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

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ABBREVIATIONS

AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MTD	maximum tolerated dose
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
POD	point of departure
$POD_{[ADJ]}$	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
U.S.	United States

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PREFACE

This Toxicological Review critically reviews the publicly available studies on *tert*-butyl alcohol (*tert*-butanol) to identify its adverse health effects and to characterize exposure-response relationships. The assessment examined all effects by oral and inhalation routes of exposure and includes an oral noncancer reference dose (RfD), an inhalation noncancer reference concentration (RfC), a cancer weight of evidence descriptor, and a cancer dose-response assessment. It was prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) program. This is the first IRIS assessment for this chemical.

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

- *tert*-Butanol is one of the primary metabolites of ETBE, and some of the toxicological effects of ETBE are attributed to *tert*-butanol. Therefore, data on ETBE are considered informative for the hazard identification and dose-response assessment of *tert*-butanol, and vice versa.
- The scientific literature for the two chemicals includes data on alpha2u-globulin -related nephropathy; therefore, a common approach was employed to evaluate these data as they relate to the mode of action for kidney effects.
- A combined physiologically based pharmacokinetic (PBPK) model for *tert*-butanol and ETBE in rats was applied to support the dose-response assessments for these chemicals.

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.

A public science meeting was held on June 30, 2016 to provide the public an opportunity to engage in early discussions on the draft IRIS toxicological review and the draft charge to the peer review panel prior to release for external peer review. The complete set of public comments, including the slides presented at the June 2016 public science meeting, is available on the docket at http://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2013-1111).

Organ/system-specific reference values are calculated based on kidney and thyroid toxicity data. These reference values could be useful for cumulative risk assessments that consider the combined effect of multiple agents acting on the same biological system.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to IRIS Toxicological Reviews. The findings of this assessment and related documents produced during its development are available on the IRIS website (http://www.epa.gov/iris). Appendices for toxicokinetic information, PBPK modeling, genotoxicity study summaries, dose-response modeling, and other information are provided as Supplemental Information to this Toxicological Review. For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), 202-566-1749 (fax), or hotline.iris@epa.gov.

Uses

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

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

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

Fate and Transport

Soil

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

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

(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 occurs over weeks to months and in anaerobic aquatic conditions, the biodegradation rate decreases. Bioconcentration of *tert*-butanol in aquatic organisms is low (HSDB, 2007).

Air

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

Occurrence in the Environment

The Toxics Release Inventory (TRI) Program National Analysis Report estimated that more than 1 million pounds of *tert*-butanol has been released into the soil from landfills, land treatment, underground injection, surface impoundments, and other land disposal sources. In 2014, the TRI program also reported 1,845,773 pounds of *tert*-butanol released into the air, discharged to bodies of water, disposed at the facility to land, and disposed in underground injection wells (U.S. EPA, 2016). Total off-site disposal or other releases of *tert*-butanol amounted to 67,060 pounds (U.S. EPA, 2016). 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).

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

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

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

General Population Exposure

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

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

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

Assessments by Other National and International Health Agencies

Toxicity information on *tert*-butanol has been evaluated by the National Institute for Occupational Safety and Health (NIOSH, 2007), the Occupational Safety and Health Administration (OSHA, 2006), and the Food and Drug Administration (FDA, 2015, 2011). The results of these assessments are presented in Appendix A of the Supplemental Information to this Toxicological Review. Of importance to recognize is that these earlier assessments could have been prepared for different purposes and might use different methods. In addition, newer studies have been included in the IRIS assessment.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

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

1. Objectives and Scope of the IRIS Program

Soon after EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in support of actions to protect human health and the environment. EPA's IRIS program[3] contributes to this endeavor by reviewing epidemiologic and experimental studies of chemicals in the environment to identify adverse health effects and characterize exposure–response relationships. Health agencies worldwide use IRIS assessments, which are also a scientific resource for researchers and the public.

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

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

IRIS assessments follow EPA guidance[4] and standardized practices of systematic review. This Preamble summarizes and does not change IRIS operating procedures or EPA guidance.

Periodically, the IRIS program asks for nomination of agents for future assessment or reassessment. Selection depends on EPA's priorities, relevance to public health, and availability of pertinent studies. The IRIS multiyear agenda[5] lists upcoming assessments. The IRIS program may also assess other agents in anticipation of public health needs.

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

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

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

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

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

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

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

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

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

3. Identifying and Selecting Pertinent Studies

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

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

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

The IRIS program posts initial protocols for literature searches on its website and adds search results to EPA's HERO database.[6] Then the IRIS program takes extra steps to

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

ensure identification of pertinent studies: by encouraging the scientific community and the public to identify additional studies and ongoing research; by searching for data submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act; and by considering late-breaking studies that would impact the credibility of the conclusions, even during the review process.[7]

4. Evaluating Study Methods and Quality

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

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

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

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

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

5. Integrating the Evidence of Causation for Each Health Outcome

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

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

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

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

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

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

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

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

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

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

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

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

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

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

6. Selecting Studies for Derivation of Toxicity Values

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

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

Derivation of toxicity values begins with a new evaluation of studies, as some studies used qualitatively for hazard identification may not be useful quantitatively for exposure–response assessment. Quantitative analyses require quantitative measures of exposure and response. An assessment weighs the merits of the human and animal studies, of various animal models, and of different routes and durations of exposure (U.S. EPA, 1994b). Study selection is not reducible to a formula, and each assessment explains its approach.

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

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

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

7. Deriving Toxicity Values

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

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

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

For human studies, an assessment may develop exposure–response models that reflect the structure of the available data (U.S. EPA, 2005b). For animal studies, EPA has developed a set of empirical ("curve-fitting") models[8] that can fit typical data sets (U.S. EPA, 2005b). Such modeling yields a *point of departure*, defined as a dose near the lower end of the observed range, without significant extrapolation to lower levels (e.g., the estimated dose associated with an extra risk of 10% for animal data or 1% for human data, or their 95% lower confidence limits)(U.S. EPA, 2005b), (U.S. EPA, 2012b).

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

For a group of agents that act at a common site or through common mechanisms, an assessment may derive relative potency factors based on relative toxicity, rates of absorption or metabolism, quantitative structure–activity relationships,

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

or receptor-binding characteristics (U.S. EPA, 2005b).

Extrapolation: slope factors and unit risks. An *oral slope factor* or an *inhalation unit risk* facilitates subsequent estimation of human cancer risks. Extrapolation proceeds linearly (i.e., risk proportional to dose) from the point of departure to the levels of interest. This is appropriate for agents with direct mutagenic activity. It is also the default if there is no established mode-of-action (U.S. EPA, 2005b).

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

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

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

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

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

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

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

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

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

8. Process for Developing and Peer-Reviewing IRIS Assessments

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

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

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

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

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

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

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

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

9. General Structure of IRIS Assessments

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

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

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

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

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

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

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

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

August 2016

10. Preamble References

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U.S. EPA. (2012). Benchmark dose technical guidance (pp. 1-99). (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S. EPA. (2014). Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation. (EPA/100/R-14/002F). Washington, DC: Risk Assessment Forum, Office of the Science Advisor. http://www.epa.gov/raf/DDEF/pdf/ddef-final.pdf

EXECUTIVE SUMMARY

Summation of Occurrence and Health Effects

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

Animal studies demonstrate that chronic oral exposure to *tert*-butanol is associated with kidney and thyroid effects. No chronic inhalation exposure studies have been conducted. Evidence is suggestive of carcinogenic potential for *tert*-butanol, based on thyroid tumors in male and female mice and renal tumors in male rats.

Effects Other Than Cancer Observed Following Oral Exposure

Kidney effects are a potential human hazard of oral exposure to *tert*-butanol. Kidney toxicity was observed in males and females in two strains of rats. Kidney weights were increased in male and female rats after 13 weeks or 15 months of treatment. Histopathological examination in male and female rats showed increased incidence or severity of nephropathy after 13 weeks of oral exposure, increases in severity of nephropathy after 2 years of oral exposure, and increased transitional epithelial hyperplasia after 2 years of oral exposure. Additionally, increased suppurative inflammation was noted in females after 2 years of oral exposure. In one strain of mice, the only kidney effect observed was an increase in kidney weight (absolute or relative) in female mice after 13 weeks, but no treatment-related histopathological lesions were reported in the kidneys of male or female mice at 13 weeks or 2 years. A mode of action (MOA) analysis determined that *tert*-butanol exposure induces a male rat-specific alpha2u-globulin -associated nephropathy. *tert*-Butanol, however, is a weak inducer of alpha2u-globulin nephropathy, which is not the sole process contributing to renal tubule nephropathy. Chronic progressive nephropathy (CPN) might also be involved in some noncancer effects, but the data are complicated by alpha2u-globulin nephropathy in males. Effects attributable to alpha2u-globulin nephropathy in males were not considered for kidney hazard identification. Females are not affected by alpha2u-globulin nephropathy, so changes in kidney weights in female rats, transitional epithelial hyperplasia in female rats, suppurative inflammation in female rats, and severity and incidence of nephropathy in female rats are considered to result from *tert*-butanol exposure and are appropriate for identifying a hazard to the kidney.

At this time, evidence of selective developmental toxicity, neurodevelopmental toxicity, and reproductive system toxicity following *tert*-butanol exposure is inadequate with minimal effects observed at otherwise toxic dose levels. The available information also is inadequate to draw conclusions regarding liver and urinary bladder toxicity, respectively, because of lack of consistency and lack of progression.

Oral Reference Dose (RfD) for Effects Other Than Cancer

Kidney toxicity, represented by increases in severity of nephropathy in female rats, was chosen as the basis for the overall oral reference dose (RfD) (see Table ES-1). The kidney effects observed in female rats in the chronic study by NTP (1995) were used to derive the RfD. Increased severity of nephropathy was selected as the critical effect because it was observed in female rats consistently, it is an indicator of kidney toxicity, and it was induced in a dose-responsive manner. Dose-response data were not amenable to modeling; accordingly, the point of departure was derived from the lowest-observed-adverse-effect level (LOAEL) of 43 mg/kg-day (U.S. EPA, 2011a).

