1-10) (Daniel and Evans, 1982). Results were only presented in figures and were not compared with controls.

Table 1-10. Evidence pertaining to neurodevelopmental effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Daniel and Evans (1982) Liquid diet (0, 0.5, 0.75, or 1.0%, w/v); GD6–20; Swiss Webster (Cox) mouse; 15 pregnant dams/treatment; 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 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 Analytical concentration: 0, 6,000, or 12,000 mg/m^3; (dynamic whole body chamber) 7 hr/d GD 1–19 Generation method, analytical concentration and method were reported	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) 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

Table 1-10. Evidence pertaining to neurodevelopmental effects in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results
Nelson et al. (1991) adult male Sprague-Dawley rats (18/treatment) mated to untreated females Analytical concentration: 0, 6,000, or 12,000 mg/m^3; (dynamic whole body chamber) 7 hr/d for 6 wk Generation method, analytical concentration and method were reported	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.
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 or developmental effects, including neurodevelopmental effects.

Summary of Reproductive and Developmental Toxicity

EPA concluded that the evidence does not support reproductive effects as a potential human hazard of *tert*-butanol exposure. There are no two-generation reproductive studies available by oral or inhalation exposure. Two oral exposure studies (Lyondell Chemical Co., 2004; NTP, 1995) and one subchronic inhalation study (NTP, 1997) are available. Overall, reproductive effects observed due to exposure to *tert*-butanol were limited to altered length of estrous cycle (NTP, 1995), but there is no available information to infer how this effect may influence reproductive ability.

EPA identified suggestive evidence of developmental effects as a potential human hazard of *tert*-butanol exposure. Exposure during gestation resulted in increased fetal loss, decreased fetal body weight, and possible increases in skeletal variations in exposed offspring or pups, although effects were not always consistent across exposure routes (oral and inhalation). Dams had decreased body weight and/or body weight gains, decreased food consumption, and/or clinical signs of intoxication at the same doses that *tert*-butanol caused fetal effects. Neurodevelopmental

effects including decreased brain weight, changes in brain biochemistry, and changes in behavioral performances have also been observed. Each of the neurodevelopmental studies, however, had limitations in the study design and/or reporting. In addition, results from the neurodevelopmental studies were not always consistent between studies or across dose.

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

Synthesis of Carcinogenicity Data (Other than in the Kidney or Thyroid)

This section reviews the studies that investigated whether exposure to *tert*-butanol can cause cancers (other than in the kidney or thyroid) in humans or animals. The database examining carcinogenicity following *tert*-butanol exposure contains no human data and two chronic studies, one in rats and one in mice. As mentioned in the Study Selection, the studies providing data on carcinogenicity exposed animals via drinking water for ≥30 days. Shorter duration studies do not generally evaluate carcinogenicity, but any shorter duration studies that examined carcinogenicity are discussed in the text if they provide data to support mode of action or hazard identification. No methodological concerns were identified that would lead one or more studies to be considered less informative for assessing human health hazard.

Kidney and thyroid tumors are presented above with the specific organ hazard identification. No other treatment-related changed in tumors in other organs were noted in the 2-year oral rat or mouse studies conducted by NTP (1995), which evaluated a comprehensive set of tissues/organs. There is no 2-year inhalation study.

Mechanistic Evidence

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

Summary of Carcinogenicity Evidence

There are limited data available on the carcinogenicity of *tert*-butanol. There are 2-year oral studies in one strain of rats and one strain of mice, but no 2-year inhalation studies. EPA identified suggestive evidence of kidney and thyroid tumors as a potential human hazard.

1.1.5. Other Toxicological Effects

Synthesis of Other Toxicity Data

The database for effects other than kidney, thyroid, reproductive, developmental (including neurodevelopmental) and cancer contain only 14 rodent studies. As previously mentioned in the Study Selection, all selected studies employed inhalation, oral gavage, or drinking water exposures for ≥30 days. Studies employing short term and acute exposures that examined other toxicological effects are not included in the evidence tables; however, they are discussed in the text if they

provide data to support mode of action or hazard identification. No studies were removed for methodological concerns.

tert-Butanol also has been found to have CNS effects similar to ethanol in terms of animals appearing intoxicated and having withdrawal symptoms after cessation of oral or inhalation exposure. Severity of CNS symptoms such as withdrawal increased with dose and duration of exposure. However, study quality concerns (e.g., short exposure durations, lack of data reporting, small number of animals per treatment group) associated with all of the studies in the database preclude a clear understanding of potential neurotoxicity following *tert*-butanol exposure, and therefore, CNS studies are not presented in evidence tables.

Effects in other tissues were observed with less consistency. These include decreased body weight, liver effects, and urinary bladder effects.

Body weight

Body weight was decreased by >10% in both rats and mice with subchronic and chronic exposure, with males generally more affected than females (Table 1-11). The concentrations used in the subchronic inhalation study did not decrease body weights. However, a short-term (i.e., 18-day) inhalation study in rats observed a >10% decrease in body weight at concentrations about threefold higher (in mg/m^3) than the highest concentration used in the subchronic study. The same concentrations did not have any effect on the body weight in mice with short-term inhalation exposure.

Liver effects

Although some rodent studies observed statistically significant changes in relative liver weight with *tert*-butanol exposure, absolute liver weight was significantly increased only in female rats after subchronic oral exposure (Table 1-12). The results pertaining to histopathology changes were inconsistent (Table 1-13). The oral NTP (1995) subchronic and chronic studies did not observe treatment-related effects on liver histopathology in both sexes of F344 rats, but in a 10-week study in a different rat strain (Wistar rats), several liver lesions (including necrosis) and increased liver glycogen were seen in male rats (no females were included in the study) with the only dose used (Acharya et al., 1997; Acharya et al., 1995). The study did not provide any incidence or severity data. The dose used in this study was in the range of the lower doses used in the NTP (1995) study. In the developmental study by Lyondell Chemical Co. (2004), the F1 SD rats treated by *tert*-butanol for at least 9 weeks did not show any liver effects. An increased incidence of fatty liver was observed in the male mice of the highest dose group in the 2-year mouse bioassay, but no histopathologic changes were seen in the subchronic mouse study. No changes in liver histopathology were observed in the NTP (1997) subchronic inhalation study.

Urinary bladder effects

Several studies also reported effects in the urinary bladder (Table 1-14). Transitional epithelial hyperplasia was observed in male rats and mice after 13 weeks of exposure at doses of 3,610 mg/kg-day (male rats) and ≥3940 mg/kg-day (male mice). Male mice exposed at doses of 2,070 mg/kg-day for 2 years of also exhibited transitional epithelial hyperplasia. Neither female rats nor female mice showed increased incidences of transitional epithelial hyperplasia. Both sexes of mice demonstrated incidence of inflammation in the urinary bladder after both subchronic and chronic exposures, with a greater incidence in males compared to females.

An exposure-response array of these effects in body weight, liver, and urinary bladder is provided in Figure 1-8 and Figure 1-9 for oral and inhalation studies, respectively.

Mechanistic Evidence

No mechanistic evidence is available for these other toxicological effects.

Summary of Other Toxicity Data

EPA concluded that the evidence does not support body weight changes, liver effects, and urinary bladder effects as potential human hazards of *tert*-butanol exposure.

Table 1-11. Evidence pertaining to effects on body weight in animals following exposure to *tert*-butanol

Reference and study design	Results
Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	Body weight in treated animals lower than controls by ~7% ($p < 0.05$); (results only provided in a figure)
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	Percent change compared to control: **F0 Males**: Dose (mg/kg-d) / Body weight: 0 / 0; 64 / −2; 160 / −4; 400 / +2; 1,000 / −7 **F0 Females**: Dose (mg/kg-d) / Body weight: 0 / 0; 64 / 0; 160 / −2; 400 / +1; 1,000 / +4
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	Percent change compared to control: **Males**: Dose (mg/kg-d) / Body weight: 0 / 0; 230 / −4; 490 / −5*; 840 / −12*; 1,520 / −17*; 3,610 / All dead **Females**: Dose (mg/kg-d) / Body weight: 0 / 0; 290 / +2; 590 / +1; 850 / +1; 1,560 / −2; 3,620 / −21*
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 F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males**: Dose (mg/kg-d) / Body weight: 0 / 0; 350 / −1; 640 / +1; 1,590 / −4; 3,940 / −14*; 8,210 / −24* **Females**: Dose (mg/kg-d) / Body weight: 0 / 0; 500 / +3; 820 / −1; 1,660 / +4; 6,430 / −6; 11,620 / −15* High-dose females had a significantly lower initial weight, but also had a significantly lower body weight gain indicating that there was some effect of treatment

Table 1-11. Evidence pertaining to effects on body weight in animals following exposure to *tert*-butanol (*continued*)