The overall RfD was calculated by dividing the POD for increases in severity of nephropathy by a composite uncertainty factor (UF) of 100 to account for the extrapolation from animals to humans (3), derivation from a LOAEL (3), and for interindividual differences in human susceptibility (10).

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

Hazard	Basis	Point of departure* (mg/kg-day)	UF	Chronic RfD (mg/kg-day)	Study exposure description	Confidence
Kidney	Increases in severity of nephropathy	43.2	100	4×10^{-1}	Chronic	Medium
Overall RfD	**Kidney**	**43.2**	**100**	**4×10^{-1}**	**Chronic**	**Medium**

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

Effects Other Than Cancer Observed Following Inhalation Exposure

Kidney effects are a potential human hazard of inhalation exposure to *tert*-butanol. Although no effects were observed in mice, kidney weights were increased in male and female rats following 13 weeks of inhalation exposure. In addition, the severity of nephropathy increased in male rats. No human studies are available to evaluate the effects of inhalation exposure. As discussed above for oral effects, endpoints in males specifically related to alpha2u-globulin nephropathy were not considered for kidney hazard identification. Changes in kidney weights and severity of nephropathy in females, however, are considered a result of *tert*-butanol exposure and are appropriate for identifying a hazard to the kidney.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

Kidney toxicity, represented by increases in severity of nephropathy, was chosen as the basis for the RfC (see Table ES-2). Although endpoints from a route-specific study were considered, the availability of a physiologically based pharmacokinetic (PBPK) model for *tert*-butanol in rats (Borghoff et al., 2016) allowed for more specific and sensitive equivalent inhalation PODs derived from a route-to-route extrapolation from the PODs of the oral NTP (1995) study. The POD adjusted for the human equivalent concentration (HEC) was 491 mg/m^3 based on increases in severity of nephropathy.

As discussed in Section 2.2.2, it is recognized that there is uncertainty in route-to-route extrapolation because actual risk may not correlate exactly with the internal dose metric used for the extrapolation (in this case, average blood concentration of *tert*-butanol). The U.S. EPA is not aware of a quantitative analysis of such uncertainty, which would involve comparison of cross-route extrapolation to toxicity data for a number of chemicals and endpoints sufficient to characterize the accuracy of the approach. Such an analysis is beyond the scope of this assessment. However, it is the U.S. EPA's judgment that this uncertainty is less than the uncertainty of the alternative, which would be to base the RfC on the subchronic toxicity data. In particular, toxicity to the kidney requires that *tert*-butanol be systemically distributed in the blood, hence must be correlated with some measure of blood concentration. The uncertainty in the extrapolation occurs because the metric used might not accurately predict the effect, vs. other possible metrics such as peak concentration.

The RfC was calculated by dividing the POD by a composite UF of 100 to account for toxicodynamic differences between animals and humans (3), derivation from a LOAEL (3), and interindividual differences in human susceptibility (10).

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

Hazard	Basis	Point of departure* (mg/m^3)	UF	Chronic RfC (mg/m^3)	Study exposure description	Confidence
Kidney	Increases in severity of nephropathy	491	100	5×10^0	Chronic	Medium
Overall RfC	**Kidney**	**491**	**100**	**5×10^0**	**Chronic**	**Medium**

*Continuous inhalation HEC that leads to the same average blood concentration of *tert*-butanol as drinking water exposure to the rat at the BMDL.

Evidence of Human Carcinogenicity

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

Quantitative Estimate of Carcinogenic Risk from Oral Exposure

In accordance with EPA's guidance on alpha2u-globulin (U.S. EPA, 1991b), rat kidney tumors are unsuitable for quantitative analysis because not enough data are available to determine the relative contribution of alpha2u-globulin nephropathy and other processes to the overall kidney tumor response. A quantitative estimate of carcinogenic potential from oral exposure to *tert*-butanol was based on the increased incidence of thyroid follicular cell adenomas in female $B6C3F_1$ mice and thyroid follicular cell adenomas and carcinomas in male $B6C3F_1$ mice (NTP, 1995). The study included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting of methods and results.

Although *tert*-butanol was considered to have only "suggestive evidence of carcinogenic potential," the NTP study was well conducted and suitable for quantitative analysis. Slope factors were derived for thyroid tumors in female or male mice. The modeled *tert*-butanol POD was scaled to HEDs according to EPA guidance by converting the $BMDL_{10}$ on the basis of (body weight) $^{3/4}$ scaling (U.S. EPA, 2011a, 2005a). Using linear extrapolation from the $BMDL_{10}$, a human equivalent oral slope factor was derived (slope factor = $0.1/BMDL_{10}$). The resulting oral slope factor is **5×10^{-4} per mg/kg-day**.

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

No chronic inhalation studies of exposure to *tert*-butanol are available. Although the mouse thyroid tumors served as the basis for the oral slope factor, route-to-route extrapolation is not possible for these thyroid effects in mice because the only PBPK model available is for rats. Therefore, no quantitative estimate of carcinogenic risk could be determined for inhalation exposure.

Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

Information is inadequate to identify any populations or lifestages that might be especially susceptible to *tert*-butanol.

Key Issues Addressed in Assessment

The human relevance of the kidney effects observed in male and female rats was analyzed in the assessment, particularly as they relate to alpha2u-globulin nephropathy and the exacerbation of chronic progressive nephropathy. An evaluation of whether *tert*-butanol caused alpha2u-globulin -associated nephropathy was performed using the EPA 1991 and IARC 1999 frameworks evaluated (U.S. EPA, 1991a; Capen, 1999, 699905). The presence of alpha2u-globulin in the hyaline droplets was confirmed in male rats by alpha2u-globulin immunohistochemical staining. Linear mineralization and tubular hyperplasia were reported in male rats, although only in the chronic study. Other subsequent steps in the pathological sequence, including necrosis, exfoliation, and granular casts, either were absent or inconsistently observed across subchronic or chronic studies. None of these effects occurred in female rats or in either sex of mice, although these endpoints were less frequently evaluated in these models. Evidence implies that an alpha2u-globulin MOA is operative, although it is relatively weak in response to *tert*-butanol and is not solely responsible for the renal tubule nephropathy observed in male rats. CPN also plays a role in the exacerbation of nephropathy in both male and female rats, however the MOA of CPN is unknown, and therefore, its potential relevance to humans cannot be ruled out (NIEHS, 2019). Several other effects in the kidney unrelated to alpha2u-globulin were observed in female rats, including suppurative inflammation, transitional epithelial hyperplasia, and increased kidney weights (NTP, 1997, 1995). These specific effects observed in female rats, not confounded by alpha2u-globulin related processes, are considered the result of *tert*-butanol exposure and therefore relevant to humans.

Concerning cancer, alpha2u-globulin accumulation is indicated as relatively weak in response to *tert*-butanol exposure and not the sole mechanism responsible for the renal tubule carcinogenicity observed in male rats. CPN and other effects induced by both alpha2u-globulin processes and *tert*-butanol play a role in renal tubule nephropathy, and the evidence indicates that CPN augments the renal tubule tumor induction associated with *tert*-butanol exposure in male rats. Poor dose-response relationships between alpha2u-globulin processes and renal tumors in male rats and a lack of renal tumors in female rats despite increased CPN severity, however, suggest that other, unknown processes contribute to renal tumor development. Based on this analysis of available MOA data, these renal tumors are considered relevant to humans.

In addition, an increase in the incidence of thyroid follicular cell adenomas was observed in male and female mice in a 2-year drinking water study (NTP, 1995). Thyroid follicular cell hyperplasia was considered a preneoplastic effect associated with the thyroid tumors, and the incidences of follicular cell hyperplasias were elevated in both male and female B6C3F$_1$ mice following exposure. U.S. EPA (1998b) describes the procedures the Agency uses in evaluating chemicals that are animal thyroid carcinogens. The available evidence base is inadequate for concluding that an antithyroid MOA is operating in mouse thyroid follicular cell tumorigenesis. No other MOAs for thyroid tumors were identified, and the mouse thyroid tumors are considered relevant to humans (U.S. EPA (1998a).

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

A literature search and screening strategy was 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 using the most common synonyms and trade names 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 core online scientific databases, PubMed, Web of Science, and Toxline. as well as TSCATS through December 2016, using the keywords and limits described in Table LS-1. The overall literature search approach is shown graphically in Figure LS-1. Eight more citations were obtained using additional search strategies described in Table LS-2. After electronically eliminating duplicates from the citations retrieved through these databases, 3,138 unique citations were identified.

The resulting 3,138 citations were screened for pertinence and separated into categories as presented in Figure LS-1 using the title and either abstract or full text, or both, to examine the health effects of *tert*-butanol exposure. The inclusion and exclusion criteria used to screen the references and identify sources of health effects data are provided in Table LS-3.

- 12 references were identified as “Sources of Health Effects Data” and were considered for data extraction to evidence tables and exposure-response arrays.
- 202 references were identified as “Sources of Mechanistic and Toxicokinetic Data” and “Sources of Supporting Health Effects Data”; these included 41 studies describing physiologically based pharmacokinetic (PBPK) models and other toxicokinetic information, 73 studies providing genotoxicity and other mechanistic information, 1 human case report, 74 irrelevant exposure paradigms (including acute, dermal, eye irritation, and injection studies), 6 preliminary toxicity studies, and 7 physical dependency studies. Information from these studies was not extracted into evidence tables; however, these studies were considered as support for assessing *tert*-butanol health effects, for example, evaluation of mode of action and extrapolation of experimental animal findings to humans. Additionally, although still considered sources of health effects information, studies investigating the effects of acute and direct chemical exposures are generally less pertinent for characterizing health hazards associated with chronic oral and inhalation exposure. Therefore, information from these studies was not considered for extraction into evidence tables. Nevertheless, these studies were still evaluated as possible sources of supplementary health effects information.

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

- 2,796 references were identified as not being pertinent (not on topic) to an evaluation of the health effects of *tert*-butanol and were excluded from further consideration (see Figure LS-1 for exclusion categories and Table LS-3 for exclusion criteria). For example, health effect studies of gasoline and *tert*-butanol mixtures were not considered pertinent to the assessment because the separate effects of the gasoline or other chemical components could not be determined. Retrieving a large number of references that are not on topic is a consequence of applying an initial search strategy designed to cast a wide net and to minimize the possibility of missing potentially relevant health effects data.

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

Selection of Studies for Inclusion in Evidence Tables

To summarize the important information systematically from the primary health effects studies in the *tert*-butanol evidence base, evidence tables were constructed in a standardized tabular format as recommended by NRC (2011). Studies were arranged in evidence tables by effect, species, duration, and design, and not by quality. Of the studies retained after the literature search and screen, 12 studies were identified as "Sources of Health Effects Data" and were considered for extraction into evidence tables for hazard identification in Chapter 1. Initial review found two references (Cirvello et al., 1995; Lindamood et al., 1992) to be publications of the NTP (1995) data prior to the release of the final National Toxicology Program (NTP) report. One publication (Takahashi et al., 1993) in the "Supplementary Studies" category also was based on data from the NTP report. The interim publications and the final NTP report differed. The finalized NTP (1995) report was considered the more complete and accurate presentation of the data; therefore, this report was included in evidence tables and Cirvello et al. (1995), Takahashi et al. (1993), and Lindamood et al. (1992) were not. Data from the remaining 10 references in the "Sources of Health Effects Data" category were extracted into evidence tables.

Supplementary studies that contain pertinent information for the toxicological review and augment hazard identification conclusions, such as genotoxic and mechanistic studies, studies describing the kinetics and disposition of *tert*-butanol absorption and metabolism, pilot studies, and one case report, were not included in the evidence tables. Short-term and acute studies (including an 18-day study and a 14-day study by NTP), which used oral and inhalation exposures performed primarily in rats, did not differ qualitatively from the results of the longer studies (i.e., ≥30-day exposure studies). These were grouped as supplementary studies, however, because the evidence base of chronic and subchronic rodent studies was considered sufficient for evaluating chronic health effects of *tert*-butanol exposure. Additionally, studies of effects from chronic exposure are most pertinent to lifetime human exposure (i.e., the primary characterization provided by IRIS assessments) and are the focus of this assessment. Such supplementary studies are discussed in the narrative sections of Chapter 1 and are described in sections such as the "Mode of Action Analysis" to augment the discussion or presented in appendices, if they provide additional information.

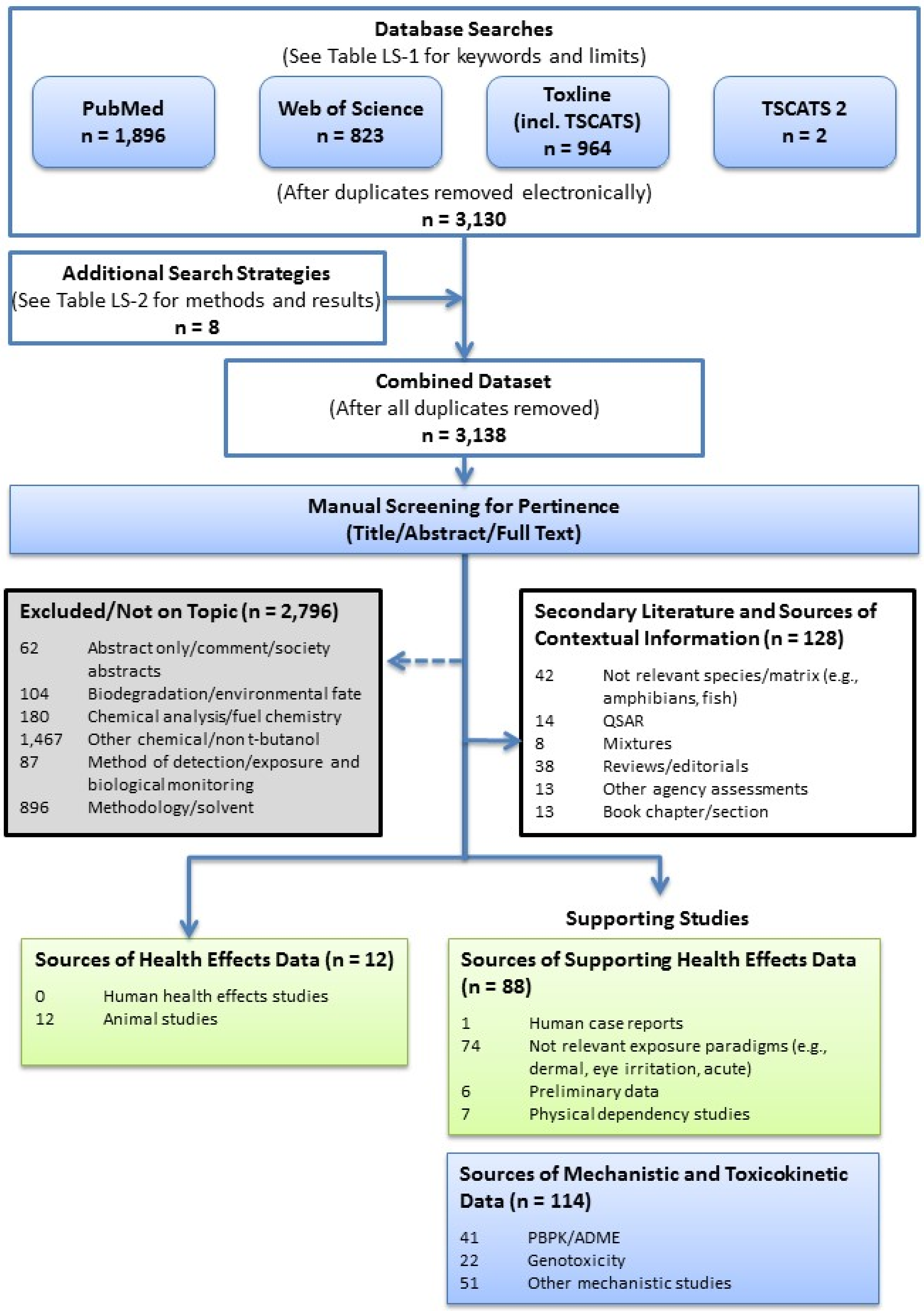


Figure LS-1. Summary of literature search and screening process for *tert*-butanol.

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

Database (Search date)	Keywords	Limits
PubMed (12/20/2012) (4/17/2014) (5/13/2015) (12/31/2016) (7/5/2019)*	*tert*-butanol *OR 75-65-0[rn] OR "t-butyl hydroxide" OR "2-methyl-2-propanol" OR "trimethyl carbinol" OR "t-butyl alcohol" OR tert-butanol OR "tert-butyl alcohol" OR tert-butyl alcohol[mesh]*	None
Web of Science (12/20/2012) (4/17/2014) (5/13/2015) (12/31/2016) (7/5/2019)*	Topic = *(tert-butanol OR 75-65-0 OR "t-butyl hydroxide" OR "2-methyl-2-propanol" OR "trimethyl carbinol" OR "t-butyl alcohol" OR "tert-butanol" OR "tert-butyl alcohol")*	Refined by: Research Areas = (cell biology OR respiratory system OR microscopy OR biochemistry molecular biology OR gastroenterology OR hepatology OR public environmental occupational health OR oncology OR physiology OR cardiovascular system cardiology OR toxicology OR life sciences biomedicine other topics OR hematology OR pathology OR neurosciences neurology OR developmental biology)
Toxline (includes TSCATS) (1/11/2013) (4/17/2014) (5/13/2015) (12/31/2016) (7/5/2019)*	*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-butyl alcohol*	Not PubMed
TSCATS2 (1/4/2013) (4/17/2014) (5/13/2015) (12/31/2016)	75-65-0	None

***:** See post-peer-review literature search update section

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 and public comments	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

Approach used	Source(s)	Date performed	Number of additional references identified
	Public comment article: (Borghoff et al., 2016)	10/2016	1
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: Occupational Safety and Health Administration.	1/2013	None

Table LS-3. Inclusion-exclusion criteria

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

	Inclusion criteria	Exclusion criteria/Supplemental material*
		• Chemical, physical, or fuel chemistry studies • Analytical methods for measuring/detecting/ remotely sensing *tert*-butanol • Use of *tert*-butanol as a solvent or methodology for testing unrelated to *tert*-butanol • Not chemical specific: Studies that do not involve testing of *tert*-butanol • Foreign language studies that were not considered further because, based on title or abstract, judged not potentially relevant • QSAR studies

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

Evidence base Evaluation

For this draft assessment, 12 references reported on experimental animal studies that comprised the primary sources of health effects data; no studies were identified that evaluated humans exposed to *tert*-butanol (e.g., cohort studies, ecological studies). The animal studies were evaluated using the study quality considerations outlined in the Preamble, considering aspects of design, conduct, or reporting that could affect the interpretation of results, overall contribution to the synthesis of evidence, and determination of hazard potential as noted in various EPA guidance documents (U.S. EPA, 2005a, 1998d, 1996b, 1991b). The objective was to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design. As stated in the Preamble, studies were evaluated to identify the suitability of the study based on:

- Study design
- Nature of the assay and validity for its intended purpose
- Characterization of the nature and extent of impurities and contaminants of *tert*-butanol administered, if applicable
- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects
- Sample sizes and statistical power to detect dose-related differences or trends
- Ascertainment of survival, vital signs, disease or effects, and cause of death
- Control of other variables that could influence the occurrence of effects

Additionally, several general considerations, presented in Table LS-4, were used in evaluating the animal studies. Much of the key information for conducting this evaluation can be determined based on study methods and how the study results were reported. Importantly, the evaluation at this stage does not consider the direction or magnitude of any reported effects.