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, 10 mg/mL) M: 0, 90, 200, 420[a] mg/kg-d F: 0, 180, 330, 650[a] mg/kg-d 2 years	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Body weight / Dose (mg/kg-d) / Body weight 0 / 0 / 0 / 0 90 / −15 / 180 / −2 200 / −18 / 330 / −5 420 / −24 / 650 / −21 Only animals that survived at the end of 2 years were evaluated for body weight. Note: statistical significance not determined by study authors.
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, 20 mg/mL); M: 0, 540, 1,040, 2,070[a] mg/kg-d F: 0, 510, 1,020, 2,110 mg/kg-d 2 years	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Body weight / Dose (mg/kg-d) / Body weight 0 / 0 / 0 / 0 540 / +1 / 510 / −2 1,040 / −2 / 1,020 / −3 2,070 / −1 / 2,110 / −12 Only animals that survived at the end of 2 years were evaluated for body weight. Note: statistical significance not determined by study authors.
NTP (1997) F344/N rat; 10/sex/treatment 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	Percent change compared to control: Concentration (mg/m^3) / **Males** Body weight / **Females** Body weight 0 / 0 / 0 406 / −1 / −5 824 / −2 / −1 1,643 / +2 / 0 3,273 / +3 / 0 6,368 / +2 / −3
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment 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	Percent change compared to control: Concentration (mg/m^3) / **Males** Body weight / **Females** Body weight 0 / 0 / 0 406 / +4 / +3 824 / −2 / −3 1,643 / 0 / +3 3,273 / −4 / −6

Table 1-11. Evidence pertaining to effects on body weight in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results		
method were reported	6,368	0	−8

[a]There was a significant decrease in survival in the high-dose group.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

Table 1-12. Changes in liver weight in animals following exposure to *tert*-butanol

Reference and study design	Results
Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	No significant treatment-related effects (results were only provided in a figure)
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d Males: 9 weeks beginning 4 weeks prior to mating Females: 4 weeks prior to mating through PND21	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 64 / −1 / 0 160 / −3 / +1 400 / −2 / −1 1,000 / +8 / +16* **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 64 / −4 / −4 160 / −7 / −5 400 / +2 / +1 1,000 / +8 / +3
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	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 230 / −2 / +4 490 / +1 / +8* 840 / +5 / +20* 1,520 / +8 / +31* 3,610 / All dead / All dead **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 290 / +11* / +9* 590 / +10* / +9* 850 / +12* / +11* 1,560 / +15* / +16* 3,620 / +9* / +41*
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 F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 350 / +2 / +3 640 / −1 / −2 1,590 / −1 / +5 3,940 / 0 / +14* 8,210 / −16 / +22* **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 500 / −1 / −4 820 / −5 / −3 1,660 / −8 / −9* 6,430 / −2 / +6 11,620 / −6 / +13*

Table 1-12. Changes in liver weight in animals following exposure to *tert*-butanol (*continued*)

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	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Absolute weight / Relative weight / Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 / 0 / 0 / 0 90 / +2 / +7 / 180 / −14* / −8 200 / +8 / +11 / 330 / −3 / −1 420 / +1 / +14* / 650 / −6 / +9* Only animals sacrificed at 15 months were evaluated for organ weights. Organ weights were not measured in the 2-year mouse study
NTP (1997) F344/N rat; 10/sex/treatment 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	Percent change compared to control: **Males** / **Females** Concentration (mg/m^3) / Absolute weight / Relative weight / Absolute weight / Relative weight 0 / 0 / 0 / 0 / 0 406 / −8 / −8 / 0 / +3 824 / −2 / −1 / 0 / 0 1,643 / +1 / −1 / +3 / +2 3,273 / +10 / +7 / +9 / +9* 6,368 / +5 / +5 / +4 / +8*
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment 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	Percent change compared to control: **Males** / **Females** Concentration (mg/m^3) / Absolute weight / Relative weight / Absolute weight / Relative weight 0 / 0 / 0 / 0 / 0 406 / −1 / 0 / +1 / −4 824 / +4 / +9 / +1 / +5 1,643 / +7 / +5 / +5 / +1 3,273 / −8 / -2 / +2 / +9* 6,368 / +5 / +7 / +8 / +21*

[a]The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.

Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

Table 1-13. Changes in liver histopathology in animals following exposure to *tert*-butanol

Reference and study design	Results
Acharya et al. (1997; 1995) Wistar rat; 5–6 males/treatment Drinking water (0, 0.5%), 0, 575 mg/kg-d 10 weeks	↑ liver glycogen (~ 7 fold)* ↑incidence of centrilobular necrosis, vacuolation of hepatocytes, loss of hepatocyte architecture, peripheral proliferation, and lymphocyte infiltration (incidences and results of statistical tests not reported)
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d Males: 9 weeks beginning 4 weeks prior to mating Females: 4 weeks prior to mating through PND21	No treatment-related effects 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 F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Histopathology data for the 13-week study were not provided, but the liver was evaluated indicating that no changes in liver histopathology were observed in the 13-week study.
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Histopathology data for the 13-week study were not provided, but the liver was evaluated indicating that no changes in liver histopathology were observed in the 13-week study.
NTP NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, 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	No treatment-related effects observed.

Table 1-13. Changes in liver histopathology in animals following exposure to *tert*-butanol (*continued*)

Reference and study design	Results			
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, 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	**Males**		**Females**	
	Dose (mg/kg-d)	Incidence of fatty change	Dose (mg/kg-d)	Incidence of fatty change
	0	12/59	0	11/60
	540	5/60	510	8/60
	1,040	8/59	1,020	8/60
	2,070	29/59*	2,110	6/60
NTP (1997) F344/N rat; 10/sex/treatment 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	Histopathology data for the 13-week study were not provided, but the liver was evaluated in control and high-dose group indicating that no changes in liver histopathology were observed in the 13-week study.			
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment 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	Authors stated that there were no treatment-related microscopic observations, but data were not provided.			

[a]The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.

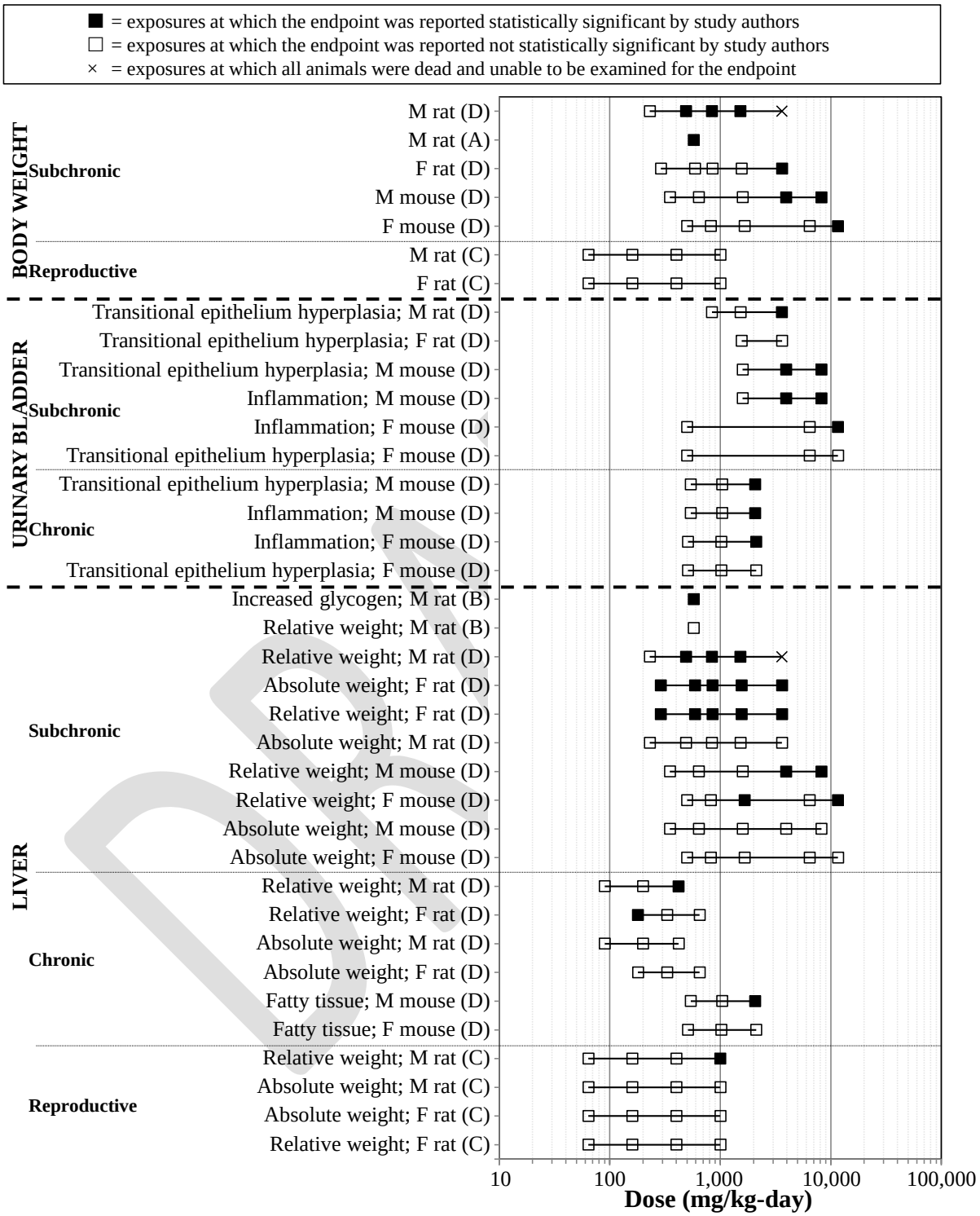
Table 1-14. Changes in urinary bladder histopathology in animals following oral exposure to *tert*-butanol