EPA considered statistical tests to evaluate whether the observations might be due to chance. The standard for determining statistical significance of a response is a trend test or comparison of outcomes in the exposed groups against those of concurrent controls. Studies that did not report statistical testing were identified and, when appropriate, statistical tests were conducted by EPA.

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

Experimental Animal Studies

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

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

Methodological feature	Considerations (relevant information extracted into evidence tables)
Test animal	Suitability of the species, strain, sex, and source of the test animals
Experimental design	Suitability of animal age/lifestage at exposure and endpoint testing; periodicity and duration of exposure (e.g., hr/day, day/week); timing of endpoint evaluations; and sample size and experimental unit (e.g., animals, dams, litters)
Exposure	Characterization of test article source, composition, purity, and stability; suitability of the control (e.g., vehicle control); documentation of exposure techniques (e.g., route, chamber type, gavage volume); verification of exposure levels (e.g., consideration of homogeneity, stability, analytical methods)
Endpoint evaluation	Suitability of specific methods for assessing the endpoint(s) of interest
Results presentation	Data presentation for endpoint(s) of interest (including measures of variability) and for other relevant endpoints needed for results interpretation (e.g., maternal toxicity, decrements in body weight relative to organ weight)

Table LS-5. Summary of experimental animal evidence base

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

Post-Peer-Review Literature Search Update

A post-peer-review literature search update was conducted in PubMed, Web of Science, and Toxline for the period January 2017 to July 2019 using a search strategy consistent with previous literature searches (see LS-1).

Consistent with the IRIS Stopping Rules (https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf), manual screening of the literature search update focused on identifying new studies that might change a major conclusion of the assessment. No references were identified in the post-peer-review literature search which would change any major conclusions in the assessment.

The documentation and results for the literature search and screen, including the specific references identified using each search strategy and tags assigned to each reference based on the manual screen, can be found on the HERO website on the *tert*-butanol project page at: (https://hero.epa.gov/hero/index.cfm/project/page/project_id/1543).

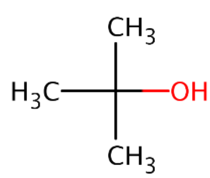
1 HAZARD IDENTIFICATION

1.1 OVERVIEW OF CHEMICAL PROPERTIES AND TOXICOKINETICS

1.1.1 Chemical Properties

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

Table 1-1. Chemical identity and physicochemical properties of *tert*-Butanol as curated by EPA's CompTox Chemicals Dashboard

Characteristic or property	Value	
Chemical structure		
CASRN	75-65-0	
Synonyms	2-Propanol, 2-methyl- , 1,1-Dimethylethanol , 2-Methylpropan-2-ol, 2-Methylpropan-2-ol(other name: tert-Butylalcohol), 2-methylpropane-2-ol, 2-metilpropan-2-ol, t-Butanol, Trimethyl carbinol, Trimethylcarbinol, Trimethylmethanol, Arconol, t-Butyl hydroxide, tert-Butanol, (see https://comptox.epa.gov/dashboard for additional synonyms)	
Molecular formula	$C_4H_{10}O$	
Molecular weight ($g.mol^{-1}$)	74.123	
	Average experimental value[a]	**Average predicted value[a]**
Flash point (°C)	—	17.5
Boiling point (°C)	82.8	79.7
Melting point (°C)	25.2	-30.4
Log K_{OW}	3.50	3.78
Water solubility (mol/L)	13.5	3.61
Density (g/cm^3)	—	0.833
Henry's law constant ($atm\text{-}m^3/mole$)	9.05×10^{-6}	9.01×10^{-6}

Vapor pressure (mm Hg at 20°C)	40.7	52.7

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

1.1.2 Toxicokinetics

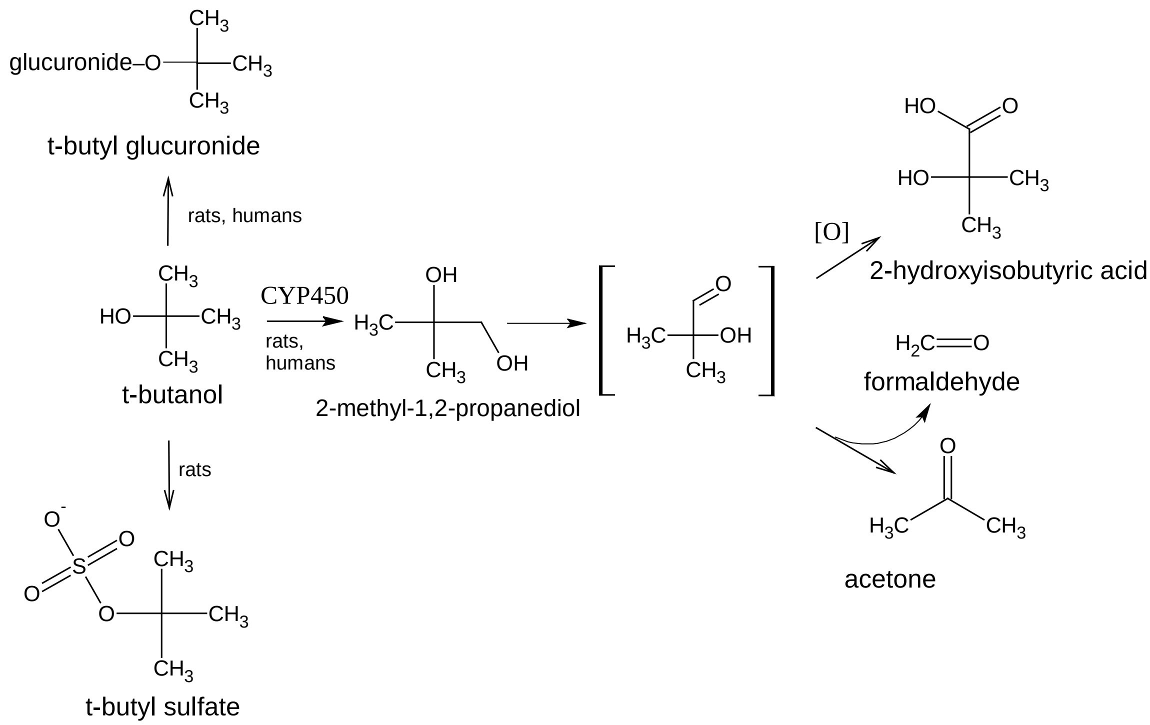
tert-Butanol is rapidly absorbed following exposure by oral and inhalation routes (see Appendix B, Section B.1.1). Studies in experimental animals indicate that 99% of the compound was absorbed after oral administration. Comparable blood levels of *tert*-butanol and its metabolites also have been observed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

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

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

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

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



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

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

1.1.3 Description of Toxicokinetic Models

While no models of *tert*-butanol have been created independently of other chemicals from which it arises as a metabolite (e.g., MTBE, ETBE), *tert*-butanol sub-models have been adapted specifically to estimate internal doses for administration of *tert*-butanol. In particular, some of these *tert*-butanol sub-models were parameterized using pharmacokinetic studies with *tert*-butanol exposures and three physiologically based pharmacokinetic (PBPK) models have been developed. These PBPK models can be used to simulate direct administration of *tert*-butanol in rats, in addition to exposure to the parent compound for each: Leavens and Borghoff (2009); Salazar et al. (2015), and Borghoff et al. (2016). Other models have incorporated *tert*-butanol as a sub-model following MTBE administration but were not considered further because they do not include terms for direct exposure to *tert*-butanol (e.g., Rao and Ginsberg, 1007). In Leavens and Borghoff (2009), *tert*-butanol is incorporated as a metabolite of MTBE; in Salazar et al. (2015) and Borghoff et al. (2016), it is incorporated as a metabolite of ETBE. With all three models, inhalation and oral exposure to *tert*-butanol can be simulated in rats; i.e. with exposure to the parent MTBE or ETBE set to zero. A more detailed summary and evaluation of the toxicokinetic models is provided in Appendix B of the Supplemental Information (Sections B.1.5. and B.1.7.).

1.1.4 Chemicals Extensively Metabolized to *tert*-Butanol

tert-Butanol is a metabolite of other compounds, including ETBE, MTBE, and *tert*-butyl acetate. Some of the toxicological effects observed for these compounds are attributed to *tert*-butanol. Animal studies demonstrate that chronic exposure to ETBE is associated with noncancer kidney effects, including increased kidney weights in male and female rats accompanied by increased chronic progressive nephropathy (CPN), urothelial hyperplasia (in males), and increased blood concentrations of total cholesterol, blood urea nitrogen, and creatinine (Saito et al., 2013; Suzuki et al., 2012). In these studies, increased liver weight and centrilobular hypertrophy also were observed in male and female rats exposed to ETBE. Liver adenomas and carcinomas were increased in male rats following 2-year inhalation exposure (Saito et al., 2013).

In 1996, the U.S. Agency for Toxic Substances and Disease Registry's (ATSDR) *Toxicological Profile for MTBE* (ATSDR, 1996) identified cancer effect levels of MTBE based on carcinogenicity data in animals. ATSDR reported that inhalation exposure was associated with kidney cancer in rats and liver cancer in mice. ATSDR concluded that oral exposure to MTBE might cause liver and kidney damage and nervous system effects in rats and mice. The chronic inhalation minimal risk level was derived based on incidence and severity of chronic progressive nephropathy in female rats (ATSDR, 1996). In 1997, EPA's Office of Water concluded that MTBE is carcinogenic to animals and poses a potential carcinogenic potential to humans based on an increased incidence of Leydig cell adenomas of the testes, kidney tumors, lymphomas, and leukemia in exposed rats (U.S. EPA, 1997). In 1998, the International Agency for Research on Cancer (IARC) found "limited evidence" of MTBE carcinogenicity in animals and placed MTBE in Group 3 (i.e., not classifiable as to carcinogenicity in humans) (IARC, 1999). IARC reported that oral exposure in rats resulted in testicular tumors in males and lymphomas and leukemias (combined) in females; inhalation exposure in male rats resulted in renal tubule adenomas; and inhalation exposure in female mice resulted in hepatocellular adenomas (IARC, 1999).

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

1.2 PRESENTATION AND SYNTHESIS OF EVIDENCE BY ORGAN/SYSTEM

1.2.1 Kidney Effects

Synthesis of Effects in Kidney

This section reviews the studies that investigated whether subchronic or chronic exposure to *tert*-butanol can affect kidneys in humans or animals. The evidence base examining kidney effects following *tert*-butanol exposure contains eight studies (from five references) performed in rats or mice (Huntingdon Life Sciences, 2004; Acharya et al., 1997; NTP, 1997; Acharya et al., 1995; NTP, 1995) and a reevaluation of the rat data from NTP (1995), published by Hard et al. (2011) and Hard et al. (2019); no human data are available. Studies using short-term and acute exposures that examined kidney effects are not included in the evidence tables; they are discussed in the text, however, if they provide data to inform mode of action (MOA) or hazard identification. *tert*-Butanol exposure resulted in kidney effects after both oral (drinking water) and inhalation exposure in both sexes of rats (Table 1-1, Table 1-2, Figure 1-1, and Figure 1-2); studies are arranged in the evidence tables first by effect, then by route, and then duration.

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

Kidney weight. Kidney weight was observed to increase in male and female F344 rats following exposures of 13 weeks (oral and inhalation) (NTP, 1997) and 15 months (oral) (NTP, 1995). Huntingdon Life Sciences (2004) also reported increased kidney weight in Sprague-Dawley rats administered *tert*-butanol orally for approximately 10 weeks (tabular data presented in the Supplemental Information to this Toxicological Review). Dose-related increase in kidney weight was also observed in both male and female rats (Spearman's rank coefficient > 0.72) following either oral or inhalation exposures (Figure 1-3), and in female mice following inhalation exposure (Spearman's rank coefficient = 0.9).

Measures of relative, as opposed to absolute, organ weight are sometimes preferred because they account for changes in body weight that might influence changes in organ weight (Bailey et al., 2004), although potential impact should be evaluated. However, for *tert*-butanol, body weight in exposed animals noticeably decreased at the high doses relative to controls in the oral 13-week and 2-year studies (NTP, 1995). Thus, use of relative organ weight change would not be a reliable measure of kidney weight change for this assessment. Although relative and absolute kidney weight data are both presented in exposure-response arrays (and in evidence tables in Appendix B of the Supplemental Information), the absolute measures were considered more informative for determining *tert*-butanol hazard potential. Support for this judgement can be found in a 2014 analysis which indicates that increased absolute, but not relative, subchronic kidney weights are significantly correlated with chemically induced histopathological findings in the kidney in chronic and subchronic studies (Craig et al., 2014).

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

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

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

The lesions collectively described by NTP (1997, 1995) as nephropathy and noted as common spontaneous lesions in rats are consistent with CPN. CPN is not a specific diagnosis per se but, rather, an aggregate term describing a spectrum of effects. The morphological spectrum observed in CPN in male rats does not appear to have a human analogue in the aging kidney (NTP 2019). However, several individual lesions noted in CPN (e.g. tubule atrophy, tubule dilation, thickening of tubular basement membranes, glomerulosclerosis) also occur in the human kidney

(Lusco et al., 2016; Zoja et al., 2015; Frazier et al., 2012; Satirapoj et al., 2012; NIEHS, 2019). Therefore, exacerbation of one or more of these lesions following *tert*-butanol exposure may reflect some type of cell injury or inflammatory process, which is relevant to the human kidney.

Several factors including genetic predisposition, increased glomerular permeability, elevated protein loads, and hemodynamic changes in the kidney may play a role in the progression of CPN; however, no etiological factors have been clearly identified (NIEHS, 2019). The effects characterized as CPN which are related to age (increased severity and incidence) and strain (higher in Sprague-Dawley rats compared to other strains) incidence are not considered histopathological manifestations of chemically-induced toxicity (NIEHS, 2019) [see U.S. EPA (1991a), p. 35 for further details and a list of the typical, observable histopathological features of CPN]. These lesions, however, are frequently exacerbated by *tert*-butanol treatment (NTP, 1997), as evidenced by the dose-related increases in severity of the nephropathy compared to female and male rat controls. The chemical-related changes in increased severity of nephropathy are included in the consideration of hazard potential.

NTP (1995) observed other kidney lesions, described as being associated with nephropathy but diagnosed separately. Renal mineralization is defined by NTP (1995) as "focal mineral deposits primarily at the corticomedullary junction." Renal (corticomedullary) mineralization was observed in essentially all female rats at all reported treatment durations. NTP (1995) describes focal, medullary mineralization as being associated with CPN but notes that focal mineralization is "usually more prominent in untreated females than in untreated males," which is consistent with the widespread appearance of this lesion in females. Corticomedullary mineralization (also referred to as nephrocalcinosis) in the rat is a common (especially in females) background/incidental finding that is not generally considered to be clinically important to rats or relevant to human health (Frazier et al., 2012). Thus, corticomedullary mineralization was not included in the consideration of hazard potential.

A dose-related, increased incidence of renal mineralization was reported in male rats at the end of the 13-week, 15-month, and 2-year oral evaluations (NTP, 1995). This mineralization is distinct from linear mineralization, which is considered a lesion characteristic of alpha2u-globulin nephropathy (for further discussion of this particular lesion, see *Mode of Action Analysis—Kidney Effects*). Linear mineralization is characterized as distinct linear deposits along radiating medullary collecting ducts. An increased incidence of linear mineralization was limited to exposed males in the 2-year oral study (NTP, 1995). Linear mineralization was not included in the consideration of hazard potential.

Two other histological kidney lesions observed in male and female rats are suppurative inflammation and transitional epithelial hyperplasia (also known as urothelial hyperplasia). These lesions were observed in the 2-year oral NTP (1995) study. NTP (1995) and Frazier et al. (2012) describe these lesions as related to the nephropathy (characterized above as common and spontaneous and considered CPN). However, suppurative inflammation and urothelial hyperplasia are typically not related to CPN or are noted as secondary changes to CPN and not a direct result of CPN (NIEHS, 2019). Incidence of suppurative inflammation in female rats was low in the control group and increased with dose, with incidences ≥24% in the two highest dose groups, compared with controls. In comparison, 20% of the control males exhibited suppurative inflammation, and the changes in incidence were not dose related (incidences ranging from 18 to 36%). To determine if the severity of these lesions was positively associated with the severity of nephropathy, contingency tables comparing the occurrence of suppurative inflammation with nephropathy in individual rats were arranged by severity and analyzed with Spearman's rank correlation tests to determine strength of associations for each comparison (Table 1-4 and Table 1-5). Suppurative inflammation and nephropathy were moderately correlated in females (rho = 0.47) and weakly correlated in males (rho = 0.17). The data indicate that CPN correlates with the induction of suppurative inflammation; however, the inflammation in female rats is also treatment related. Given that CPN is also dose-dependently increased in male and female rats (Salazar et al., 2015), disentangling the relative contribution of CPN and *tert*-butanol in the exacerbation of suppurative inflammation is problematic.

Transitional epithelial hyperplasia (also known as urothelial hyperplasia) was observed in both male and female rats exposed orally (NTP, 1995). In the control males, 50% of the animals exhibited transitional epithelial hyperplasia and the incidence and severity increased with dose. Only the mid- and high-dose females, however, exhibited dose-related increases in incidence and severity of transitional epithelial hyperplasia. This lesion was not reported in the control or low-dose females. NTP (1995) described transitional epithelial hyperplasia as increased layers of the transitional epithelial lining of the renal pelvis; study authors noted no progression of this hyperplastic lesion to neoplasia. To determine if the severity of the hyperplasia was positively associated with the severity of nephropathy, contingency tables comparing the occurrence of transitional epithelial hyperplasia with nephropathy in individual rats were arranged by severity and analyzed with Spearman's rank correlation tests to determine strength of associations for each comparison (Table 1-6 and Table 1-7). Transitional epithelial hyperplasia and nephropathy were strongly correlated (Spearman's rank coefficient = 0.66) in males and moderately correlated (Spearman's rank coefficient = 0.44) in females. The transitional epithelial hyperplasia observed in male and female rats is consistent with advanced CPN (Frazier et al., 2012). Similar to suppurative inflammation, transitional epithelial hyperplasia is both increased by dose and correlated with nephropathy, which is also dose related. Thus, disentangling the contributions of dose and nephropathy in the development of transitional epithelial hyperplasia is not possible. Transitional epithelial hyperplasia should not be confused with another lesion noted in the 2-year evaluation, renal tubule hyperplasia, which was considered preneoplastic (for further details regarding this type of hyperplasia, see the discussion under *Kidney tumors*, below).