Reference and study design	Results
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 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) / Transitional epithelial hyperplasia 0 / 0/10 230 / not evaluated 490 / not evaluated 840 / 0/10 1,520 / 1/10 (3.0) 3,610 / 7/10* (2.9) **Females** Dose (mg/kg-d) / Transitional epithelial hyperplasia 0 / 0/10 290 / not evaluated 590 / not evaluated 850 / not evaluated 1,560 / 0/10 3,620 / 3/10 (2.0) Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Incidence (severity): **Males** Dose (mg/kg-d) / Transitional epithelial hyperplasia / Inflammation 0 / 0/10 / 0/10 350 / not evaluated 640 / not evaluated 1,590 / 0/10 / 0/10 3,940 / 6/10* (1.3) / 6/10* (1.3) 8,210 / 10/10* (2.0) / 10/10* (2.3) **Females** Dose (mg/kg-d) / Transitional epithelial hyperplasia / Inflammation 0 / 0/10 / 0/10 500 / 0/1 / 0/1 820 / not evaluated 1,660 / not evaluated 6,430 / 0/10 / 0/10 11,620 / 3/9 (2.0) / 6/9* (1.2) Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
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, 420[a] mg/kg-d F: 0, 180, 330, 650[a] mg/kg-d 2 years	No treatment-related effects observed

Table 1-14. Evidence pertaining to urinary bladder effects in animals following oral exposure to *tert*-butanol (*continued*)

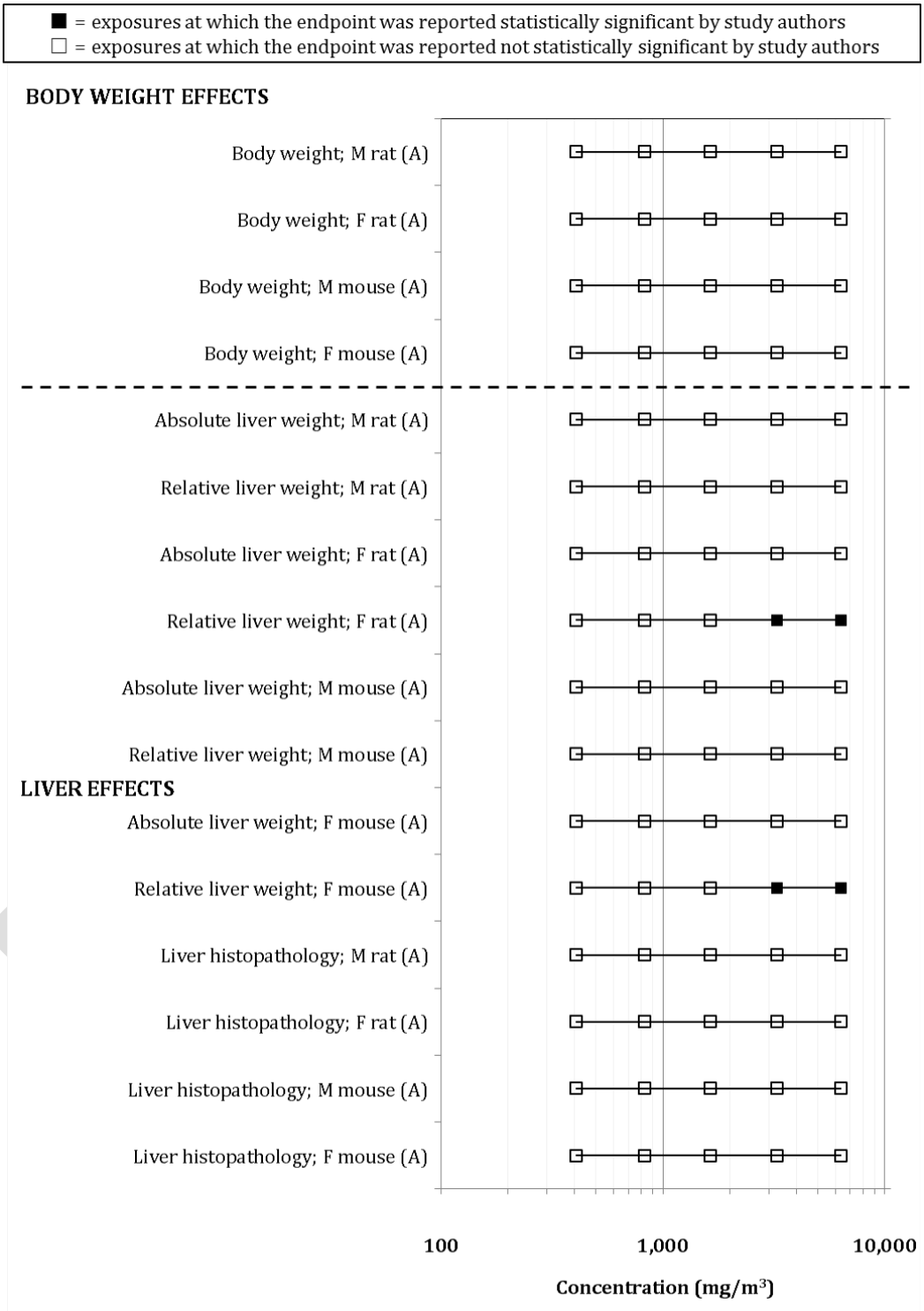
Reference and study design	Results					
NTP (1995) $B6C3F_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, 2,070[a] mg/kg-d F: 0, 510, 1,020, 2,110 mg/kg-d 2 years	Incidence (severity):					
	Males			**Females**		
	Dose (mg/kg-d)	Transitional epithelial hyperplasia	Inflam-mation	Dose (mg/kg-d)	Transitional epithelial hyperplasia	Inflam-mation
	0	1/59 (2.0)	0/59	0	0/59	0/59
	540	3/59 (1.7)	3/59 (1.7)	510	0/60	0/60
	1,040	1/58 (1.0)	1/58 (1.0)	1,020	0/59	0/59
	2,070	17/59* (1.8)	37/59* (2.0)	2,110	3/57 (1.0)	4/57* (2.0)
	Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked					

[a]The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.



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

Figure 1-8. Exposure-response array of other effects following oral exposure to *tert*-butanol.



Source: (A) NTP (1997)

Figure 1-9. Exposure-response array of other effects following inhalation exposure to *tert*-butanol.

1.2. INTEGRATION AND EVALUATION

1.2.1. Effects Other Than Cancer

The strongest evidence following *tert*-butanol exposure is for kidney, with toxicity observed after oral exposure in two strains of rats and in one strain of mice and in both sexes. In mice, the only kidney effect observed was an increase in kidney weight (absolute and/or relative) in both sexes of mice in the 13-week study, but no treatment-related histopathological lesions were reported in the kidneys of mice at the 13-week or 2-year time points (NTP, 1995). In male rats, effects related to the accumulation of α_{2u}-globulin in the kidney have been reported, including precursors to granular casts, linear mineralization, and tubular hyperplasia, but these are not considered relevant to humans (Hard et al., 2011; Cirvello et al., 1995; NTP, 1995; Lindamood et al., 1992). However, several other effects in the kidney unrelated to α_{2u}-globulin were observed in female and/or male rats. Absolute and relative kidney weights were increased in both male and female rats after both 13 weeks and 15 months of treatment (NTP, 1995). Histopathological examination also indicated kidney toxicity in both male and female rats, with increased incidence of nephropathy after 13 weeks of oral exposure and transitional epithelium hyperplasia observed after 2 years of oral exposure (NTP, 1995). Additionally, increased inflammation (suppurative) was noted in females after 2 years oral exposure (NTP, 1995). EPA identified kidney effects as a human hazard of *tert*-butanol oral exposure.

Fewer and less severe kidney effects were observed via inhalation than via oral exposure, likely due to the differing levels of internal doses achieved via the different routes. Specifically, available inhalation studies (NTP, 1997) were conducted at concentrations that are comparable, in terms of *tert*-butanol blood concentration, to the lower range of doses in oral studies. Moreover, there is convincing toxicokinetic data to indicate that *tert*-butanol is absorbed by both routes, and kidney effects are remote from the site of absorption. EPA identified kidney effects as a human hazard of *tert*-butanol inhalation exposure.

Thyroid follicular cell hyperplasia was observed in the mice after 2 years of exposure via drinking water (NTP, 1995); and EPA identified thyroid effects as a potential human hazard of tert-butanol exposure. However, this endpoint most likely reflects early events in the neoplastic progression of thyroid follicular cell tumors following *tert*-butanol exposure (see Section 1.1.2) and was not considered further for dose-response analysis and derivation of noncancer reference values.

EPA identified suggestive evidence of developmental effects as a potential human hazard of *tert*-butanol exposure. Exposure to high doses of *tert*-butanol during gestation resulted in some effects in exposed offspring or pups, although the effects were not always consistent across exposure routes (oral and inhalation). Dams exhibit effects at the same doses as fetal effects. Neurodevelopmental effects have also been observed; however, the neurodevelopmental studies had limitations in the study design and/or reporting and results were inconsistent between studies

or across dose. Thus, these effects were not considered further for dose-response analysis and derivation of reference values.

EPA concluded that the evidence does not support reproductive effects, body weight changes, liver effects, and urinary bladder effects as potential human hazards of *tert*-butanol exposure. Thus, these effects were not considered further for dose-response analysis and the derivation of reference values.

1.2.2. Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for *tert*-butanol provides "suggestive evidence of carcinogenic potential," based on a statistically significant increase in renal tumors (renal tubule adenomas and carcinomas) in male F344 rats and a statistically significant increase in thyroid follicular cell adenomas in female $B6C3F_1$ mice, all exposed to *tert*-butanol in drinking water for 2 years (Cirvello et al., 1995; NTP, 1995). There are no available studies of cancer in humans associated with exposure to *tert*-butanol.

In the NTP (1995) rodent bioassay, *tert*-butanol-exposed male rats had a significant increase in renal tumors compared to controls, a result confirmed by a PWG reevaluation (Hard et al., 2011). Although mechanistic data show that α_{2u}-globulin-related processes occur with *tert*-butanol exposure, there is insufficient evidence to support a conclusion that α_{2u}-globulin nephropathy is the sole or primary contributor to renal tumor development. Specifically, *tert*-butanol induced tumors at lower doses than those for other precursor effects such as hyperplasia and granular casts, with no further increase in tumor incidence coinciding with the induction of additional markers of α_{2u}-globulin nephropathy. Based on analysis of available mode of action data, these tumors are not attributed to α_{2u}-globulin and are considered relevant in humans (U.S. EPA, 1991a). *tert*-Butanol was negative in a variety of genotoxicity assays in different cell systems including gene mutations, sister chromatid exchanges, micronucleus formation, and chromosomal aberrations. However, DNA adducts in male Kunming mice and DNA damage in human HL-60 leukemia cells have been observed. Overall, the mode(s) of carcinogenic action for *tert*-butanol in the kidney and the thyroid are not known, and these tumor data are considered relevant to humans.

As emphasized in the Cancer Guidelines (U.S. EPA, 2005a), selection of the cancer descriptor follows a full evaluation of the available evidence. The carcinogenicity evidence for *tert*-butanol could be considered a borderline case between two cancer descriptors—"suggestive evidence of carcinogenic potential" and "likely to be carcinogenic to humans." The descriptor of "suggestive evidence of carcinogenic potential" is appropriate when a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. Exposure to *tert*-butanol produced a positive tumor response at more than one site (kidney and thyroid) and in more than one species (rat and mouse). These data appear to correspond closely to one of the examples in the Cancer Guidelines (U.S. EPA, 2005a) for the descriptor of "likely to be carcinogenic to humans;" i.e., "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." Several aspects of the data support the conclusion that these data are not sufficient to characterize *tert*-butanol as "likely to be carcinogenic to humans."

First, the renal tumors associated with *tert*-butanol exposure in the NTP (1995) rodent bioassay were predominantly benign. Based on the PWG reevaluation (Hard et al., 2011), among the three treated groups, only two of the 43 animals with tumors has carcinomas (there were no carcinomas among the 4 control animals with tumors). Additionally, no kidney tumors were observed in female rats or in either sex of mice. Furthermore, ETBE, which is rapidly metabolized to *tert*-butanol, did not induce kidney tumors in the same strain of rats at doses that resulted in similar internal concentrations of *tert*-butanol. Therefore, the level of concern raised by renal tumors associated with *tert*-butanol exposure is reduced based on the predominance of benign tumors, an increase in renal tumors in a single sex/species combination only, and the lack of coherence with the metabolically-related compound ETBE.

The thyroid tumors associated with *tert*-butanol exposure were also predominantly benign. In the NTP (1995) rodent bioassay, only female mice had a statistically significant increase in thyroid tumors; none of these were carcinomas. In males, decreased survival complicates the interpretation of thyroid tumors because male mice had an increased incidence of thyroid follicular cell hyperplasia at all exposure levels, but there was no significant increase in thyroid tumors at any exposure. Interestingly, one thyroid follicular cell carcinoma occurred in a high-dose male, but limited conclusions can be drawn from this single observation. Thyroid tumors were not observed in either sex of the rat exposed chronically to *tert*-butanol. Additionally, ETBE did not induce thyroid tumors, although only rats and not mice were tested. Therefore, the level of concern raised by thyroid tumors associated with *tert*-butanol exposure is reduced based on the predominance of benign tumors and an increase in thyroid tumors in a single sex/species combination only.

Overall, the cancer descriptor "suggestive evidence of carcinogenic potential" was selected, as some concern is raised by the positive evidence of predominantly benign renal tumors in male rats and thyroid tumors in female mice.

The Cancer Guidelines (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of *tert*-butanol via the oral route demonstrates that tumors occur in tissues remote from the site of absorption. Information on the carcinogenic effects of *tert*-butanol via the inhalation and dermal routes in humans or animals is not available. Based on the observation of systemic tumors following oral ingestion, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, there is "suggestive evidence of carcinogenic potential" from exposure to *tert*-butanol by all routes of exposure.

1.2.3. Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

No data were identified to indicate any possible susceptible populations or lifestages.

2. DOSE-RESPONSE ANALYSIS

2.1. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

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

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

EPA identified kidney effects as a human hazard of *tert*-butanol exposure. Studies within this effect category were evaluated using general study quality characteristics (as discussed in Section 6 of the Preamble) to help inform the selection of studies from which to derive toxicity values. Rationales for selecting the studies and effects to represent each of these hazards are summarized below.

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

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

Kidney Toxicity

EPA identified kidney effects as a human hazard of *tert*-butanol-induced toxicity based on findings of organ weight changes in rats and mice, as well as histopathology in rats. These findings were consistent across multiple chronic, subchronic, and short-term studies following oral and

inhalation exposure. Acharya et al. (1997; 1995) used a single exposure group and did not provide incidence or severity data, and thus was not considered for dose-response assessment. Lyondell Chemical Co. (2004) and NTP (1997) were of subchronic or shorter duration, and so were set aside given the availability of a longer duration study. Therefore, the NTP 2-year drinking water study (NTP, 1995) was identified most suitable for dose-response assessment considering the study duration, comprehensive reporting of outcomes, multiple species tested, and multiple doses tested.

In the NTP (1995) 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; male B6C3F_1 mice were exposed to approximate doses of 0, 540, 1,040, or 2,070 mg/kg-day; and female B6C3F_1 mice were exposed to approximate doses of 0, 510, 1,020, or 2,110 mg/kg-day. Reduced body weights and survival were observed and reflected in some of the effects. Kidney effects including changes in organ weight and/or histopathology were observed in both sexes in rats and mice. Effects were also observed after 13 weeks, 15 months, and 2 years of treatment (NTP, 1995). Effects were more consistent and occurred at lower doses in rats as compared to mice, so as a result, only data in the more sensitive species of rats were used for dose-response assessment. Endpoints potentially confounded by the presence of α_{2u}-globulin nephropathy in male rats, such as linear mineralization and renal tubule hyperplasia, were not used for dose-response analysis. Specific endpoints chosen for analysis were absolute and relative kidney weight (observed in males and females), kidney inflammation (observed only in females), and kidney transitional epithelial hyperplasia (observed in males and females). For most endpoints, the data at the longest duration of 2 years were selected. However, as discussed in Section 1.1.1, 2-year kidney weight data were not considered because organs were only weighed at 15 months.

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 to best empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, all models available in EPA's Benchmark Dose Software (BMDS) were evaluated. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b), the benchmark dose (BMD) and the 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) of 10% change from the control mean for organ weight data in the absence of information regarding the level of change that is considered biologically significant. Furthermore, the BMD and BMDL were estimated to facilitate a consistent basis of comparison across endpoints, studies, and assessments. A benchmark response (BMR) of 10% extra risk was considered appropriate for the quantal data on incidences of kidney inflammation and kidney transitional epithelial hyperplasia. For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and Akaike Information Criterion (AIC) value were used to select a best-fit model among models exhibiting

adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most 3-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD. The estimated BMDLs were used as points of departure (PODs). Further details including the modeling output and graphical results for the best-fit model for each endpoint can be found in Appendix C of the Supplemental Information.

In general, absolute and relative kidney weight data may both be considered appropriate endpoints for analysis (Bailey et al., 2004). However, in the NTP (1995) 2-year drinking water study, there was a noticeable decrease in body weight in exposed animals relative to controls at the 15 month interim sacrifice (see Table 1-1). In such a case, relative kidney weights are the preferred, so changes in absolute kidney weights were not analyzed.

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

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

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

where

BW_a = animal body weight

BW_h = human body weight

Using a standard BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAFs for rats is 0.24. 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 the sequence of calculations leading to the derivation of a human-equivalent POD for each data set discussed above.

Table 2-1. Summary of derivations of points of departure

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 relative kidney weight NTP (1995)	Rat/M	Exponential (M4)	10%	117	48	48	11.5
Increased relative kidney weight NTP (1995)	Rat/F	Linear	10%	158	133	133	31.9
Kidney inflammation 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, administered doses were adjusted to calculate the TWA daily doses prior to BMD modeling.
[c]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011).