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

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

Kidney tumors. The kidney is also a target organ for cancer effects (Table 1-3, Figure 1-1). Male F344 rats had an increased incidence of combined renal tubule adenomas or carcinomas in the 2-year oral bioassay (Hard et al., 2011; NTP, 1995). The increase in tumors from control was similar in the low- and high-dose groups and highest in the mid-dose group. Overall, tumor increases were statistically significant in trend testing, which accounted for mortality ($p \leq 0.018$). At the highest exposure group in male rats the mean body weight decreased by 24% raising some question as to whether the kidney tumors observed in the mid-dose group in male rats were solely the result of excessive toxicity rather than the carcinogenicity of *tert*- butanol. EPA's Cancer Guidelines (U.S. EPA, 2005a) discusses the determination of an "excessively high dose" as compared to an "adequate high dose" and describes the process as one of expert judgment which requires that "adequate data demonstrate that the effects are solely the result of excessive toxicity rather than the carcinogenicity of the tested agent" (U.S. EPA, 2005a). In the 2-year oral bioassay (NTP, 1995), the study authors did not report exposure related overt toxicity in male rats or any changes in toxicokinetics at the middle or high doses. Furthermore, the tumor incidence at the high dose in male rats, which had a final body weight reduction of 24%, was not significantly different from controls. Mortality increased with increasing exposure ($p = 0.001$) over the 2-year exposure period; however, increased mortality does not account for the highest tumor incidence occurring at the middle dose.

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

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

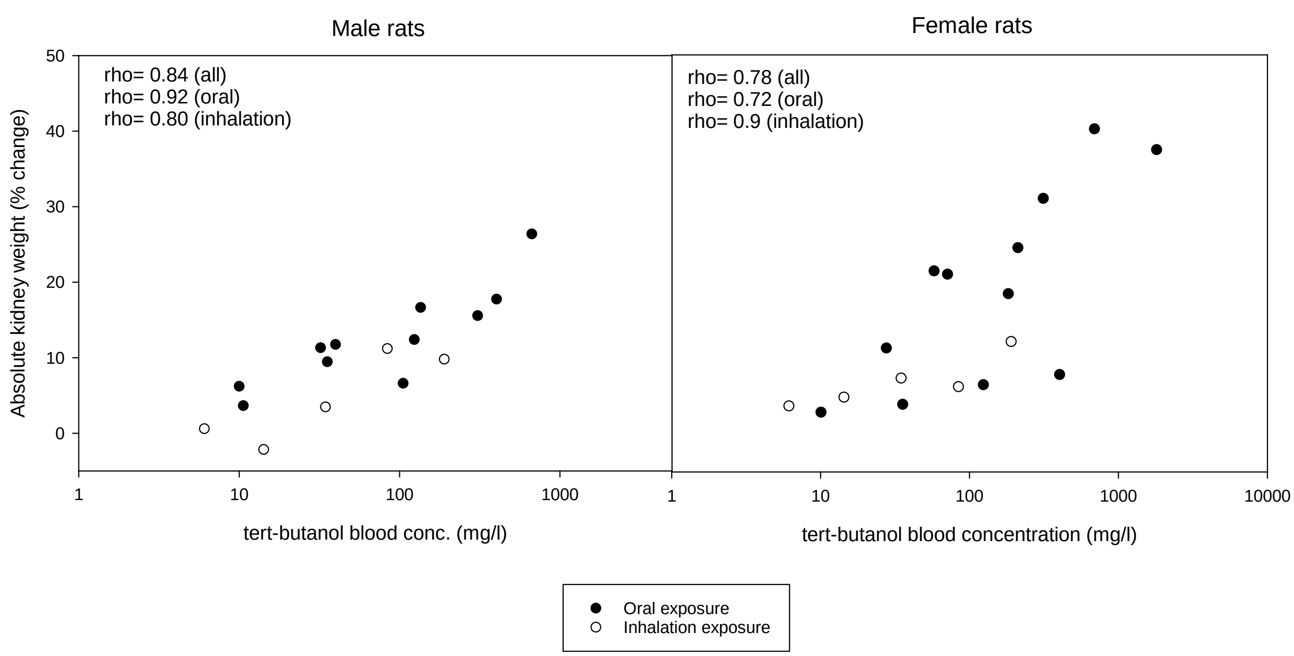


Figure 1-2. Comparison of absolute kidney weight change in male and female rats across oral and inhalation exposure based on internal blood concentration. Spearman rank correlation coefficient (rho) was calculated to evaluate the direction of a monotonic association (e.g., positive value = positive association)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

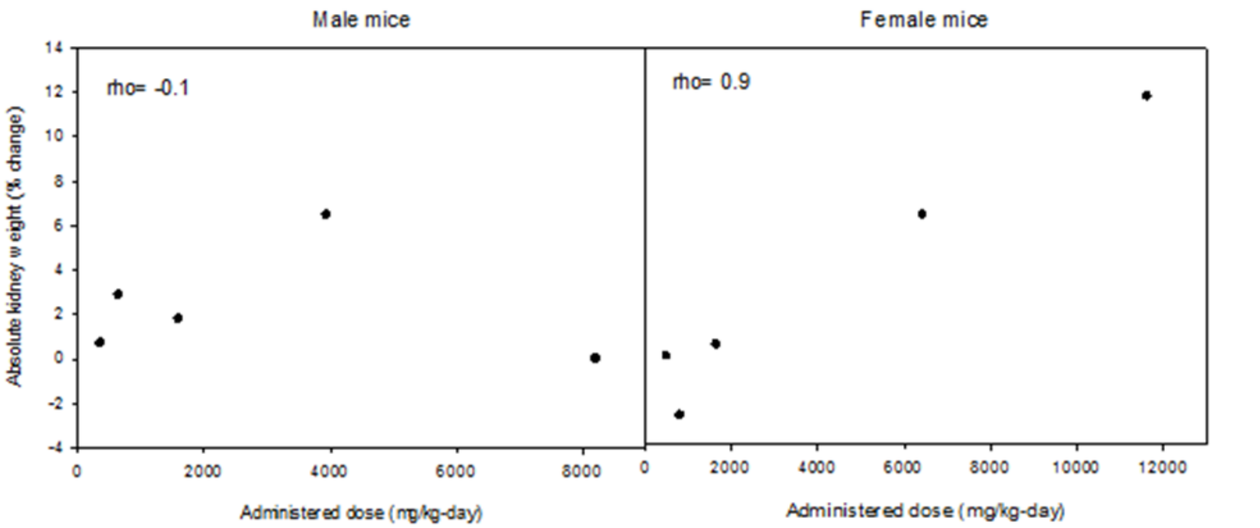


Figure 1-3. Comparison of absolute kidney weight change in male and female mice following oral exposure based on administered concentration. Spearman rank correlation coefficient (rho) was calculated to evaluate the direction of a monotonic association (e.g., positive value = positive association) and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

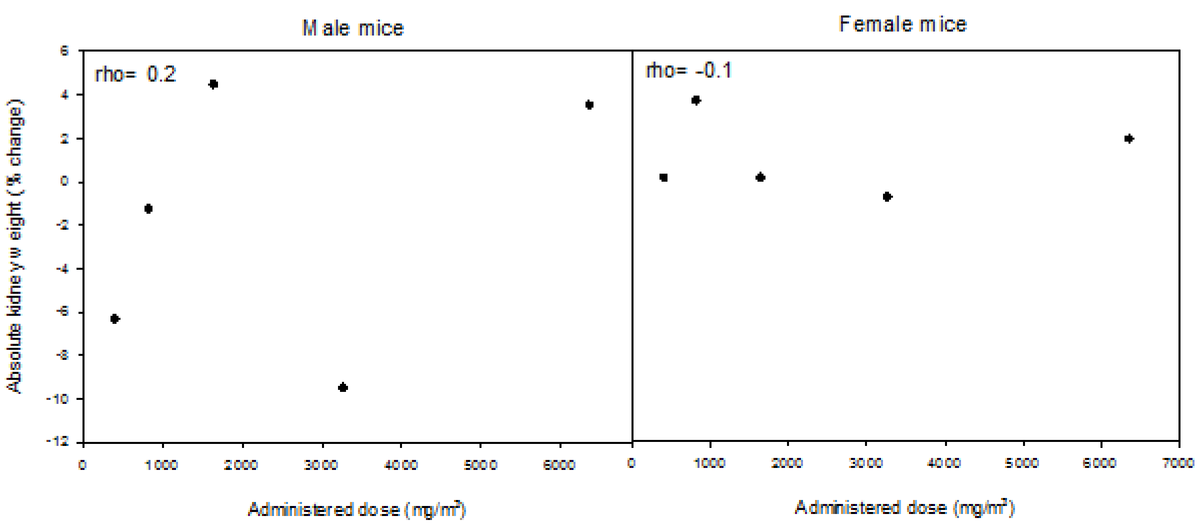


Figure 1-4. Comparison of absolute kidney weight change in male and female mice following inhalation exposure based on administered concentration. Spearman rank correlation coefficient (rho) was calculated to evaluate the direction of a monotonic association (e.g., positive value = positive association)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

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

Reference and study design	Results					
Acharya et al. (1997) Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	↑ tubular degeneration, degeneration of the basement membrane of the Bowman's capsule, diffused glomeruli, and glomerular vacuolation (no incidences reported) ↓ kidney glutathione (~40%)*					
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Incidence (severity):					
	Males			**Females**		
	Dose (mg/kg-d)	Mineralization[b]	Nephropathy[c]	Dose (mg/kg-d)	Mineralization[b]	Nephropathy[c]
	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[a]	4/10*(1.0)	7/10 (1.1)	3,620[a]	6/10 (1.2)	7/10* (1.0)
NTP (1995) $B6C3F_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Study authors indicated no treatment-related changes in kidney-related histopathology (histopathological data not provided for the 13-week study)					