2.1.3. Derivation of Candidate Values

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

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

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

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

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

A database uncertainty factor, UF_D, of 1 was applied to all PODs. The *tert*-butanol toxicity database includes a chronic toxicity study in rats and mice (NTP, 1995), a subchronic toxicity study in rats and mice (NTP, 1997), 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 a sensitive endpoint. The *tert*-butanol database contains a one-generation reproductive toxicity study in rats (Lyondell Chemical Co., 2004), though no multigenerational reproductive study has been performed. There are no immunotoxicity studies for *tert*-butanol. Information provided by studies on ETBE, which is rapidly metabolized to systemically-available *tert*-butanol, can help in considering the lack of a *tert*-butanol multigenerational reproductive study or an immunotoxicity study. No adverse effects were reported in one- and two-generation reproductive/developmental studies on ETBE (Gaoua, 2004a, b), and the database for ETBE does not indicate immunotoxicity (Banton et al., 2011; Li et al., 2011). Thus, although there are some gaps in the toxicity database for *tert*-butanol, 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.

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. The candidate values presented in the table below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative oral reference value for a specific

hazard and subsequent overall RfD for *tert*-butanol.

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

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

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 relative kidney weight; male rat NTP (1995)	12	$BMDL_{10\%}$	3	10	1	1	1	30	4×10^{-1}
Increased relative kidney weight; female rat NTP (1995)	32	$BMDL_{10\%}$	3	10	1	1	1	30	1×10^{0}
Kidney inflammation; 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}

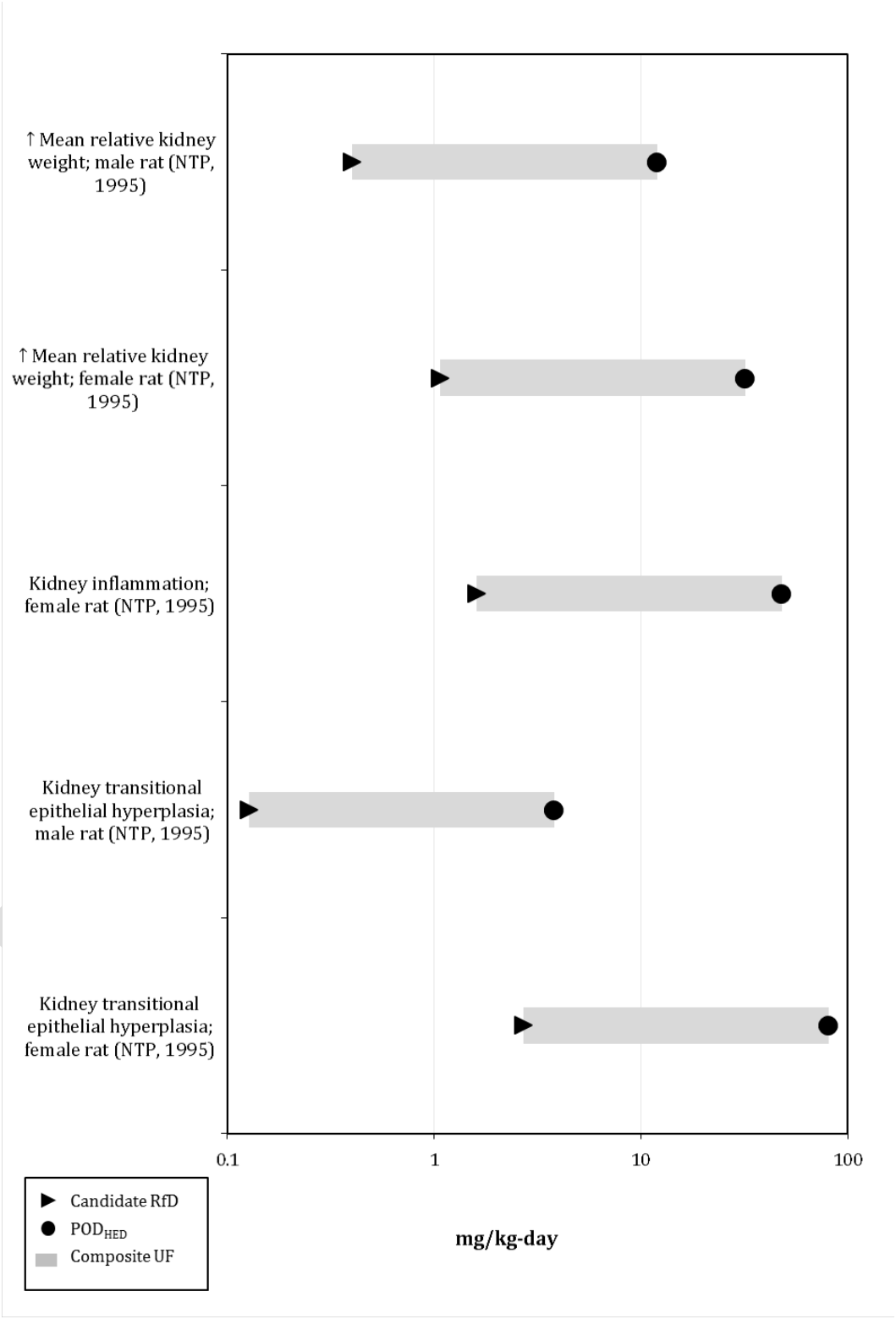


Figure 2-1. Candidate RfD values with corresponding POD and composite UF.

2.1.4. Derivation of Organ/System-Specific Reference Doses

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

Kidney Toxicity

For *tert*-butanol, candidate values were for several different effects in both sexes, spanning a range from 1×10^{-1} to 3×10^{0} mg/kg-day, for an overall thirtyfold 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**) is proposed as the kidney-specific reference dose for *tert*-butanol. Unlike kidney inflammation, this effect was observed in both sexes, with males appearing to be more sensitive than females. Additionally, it is a more specific and more sensitive indicator of kidney toxicity than the relatively non-specific endpoint of kidney weight changes. Confidence in this kidney-specific RfD is high. The PODs are based on modeled benchmark dose estimates, and the candidate values are derived from a well-conducted study, involving a sufficient number of animals per group, including both sexes, and assessing a wide range of kidney endpoints.

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

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

2.1.5. Selection of the Proposed Overall Reference Dose

For *tert*-butanol, only kidney effects were identified as a hazard; thus a single organ/system-specific reference dose was derived. Therefore, the kidney-specific RfD of **1×10^{-1} mg/kg-day** is also proposed as an estimated exposure level below which deleterious effects from *tert*-butanol exposure are not expected to occur.

The overall reference dose is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole including potentially susceptible subgroups (U.S. EPA, 2002). 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 potentially 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, no specific lifestages have been identified as a potentially susceptible subgroup.

2.1.6. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The overall confidence in this RfD is high. Confidence in the principal study (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 and high confidence in the database, confidence in the RfD is high.

2.1.7. Previous IRIS Assessment

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

2.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The inhalation reference concentration (RfC) (expressed in units of mg/m^3) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95% lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

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

EPA identified kidney effects as a human hazard of *tert*-butanol exposure. Studies within this effect category were evaluated using general study quality characteristics (as discussed in Section 6 of the Preamble) to help inform the selection of studies from which to derive toxicity values. Rationales for selecting the studies and effects to represent this hazard are summarized below.

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

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

Kidney Toxicity

EPA identified kidney effects as a human hazard of *tert*-butanol exposure based on findings of organ weight changes in rats and mice and histopathology in rats. These findings were consistent across multiple chronic, subchronic, and short-term studies following oral and inhalation exposure. Acharya et al. (1997; 1995) used a single exposure group and did not provide incidence or severity data, so was not considered for dose-response assessment. Lyondell Chemical Co. (2004) was of shorter than subchronic duration, and so was set aside given the availability of a longer duration studies. Given the availability of a chronic study, the subchronic studies of NTP (1995) and NTP (1997) would normally also be set aside for dose-response analysis. NTP (1997) is the longest duration study via the inhalation route, not requiring route-to-route extrapolation, so was kept for comparison purposes. 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 will not be reviewed here again. The NTP (1997) subchronic inhalation study 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. Briefly, groups of F344 rats and $B6C3F_1$ mice (10 per sex per species) were exposed to *tert*-butanol (>99% pure) at concentrations of 0, 409, 819, 1,637, 3,274 or 6,366 mg/m^3 by inhalation for 6 hours per day, 5 days per week, for 13 weeks (NTP, 1997). Absolute kidney weights were elevated (10–11%) in male rats exposed at ≥3,274 mg/m^3; relative kidney weights were statistically significantly elevated (~9%) in males at ≥3,274 mg/m^3 and females at 6,366 mg/m^3. Male rats exhibited an increase in the severity of chronic nephropathy (characterized as number of foci of regenerative tubules). There were few endpoints available for consideration in the subchronic study, but changes in kidney weights were also 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 thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, all models available in EPA's Benchmark Dose Software (BMDS) were evaluated. Consistent with EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b), the benchmark dose (BMD) and the 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) of 10% change from the control mean for organ weight data in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparison across endpoints, studies, and assessments. A benchmark response (BMR) of 10% extra risk was considered appropriate for the quantal data on incidences of kidney inflammation and kidney transitional epithelial hyperplasia. The estimated BMDLs were used as points of departure (PODs). Further details including the modeling output and graphical results for the best-fit model for each endpoint can be found in Appendix C of the Supplemental Information.

In general, absolute and relative kidney weight data may both be considered appropriate endpoints for analysis (Bailey et al., 2004). However, in the NTP (1995) 2-year drinking water study, there was a noticeable decrease in body weight in exposed animals relative to controls at the 15 month interim sacrifice (see Table 1-1). In such a case, relative kidney weights are preferred, so changes in absolute kidney weights from NTP (1995) were not analyzed. However, body weights were not impacted in the NTP (1997) subchronic inhalation study. Based on a historical review of 26 studies of control rats from 1-month bioassays, Bailey et al. (2004) concluded that neither absolute kidney weight nor relative kidney:body (or kidney:brain) weight are optimal for evaluating organ weight changes. Since neither approach is preferred, both were considered to be appropriate for BMD analysis of the NTP (1997) data set.

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 a 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 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 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 point of departure 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 relative kidney weight NTP (1997)	Male F344 rats	Linear	10%	6309	4821	861	861
Increased absolute kidney weight NTP (1997)	Male F344 rats	Hill	10%	1931	1705	304	304
Increased relative kidney weight NTP (1997)	Female F344 rats	No model selected	10%	--	--	--	--
Increased absolute kidney weight NTP (1997)	Female F344 rats	No model selected	10%	--	--	--	--

[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 hrs) × (days exposed per week / 7 days).
[c]POD_{HEC} calculated by adjusting the POD_{ADJ} by the DAF (=1.0) for a category 3 gas (U.S. EPA, 1994).
[d]BMD modeling failed to successfully calculate a BMD value (see Appendix C).

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

A PBPK model for *tert*-butanol in rats has been developed, as described in Appendix B. Using this model, route-to-route extrapolation of the oral BMDLs to derive inhalation PODs was performed as follows. First, the internal dose in the rat at each oral BMDL (assuming continuous exposure) was estimated using the PBPK model, to derive an "internal dose BMDL." Then, the inhalation air concentration (again, assuming continuous exposure) that led to the same internal dose in the rat was estimated using the PBPK model. The resulting BMCL was then converted to a human equivalent concentration POD 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 the 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, since the distribution from blood to kidney is independent of route. There are no data to suggest that metabolites of *tert*-butanol

mediate its renal toxicity. In the absence of evidence that would suggest otherwise, it is assumed that *tert*-butanol itself is 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 point of departure for 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 relative kidney weight NTP (1995)	Rat/M	10%	48	2.34	79.6
Mean relative kidney weight NTP (1995)	Rat/F	10%	133	7.46	231
Kidney inflammation 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.

PODs carried forth to derivation of candidate values

For the derivation of candidate values, it must be considered whether PODs from the inhalation study of NTP (1997) would provide a better basis than the route-to-route extrapolated PODs based on the oral study of NTP (1995). The only endpoint available from NTP (1997) is increased kidney weights. The corresponding PODs from this subchronic inhalation study are substantially higher than those for the same endpoint derived by route-to-route extrapolation from the chronic study (NTP, 1995), consistent with longer duration requiring a lower dose to elicit an effect. Additionally, as discussed in Section 2.1.3, kidney weight is a less-specific endpoint compared to some of the other endpoints available for analysis from the oral study (NTP, 1995). Therefore, the PODs derived from PBPK model-based route-to-route extrapolation are the preferred basis for deriving kidney-specific candidate RfCs, as they are based on a longer (chronic) duration and a more specific endpoint.

2.2.3. Derivation of Candidate Values

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

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

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

A subchronic to chronic uncertainty factor, UF_S, of 1 was applied to the PODs derived from the NTP (1995) study, as the endpoints were observed following chronic exposure. For the PODs derived from the subchronic NTP (1997) study, a UF_S of 10 was applied to account for extrapolation from subchronic to chronic duration.

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

A database uncertainty factor, UF_D, of 1 was applied to all PODs. The *tert*-butanol toxicity database includes a chronic toxicity study in rats and mice (NTP, 1995), a subchronic toxicity study in rats and mice (NTP, 1997), 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 a sensitive endpoint. The *tert*-butanol database contains a one-generation reproductive toxicity study in rats (Lyondell Chemical Co., 2004), though no multigenerational reproductive study has been performed. There are no immunotoxicity studies for *tert*-butanol. Information provided by studies on ETBE, which is rapidly metabolized to systemically-available *tert*-butanol, can help in considering the lack of a *tert*-butanol multigenerational reproductive study or an immunotoxicity study. No adverse effects were reported in one- and two-generation reproductive/developmental studies on ETBE (Gaoua, 2004a, b), and the database for ETBE does not indicate immunotoxicity (Banton et al., 2011; Li et al., 2011). Thus, although there are some gaps in the toxicity database for

tert-butanol, 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.

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.

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 relative kidney weight; male rat NTP (1997)	861	$BMCL_{10\%}$	3	10	1	10	1	300	3×10^0
Increased absolute kidney weight; male rat NTP (1997)	304	$BMCL_{10\%}$	3	10	1	10	1	300	1×10^0
Increased relative kidney weight; female rat NTP (1997)	1137	NOAEL	3	10	1	10	1	300	4×10^0
Increased absolute kidney weight; female rat NTP (1997)	1137	NOAEL	3	10	1	10	1	300	4×10^0
Increased relative kidney weight; male rat NTP (1995)	79.6	$BMCL_{10\%}$	3	10	1	1	1	30	3×10^0
Increased relative kidney weight; female rat NTP (1995)	231	$BMCL_{10\%}$	3	10	1	1	1	30	8×10^0
Kidney inflammation; 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

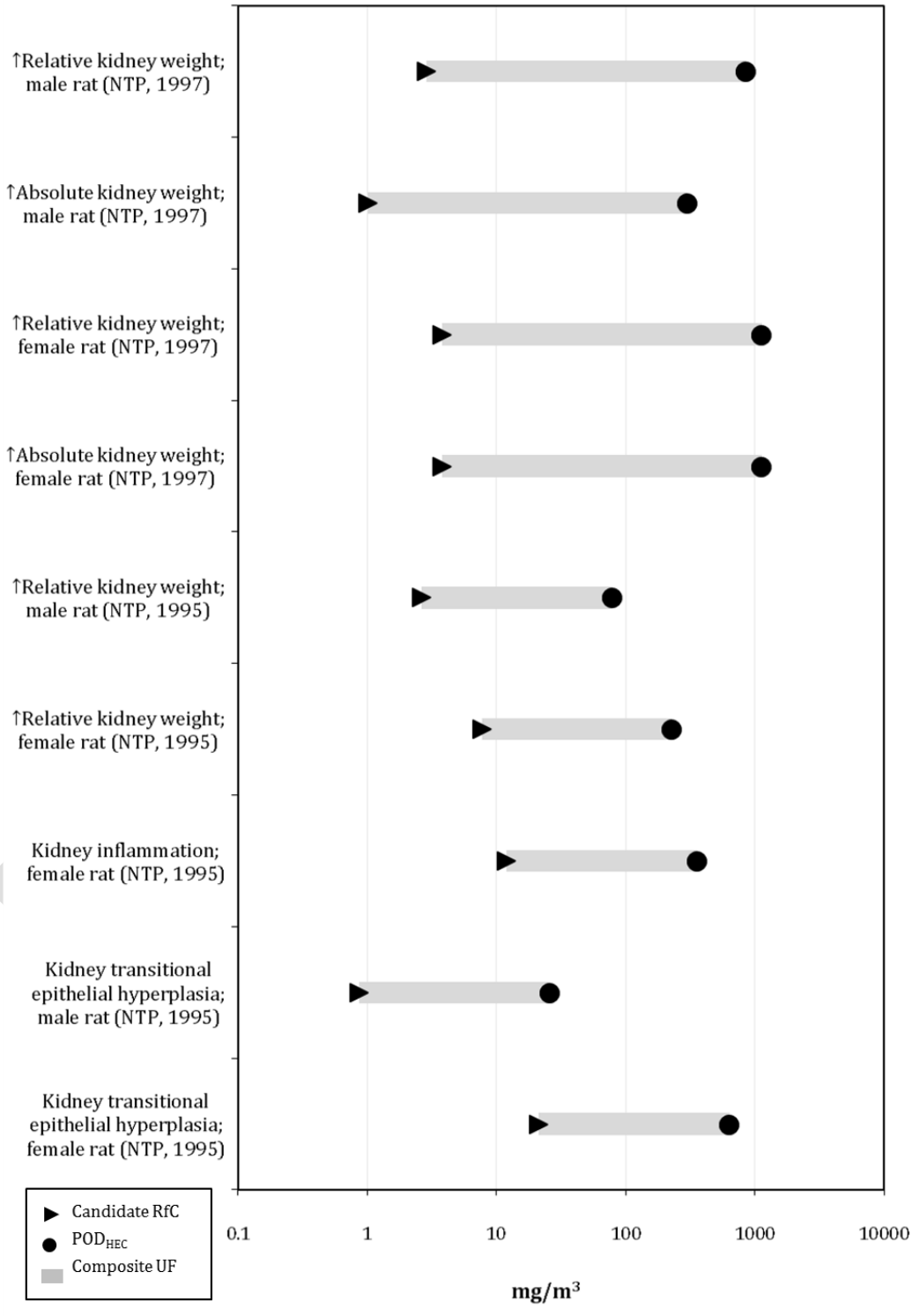


Figure 2-2. Candidate RfC values with corresponding POD and composite UF.