<table>
<tr><th>Reference and study design</th><th colspan="4">Results</th></tr>
<tr><td rowspan="25">NTP (1995)
F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months interim)
Drinking water (0, 1.25, 2.5, 5, 10 mg/mL)
M: 0, 90, 200, 420[a] mg/kg-d
F: 0, 180, 330, 650[a] mg/kg-d
2 years</td><td colspan="4">Incidence (severity):
Males</td></tr>
<tr><td>Dose (mg/kg-d)</td><td>Mineralization[b] (interim)</td><td>Mineralization[b] (terminal)</td><td>Linear mineralization[b] (terminal)</td></tr>
<tr><td>0</td><td>1/10 (1.0)</td><td>26/50 (1.0)</td><td>0/50</td></tr>
<tr><td>90</td><td>2/10 (1.0)</td><td>28/50 (1.1)</td><td>5/50* (1.0)</td></tr>
<tr><td>200</td><td>5/10 (1.8)</td><td>35/50 (1.3)</td><td>24/50* (1.2)</td></tr>
<tr><td>420[a]</td><td>9/10* (2.3)</td><td>48/50* (2.2)</td><td>46/50* (1.7)</td></tr>
<tr><td>Dose (mg/kg-d)</td><td>Transitional epithelial hyperplasia</td><td>Nephropathy[c] severity</td><td>Inflammation (suppurative) incidence</td></tr>
<tr><td>0</td><td>25/50 (1.7)</td><td>3.0</td><td>10/50</td></tr>
<tr><td>90</td><td>32/50 (1.7)</td><td>3.1</td><td>18/50</td></tr>
<tr><td>200</td><td>36/50* (2.0)</td><td>3.1</td><td>12/50</td></tr>
<tr><td>420[a]</td><td>40/50* (2.1)</td><td>3.3*</td><td>9/50</td></tr>
<tr><td colspan="4">Females</td></tr>
<tr><td>Dose (mg/kg-d)</td><td>Mineralization[b] Interim</td><td>Mineralization[b] Terminal</td><td>Inflammation (suppurative) incidence</td></tr>
<tr><td>0</td><td>10/10 (2.8)</td><td>49/50 (2.6)</td><td>2/50</td></tr>
<tr><td>180</td><td>10/10 (2.9)</td><td>50/50 (2.6)</td><td>3/50</td></tr>
<tr><td>330</td><td>10/10 (2.9)</td><td>50/50 (2.7)</td><td>13/50*</td></tr>
<tr><td>650[a]</td><td>10/10 (2.8)</td><td>50/50 (2.9)</td><td>17/50*</td></tr>
<tr><td>Dose (mg/kg-d)</td><td>Transitional epithelial hyperplasia</td><td>Nephropathy[c] severity</td><td></td></tr>
<tr><td>0</td><td>0/50</td><td>1.6</td><td></td></tr>
<tr><td>180</td><td>0/50</td><td>1.9*</td><td></td></tr>
<tr><td>330</td><td>3/50 (1.0)</td><td>2.3*</td><td></td></tr>
<tr><td>650[a]</td><td>17/50*(1.4)</td><td>2.9*</td><td></td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>NTP (1995)
B6C3F$_1$ mouse; 60/sex/treatment
Drinking water (0, 5, 10, or 20 mg/mL)
M: 0, 540, 1,040, or 2,070[a] mg/kg-d
F: 0, 510, 1,020, or 2,110 mg/kg-d
2 years</td><td>No treatment-related changes in kidney-related histopathology observed</td></tr>
<tr><td>NTP (1997)
F344/N rat; 10/sex/treatment
Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3)
(dynamic whole-body chamber)
6 hr/d, 5 d/wk
13 weeks
Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported</td><td>Male
<table>
<tr><th>Concentration (mg/m^3)</th><th>Incidence of chronic nephropathy[d]</th><th>Average severity of chronic nephropathy</th></tr>
<tr><td>0</td><td>9/10</td><td>1.0</td></tr>
<tr><td>406</td><td>8/10</td><td>1.4</td></tr>
<tr><td>824</td><td>9/10</td><td>1.4</td></tr>
<tr><td>1,643</td><td>10/10</td><td>1.6</td></tr>
<tr><td>3,273</td><td>10/10</td><td>1.9</td></tr>
<tr><td>6,368</td><td>10/10</td><td>2.0</td></tr>
</table>
Females: no treatment-related changes in kidney-related histopathology observed
Severity categories: 1 = minimal, 2= mild. No results from statistical tests reported</td></tr>
<tr><td>NTP (1997)
B6C3F$_1$ mouse; 10/sex/treatment
Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3)
(dynamic whole-body chamber)
6 hr/d, 5 d/wk
13 weeks
Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported</td><td>No treatment-related changes in kidney-related histopathology observed</td></tr>
</table>

*Statistically significant $p \leq 0.05$, as determined by the study authors.
[a]The high-dose group had an increase in mortality.
[b]Mineralization defined in NTP (1995) as focal mineral deposits, primarily at the corticomedullary junction. Linear mineralization was defined as foci of distinct linear deposits along radiating medullary collecting ducts; linear mineralization not observed in female rats.

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

Note: Conversions from drinking water concentrations to mg/kg-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 hyperplasia (standard and extended evaluation combined)	Renal tubule adenoma (single)	Renal tubule adenoma (multiple)
	0	14/50 (2.3)	7/50	1/50
	90	20/50 (2.3)	7/50	4/50
	200	17/50 (2.2)	10/50	9/50*
	420[a]	25/50* (2.8)	10/50	3/50
	Dose (mg/kg-d)	Renal tubule carcinoma	Renal tubule adenoma (single or multiple) or carcinoma	
	0	0/50	8/50	
	90	2/50	13/50	
	200	1/50	19/50*	
	420[a]	1/50	13/50	
	Female			
	Dose (mg/kg-d)	Renal tubule hyperplasia	Renal tubule adenoma (single)	Renal tubule adenoma (multiple)
	0	0/50	0/50	0/50
	180	0/50	0/50	0/50
	330	0/50	0/50	0/50
	650[a]	1/50 (1.0)	0/50	0/50

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td></td><td>
<table>
<tr><th>Dose (mg/kg-d)</th><th>Renal tubule carcinoma</th><th>Renal tubule adenoma (single or multiple) or carcinoma</th></tr>
<tr><td>0</td><td>0/50</td><td>0/50</td></tr>
<tr><td>180</td><td>0/50</td><td>0/50</td></tr>
<tr><td>330</td><td>0/50</td><td>0/50</td></tr>
<tr><td>650[a]</td><td>0/50</td><td>0/50</td></tr>
</table>
Based on standard and extended evaluations (combined). Results do not include the animals sacrificed at 15 months.
</td></tr>
<tr><td>Hard et al. (2011)
Reanalysis of the slides from male rats (all slides in controls and high-dose groups of males and females, and slides from all other males with renal tumors) in the NTP (1995) study (see above)</td><td>
<table>
<tr><th>Male

Dose (mg/kg-d)</th><th>Renal tubule adenoma (single)</th><th>Renal tubule adenoma (multiple)</th><th>Renal tubule carcinoma</th><th>Renal tubule adenoma (single or multiple) or carcinoma</th></tr>
<tr><td>0</td><td>3/50</td><td>1/50</td><td>0/50</td><td>4/50</td></tr>
<tr><td>90</td><td>9/50</td><td>3/50</td><td>1/50</td><td>13/50*</td></tr>
<tr><td>200</td><td>9/50</td><td>9/50</td><td>0/50</td><td>18/50*</td></tr>
<tr><td>420</td><td>9/50</td><td>3/50</td><td>1/50</td><td>12/50*</td></tr>
</table>
</td></tr>
<tr><td>NTP (1995)
$B6C3F_1$ mouse; 60/sex/treatment
Drinking water (0, 5, 10, or 20 mg/mL)
M: 0, 540, 1,040, or 2,070[a] mg/kg-d
F: 0, 510, 1,020, or 2,110 mg/kg-d
2 years</td><td>No increases in kidney-related tumors. Two renal tubule adenocarcinomas, one in the low-dose and one in the high-dose groups, were observed in male mice. These tumors were not considered treatment related.</td></tr>
</table>

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

Table 1-4. Comparison of nephropathy and suppurative inflammation in individual male rats from the 2-year NTP *tert*-butanol bioassay

Suppurative inflammation	Nephropathy				
	None	Minimal	Mild	Moderate	Marked
None	2	1	55	82	51
Minimal	0	0	3	23	16
Mild	0	0	1	4	2
Moderate	0	0	0	0	0
Marked	0	0	0	0	0

Spearman's rank correlation test (1-sided), $p = 0.0015$, $r_s = 0.17$

Table 1-5. Comparison of nephropathy and suppurative inflammation in individual female rats from the 2-year NTP *tert*-butanol bioassay

Suppurative inflammation	Nephropathy				
	None	Minimal	Mild	Moderate	Marked
None	7	67	90	37	4
Minimal	0	1	5	14	13
Mild	0	0	0	1	1
Moderate	0	0	0	0	0
Marked	0	0	0	0	0

Spearman's rank correlation test (1-sided), $p < 0.0001$, $r_s = 0.47$

Table 1-6. Comparison of nephropathy and transitional epithelial hyperplasia in individual male rats from the 2-year NTP *tert*-butanol bioassay

Transitional epithelial hyperplasia	Nephropathy				
	None	Minimal	Mild	Moderate	Marked
None	2	1	51	52	1
Minimal	0	0	4	26	9
Mild	0	0	2	25	42
Moderate	0	0	2	6	17
Marked	0	0	0	0	0

Spearman's rank correlation test (1-sided), $p < 0.0001$, $r_s = 0.66$

Table 1-7. Comparison of nephropathy and transitional epithelial hyperplasia in individual female rats from the 2-year NTP *tert*-butanol bioassay