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 may be 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 effects in both sexes, spanning a range from 9×10^{-1} to 2×10^{1} mg/m^3, for an overall twenty-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**) is proposed as the kidney-specific reference concentration for *tert*-butanol, consistent with the selection of the kidney-specific RfD (see Section 2.1.4). As discussed previously, unlike kidney inflammation, this effect was observed in both sexes, with males appearing to be more sensitive than females. Additionally, it is a more specific and more sensitive indicator of kidney toxicity than the relatively non-specific endpoint of kidney weight changes. Confidence in this kidney-specific RfC is medium. The PODs are based on modeled benchmark dose estimates, 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 proposed overall RfC for *tert*-butanol

Effect	Basis	RfC (mg/m^3)	Exposure description	Confidence
Kidney toxicity	Increased incidence of transitional epithelial hyperplasia	9×10^{-1}	Chronic	HIGH
Proposed overall RfC	**Increased incidence of transitional epithelial hyperplasia**	**9×10^{-1}**	**Chronic**	**HIGH**

2.2.5. Selection of the Proposed Overall Reference Concentration

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

The overall reference concentration 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, no specific lifestages have been identified has a potentially susceptible subgroup.

2.2.6. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The overall confidence in this RfC is high. Confidence in the principal study (NTP, 1995) is high. This study was well-conducted, compiled 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 and high confidence in the database, confidence in the RfC is high.

2.2.7. Previous IRIS Assessment

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

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

The following discussion identifies uncertainties associated with the RfD and RfC 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. To derive the RfC, this same approach was applied, but a PBPK model was used to extrapolate from oral to inhalation exposure. UFs were applied to the POD to account for extrapolating from an animal bioassay to human exposure, the likely existence of a diverse population of varying susceptibilities, and database deficiencies. These extrapolations are carried out with default approaches given the lack of data to inform individual steps.

The database for *tert*-butanol contains no human data on adverse health effects from subchronic or chronic exposure. Data on the effects of *tert*-butanol are derived from a small database of studies in rats and mice. The database for *tert*-butanol exposure includes one lifetime bioassay, several reproductive/developmental studies, and several subchronic 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 significant material 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. Although sufficient information is available to develop a PBPK model in rats to evaluate difference across routes of exposure, the *tert*-butanol database lacks an adequate model that would inform potential interspecies differences. 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.

2.3. ORAL SLOPE FACTOR FOR CANCER

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

2.3.1. Analysis of Carcinogenicity Data

As noted in Section 1.2.2, EPA concluded that there is "suggestive evidence of carcinogenic potential" for *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.

The only data available on potential carcinogenicity was derived from the 2-year drinking water study in rats and mice by (NTP, 1995). This study was considered suitable for dose-response analysis. It was conducted in accordance with Food and Drug Administration (FDA) Good Laboratory Practice (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 re-examined by a Pathology Working Group (Hard et al., 2011).

Dose-related increasing trends in tumors were noted at the following sites:

- Renal tubule adenomas and carcinomas in male rats; and
- Thyroid follicular adenomas in female mice.

These tumors were statistically significantly increased by pairwise comparison (Fisher exact test, $p \leq 0.05$) and by trend test (Cochran-Armitage trend test, $p \leq 0.05$). Based on analysis of mode of action data, it was concluded that processes other than α_{2u}-globulin nephropathy are likely responsible for the male rat renal tumors, so these tumors may be suitable for quantitative analysis (U.S. EPA, 1991a). Additionally, a thyroid follicular carcinoma was observed in male mice, so it is possible that the thyroid follicular adenomas in female mice could progress to malignant form. Therefore, the thyroid follicular adenomas in female mice may also be considered suitable for quantitative analysis. Considering these data along with the uncertainty associated with the suggestive nature of the weight of evidence, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk.

2.3.2. Dose-Response Analysis—Adjustments and Extrapolations Methods

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommends that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the MOA of carcinogenicity has not been established (U.S. EPA, 2005a). In the case of *tert*-butanol, the modes of carcinogenic action for renal tubule and thyroid follicular tumors are not fully understood (see Section 1.2.2). Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with *tert*-butanol exposure.

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 for mice, 0.25 kg for rats, and 70 kg for humans was used (U.S. EPA, 1988). The following formula was used for the conversion of oral BMDL to oral HED for rat endpoints:

$$\text{Scaled HED in mg/kg-day} = (\text{BMDL in mg/kg-day}) \times (\text{animal body weight}/70)^{1/4}$$
$$= (\text{BMDL in mg/kg-day}) \times 0.24$$

Details of the modeling and the model selection process can be found in Appendix C of the Supplemental Information. PODs for estimating low-dose risk were identified at doses at the lower end of the observed data, generally corresponding to 10% extra risk. Because initial modeling overestimated the control, due to the non-monotonicity of the observed dose-response, the POD

was derived after dropping the highest exposure group (U.S. EPA, 2012b). The highest exposure group also had increased mortality, which may in part explain the observed non-monotonicity.

2.3.3. Derivation of the Oral Slope Factor

The PODs estimated for each tumor site 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 = $0.1/BMDL_{10}$). This slope, a 95% upper confidence limit, represents a plausible upper bound on the true risk. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each species/tumor site combination and are listed in Table 2-8.

The oral slope factors derived from the NTP (1995) bioassay differ by twenty-fold, depending on the species and tumor site. The most sensitive endpoint of renal tumors was used to derive the oral slope factor because there are no data to support any one result as most relevant for extrapolating to humans. Two slope factors were derived for this endpoint from the NTP (1995) bioassay, one based on the original reported incidences and the other based on the Hard et al. (2011) reanalysis. The two estimates differed by less than 20%, and rounded to the same number at one significant figure. However, the Hard et al. (2011) reanalysis is considered preferable, as it is based on a PWG analysis. Therefore, the recommended slope factor for providing a sense of the magnitude of potential carcinogenic risk associated with lifetime oral exposure to *tert*-butanol is **1×10^{-2} per mg/kg-day**, based on the renal tubule tumor response in male F344 rats.

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

Tumor	Species/Sex	Selected Model	BMR	BMD (mg/kg-d)	POD= BMDL (mg/kg-d)	$BMDL_{HED}$[a] (mg/kg-d)	Slope factor[b] $(mg/kg\text{-}day)^{-1}$
Renal tubule adenoma or carcinoma	Male F344 rat; dose as administered	1° Multistage (high dose dropped)	10%	70	42	10.1	1×10^{-2}
Renal tubule adenoma or carcinoma [Hard et al. (2011) reanalysis]	Male F344 rat; dose as administered	1° Multistage (high dose dropped)	10%	54	36	8.88	1×10^{-2}
Thyroid follicular cell adenoma	Female B6C3F1 mouse	3° Multistage	10%	2002	1437	201	5×10^{-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.

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 (see Table 2-9). Uncertainty in the magnitude of the recommended oral slope factor is reflected to some extent in the range of slope factors; the oral slope factor based on the male rat data was about twenty-fold higher than the oral slope factor based on female mouse data (Table 2-9). These comparisons show that the selection of target organ, animal species, and interspecies extrapolation can impact the oral cancer risk estimate. Although the thyroid follicular cell tumors occurred in male and female mice, high mortality in high-dose male mice limited the usefulness of the data. Renal tubule tumors occurred in male rats, but not female rats. Therefore, only the data in male rats and female mice were available for deriving the oral slope factor. There are no other chronic studies to replicate these findings or that examined other animal models. There are no data in humans to support the tumors observed in animals. Although changing the methods used to derive the oral slope factor could change the results, standard practices were used due to the lack of a mouse or human PBPK model or specific MOA to indicate other methods would be preferable. Additionally, considering the uncertainty associated with the suggestive nature of the weight of evidence, the oral slope factor is recommended only for providing a sense of the magnitude of potential carcinogenic risk.

Table 2-9. Summary of uncertainties in the derivation of cancer risk values for *tert*-butanol

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of target organ ↓ oral slope factor, up to twenty-fold, if renal tumors not selected.	The kidney was selected as the target organ.	As there are no data to support any one result as most relevant for extrapolating to humans, the most sensitive result for kidney renal tubular adenomas and carcinomas was used to derive the oral slope factor. However, the overall evidence for carcinogenicity was considered "suggestive."
Selection of data set Unknown change in oral slope factor, since no other studies are available.	NTP (1995) as principal oral (drinking water) study to derive cancer risks for humans.	NTP (1995) was a well-conducted study. It was also the only bioassay available. Additional bioassays might add support to the findings or provide results for different (possibly lower) doses, which may affect the oral slope factor.
Selection of extrapolation approach (Selection of extrapolation approach could change the recommended cancer risk values.)	Oral data used for OSF.	No extrapolation methods were used.
Selection of dose metric Alternatives could ↓ or ↑ slope factor	Used administered dose converted to HED units.	Additional runs using the administered dose without conversion to HED units were also conducted, resulting in a similar oral slope

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
		factor. For rats, a PBPK model of internal dose was available, but the POD changed by less than 1.2-fold when modeling was based on internal doses. For mice, no PBPK model was available, so using a PBPK model for determining internal doses could have an unknown effect on the estimated OSF value.
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 BW2/3])	The default approach of body weight$^{3/4}$ was used.	There are no data to suggest an alternative approach. Because the dose metric was not an area under the curve, BW$^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks. While the true human correspondence is unknown, this overall approach is expected to neither over- nor underestimate human equivalent risks.
Dose-response modeling Alternatives could ↓ or ↑ slope factor	Used multistage dose-response model to derive a BMD and BMDL.	No biologically based models for *tert*-butanol were available. The multistage model has biological support and is the model most consistently used in EPA cancer assessments.
Low-dose extrapolation ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation	Linear extrapolation of risk in low-dose region used.	Linear low-dose extrapolation for agents without a known MOA is supported.
Statistical uncertainty at POD ↓ oral slope factor 1.7-fold if BMD used as the POD rather than BMDL	BMDL (preferred approach for calculating plausible upper bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure at 10% extra risk of renal 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

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

2.4. INHALATION UNIT RISK FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose

extrapolation procedure. If derived, the inhalation unit risk is a plausible upper bound on the estimate of risk per μg/m^3 air breathed.

2.4.1. Analysis of Carcinogenicity Data

As noted in Section 1.2.2, EPA concluded that there is "suggestive evidence of carcinogenic potential" for *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, it could provide a sense of the magnitude and uncertainty of potential risks, rank potential hazards, or set research priorities.

The only data available on potential carcinogenicity were from the 2-year drinking water study in rats and mice by NTP (1995), discussed previously in Section 2.3.1. Because a PBPK model for the rat is available to conduct route-to-route extrapolation (discussed below), the male rat renal tubule adenoma and carcinoma data are suitable for quantitative analysis to support an inhalation unit risk. Considering these data and uncertainty associated with the suggestive nature of the weight of evidence, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk.

2.4.2. Dose Response Analysis – Adjustments and Extrapolation Methods

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

A PBPK model for *tert*-butanol in rats has been developed, as described in Appendix B. Using this model, route-to-route extrapolation of the oral BMDL to derive an inhalation POD was performed as follows. First, the internal dose in the rat at the 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, resulting in a route-to-route extrapolated BMCL.

A critical decision in the route-to-route extrapolation is the selection of the internal dose metric to use that established "equivalent" oral and inhalation exposures. For *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, since the distribution from blood to kidney is independent of route. There are no data that suggest metabolites of *tert*-butanol mediate its renal toxicity. In the absence of evidence that would suggest otherwise, it is assumed

that *tert*-butanol itself is the active toxicological agent. Therefore, the concentration of *tert*-butanol in blood was selected as the dose metric to derive the BMCL.

The RfC methodology provides a mechanism for deriving a HEC from the BMCL 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 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 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:

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

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the MOA of carcinogenicity has not been established (U.S. EPA, 2005a). In the case of *tert*-butanol, the mode of carcinogenic action for renal tubule tumors is not fully understood (see Section 1.2.2). Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with *tert*-butanol exposure.

2.4.3. Inhalation Unit Risk Derivation

The results from route-to-route extrapolation of the male rat renal tubule tumor data are summarized in Table 2-10. The lifetime inhalation unit risk for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (inhalation unit risk = $0.1/BMCL_{10}$). This slope, a 95% upper confidence limit represents a plausible upper bound on the true risk. Using linear extrapolation from the $BMCL_{10}$, a human equivalent inhalation unit risk was derived, as listed in Table 2-10.

Two inhalation unit risks were derived from the NTP (1995) bioassay: one based on the original reported incidences and one based on the Hard et al. (2011) reanalysis. The two estimates differ by less than 20%, but the Hard et al. (2011) reanalysis is considered preferable, as it is based

on a PWG analysis. Therefore, the recommended inhalation unit risk for providing a sense of the magnitude of potential carcinogenic risk associated with lifetime inhalation exposure to *tert*-butanol is **2×10^{-3} per mg/m^3**, or **2×10^{-6} per µg/m^3**, based on the renal tubule tumor response in male F344 rats.

Table 2-10. Summary of the inhalation unit risk derivation

Tumor	Species/Sex	BMR	BMDL (mg/kg-d)	Internal Dose[a] (mg/L)	POD= $BMCL_{HEC}$[c] (mg/m^3)	Unit Risk[b] (mg/m^3)$^{-1}$
Renal tubule adenoma or carcinoma	Male F344 rat	10%	41.6	2.01	68.7	1×10^{-3}
Renal tubule adenoma or carcinoma [Hard et al. (2011) reanalysis]	Male F344 rat	10%	36.3	1.74	59.8	2×10^{-3}

[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.
[c] Human equivalent inhalation unit risk = $0.1/BMCL_{HEC}$.

2.4.4. Uncertainties in the Derivation of the Inhalation Unit Risk

There is uncertainty when extrapolating data from animals to estimate potential cancer risks to human populations from exposure to *tert*-butanol (see Table 2-11). Uncertainty in the magnitude of the recommended inhalation unit risk can be inferred to some extent from the range of oral slope factors; the oral slope factor based on the male rat data was about twenty-fold higher than the oral slope factor based on female mouse data (Table 2-9). These comparisons show that the selection of target organ, animal species, and interspecies extrapolation can impact the inhalation unit risk estimate. Although the thyroid follicular cell tumors occurred in male and female mice, high mortality in high-dose male mice limited the usefulness of the data. Additionally, no PBPK model was available in mice for use in route-to-route extrapolation, so these data could not be used to estimate an inhalation unit risk. Renal tubule tumors occurred in male rats, but not female rats. Therefore, only the data in male rats were available for deriving the inhalation unit risk. There are no other chronic studies to replicate these findings or that examined other animal models. There are no data in humans to support the tumors observed in animals. Although changing the methods used to derive the inhalation unit risk could change the results, standard practices were used due to the lack of a mouse or human PBPK model or specific MOA to indicate other methods which would be preferable. Additionally, considering the uncertainty associated with the suggestive nature of the weight of evidence, the inhalation unit risk is recommended only for providing a sense of the magnitude of potential carcinogenic risk.

Table 2-11. Summary of uncertainties in the derivation of cancer risk values for *tert*-butanol

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
Selection of target organ Inhalation unit risk may change by an unknown amount if a PBPK model to extrapolate mouse thyroid tumors to the inhalation route were available.	The kidney was selected as the target organ.	No PBPK model to extrapolation mouse thyroid tumors was available. Additionally, the overall evidence for carcinogenicity was considered "suggestive."
Selection of data set Unknown change in inhalation unit risk, since no other studies are available.	NTP (1995) as principal oral (drinking water) study to derive cancer risks for humans.	NTP (1995) was a well-conducted study. It was also the only bioassay available. Additional bioassays might add support to the findings or provide results for different (possibly lower) doses, which may affect the inhalation unit risk.
Selection of extrapolation approach Different PBPK model could ↓ or ↑ inhalation unit risk.	PBPK model-based extrapolation of oral data used for inhalation unit risk.	PBPK model accurately predicted *tert*-butanol toxicokinetics. Data and model predictions were within 2-fold of each other.
Selection of dose metric Alternatives could ↓ or ↑ inhalation unit risk.	Used *tert*-butanol concentration in blood as the dose metric for route-to-route extrapolation, converted to HEC.	In the absence of evidence that would suggest that metabolites of *tert*-butanol are responsible for carcinogenicity, it is assumed that *tert*-butanol itself is the active toxicological agent. An alternative dose metric of *tert*-butanol metabolism would result in a 1.2-fold decrease in the inhalation unit risk.
Interspecies extrapolation of dosimetry and risk Alternatives could ↓ or ↑ inhalation unit risk..	The default approach for a Category 3 gas was used.	There are no data to suggest an alternative approach. While the true human correspondence is unknown, this overall approach is expected to neither over- nor underestimate human equivalent risks.
Dose-response modeling Alternatives could ↓ or ↑ inhalation unit risk.	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.
Low-dose extrapolation ↓ cancer risk estimate would be expected with the application of nonlinear low-dose extrapolation .	Linear extrapolation of risk in low-dose region used.	Linear low-dose extrapolation for agents without a known MOA is supported.
Statistical uncertainty at POD ↓ inhalation unit risk 1.7-fold if the BMD used to derive the inhalation POD rather than BMDL.	BMDL (preferred approach for calculating plausible upper bound)	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure at 10% extra risk of renal tumors.
Sensitive subpopulations	No sensitive populations	No chemical-specific data are available to

Consideration and Impact on Cancer Risk Value	Decision	Justification and Discussion
↑ inhalation unit risk to unknown extent.	have been identified.	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.

2.4.5. Previous IRIS Assessment: Inhalation Unit Risk

A 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 applied to account for early-life exposure to carcinogens that act through a mutagenic mode of action. Because chemical-specific life-stage susceptibility data for cancer are not available, and because the mode of action for *tert*-butanol carcinogenicity is not known (see Section 1.1.4), ADAFs were not applied.

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