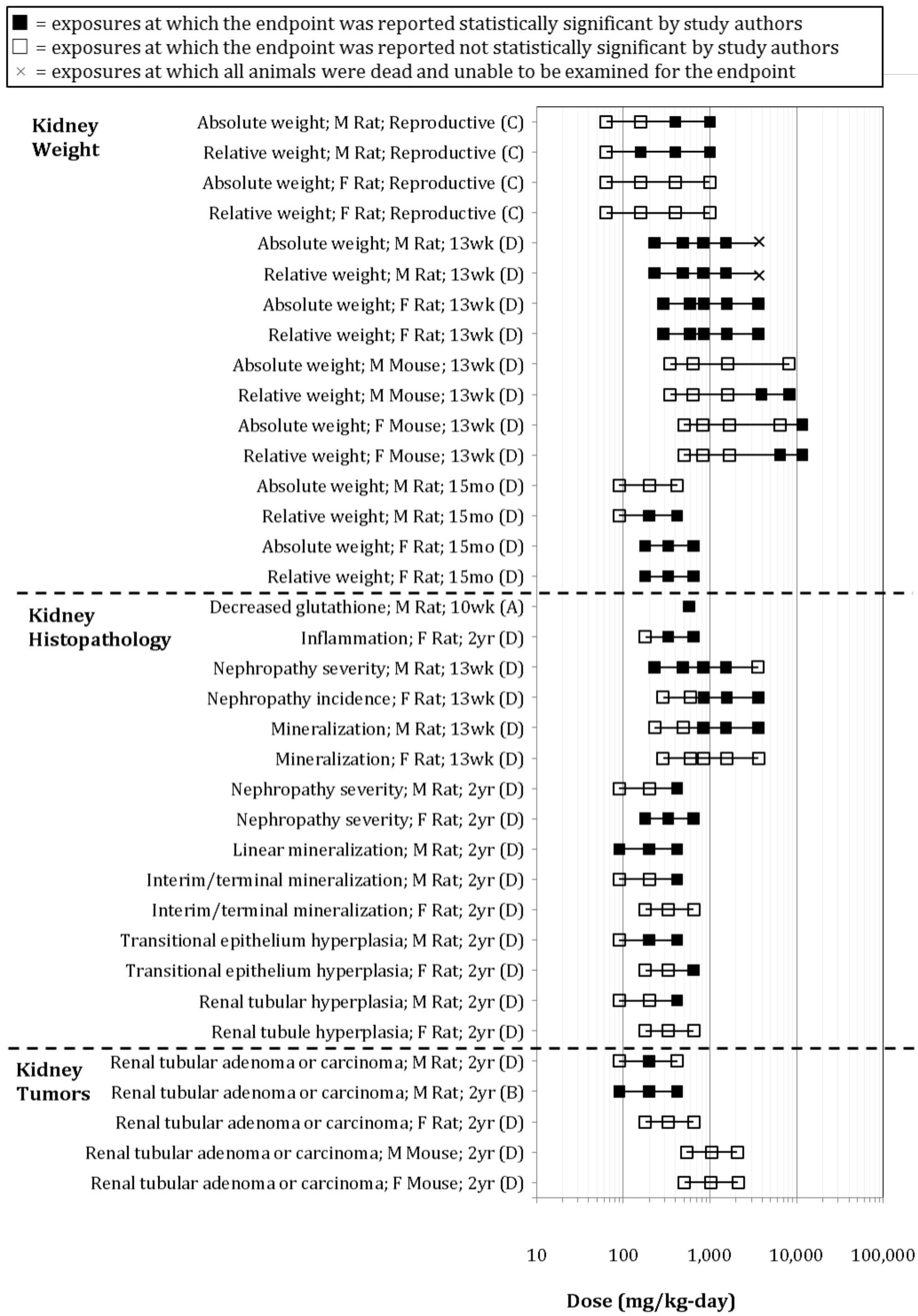
Transitional epithelial hyperplasia	Nephropathy				
	None	Minimal	Mild	Moderate	Marked
None	7	68	95	43	7
Minimal	0	0	0	8	6
Mild	0	0	0	1	5
Moderate	0	0	0	0	0
Marked	0	0	0	0	0

Spearman's rank correlation test (1-sided), $p < 0.0001$, $r_s = 0.437$

Table 1-8. Comparison of CPN and renal tubule hyperplasia with kidney adenomas and carcinomas in male rats from the 2-year NTP *tert*-butanol bioassay

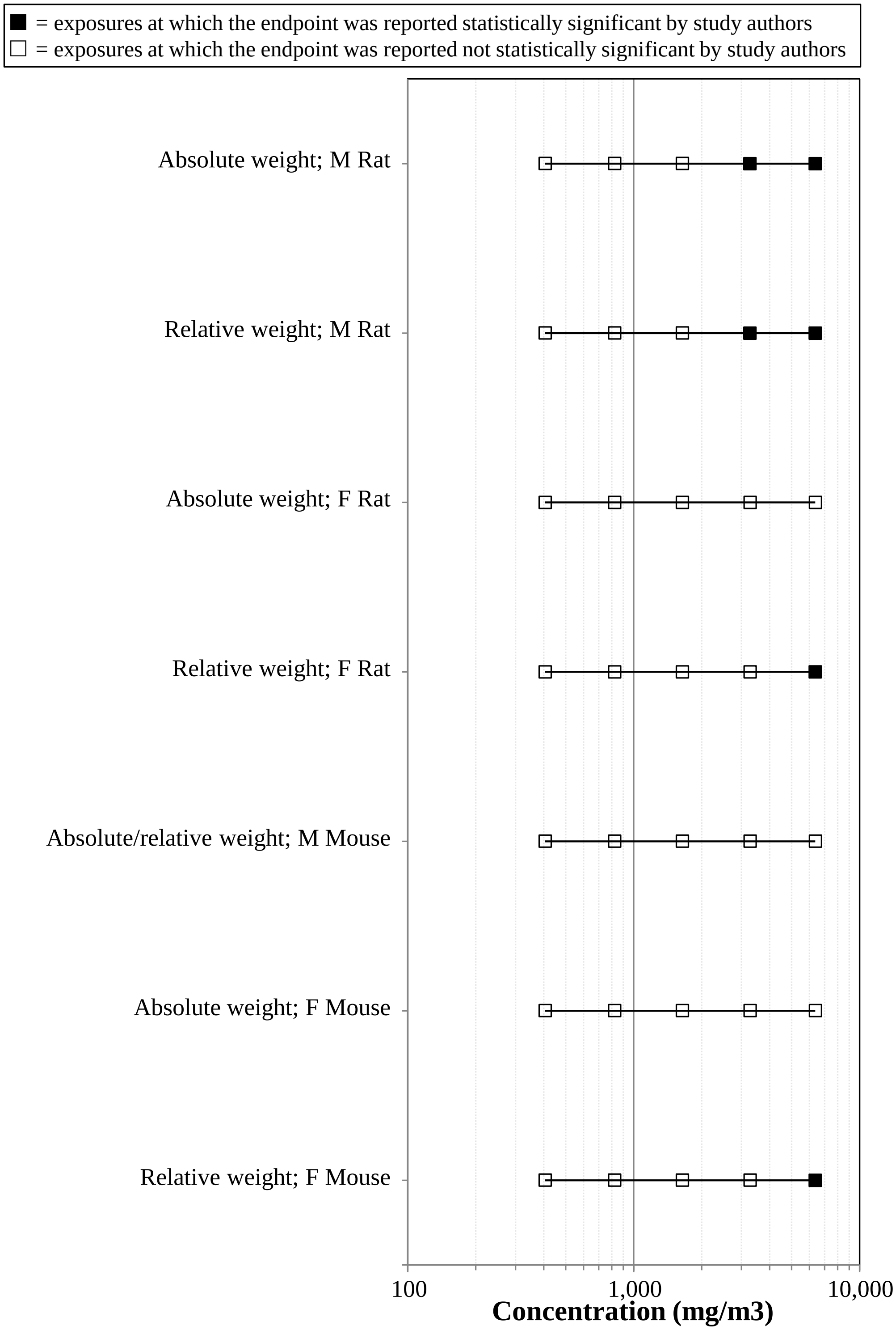
CPN	Renal Tumors Absent	Renal Tumors Present	Renal tubule hyperplasia	Renal Tumors Absent	Renal Tumors Present
None	2	0	None	133	29
Minimal	1	0	Minimal	17	2
Mild	57	2	Mild	17	13
Moderate	93	16	Moderate	10	3
Marked	34	35	Marked	10	6

Spearman's rank correlation test (1-sided): CPN, $p < 0.0001$, $r_s = 0.430$; renal tubule hyperplasia, $p = 0.01$, $r_s = 0.161$



Sources: (A) Acharya et al. (1997); (1995); (B) Hard et al. (2011)*; (C) Huntingdon Life Sciences (2004) (D) NTP (1995); *reanalysis of NTP (1995).

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



Source: NTP (1997).

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

Mode of Action Analysis—Kidney Effects

a) alpha2u-globulin -Associated Renal Tubule Nephropathy and Carcinogenicity

One disease process to consider when interpreting kidney effects in rats is related to the accumulation of alpha2u-globulin protein. alpha2u-globulin , a member of a large superfamily of low-molecular-weight proteins, was first characterized in male rat urine. Such proteins have been detected in various tissues and fluids of most mammals (including humans), but the particular isoform of alpha2u-globulin commonly detected in male rat urine is considered specific to that sex and species. Exposure to chemicals that induce alpha2u-globulin accumulation can initiate a sequence of histopathological events leading to kidney tumorigenesis. Because alpha2u-globulin -associated renal tubule nephropathy and carcinogenicity occurring in male rats are presumed not relevant for assessing human health hazards (U.S. EPA, 1991a), evaluating the data to determine if alpha2u-globulin plays a role is important. The role of alpha2u-globulin accumulation in the development of renal tubule nephropathy and carcinogenicity observed following *tert*-butanol exposure was evaluated using the U.S. EPA (1991a) Risk Assessment Forum Technical panel report, *Alpha$_{2u}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat* as well as the IARC 1999 framework (Capen, 1999). These frameworks provide specific guidance for evaluating renal tubule tumors in male rats that are related to chemical exposure for the purpose of risk assessment, based on an examination of the potential involvement of alpha2u-globulin accumulation.

Studies in the *tert*-butanol evidence base evaluated and reported effects on the kidney, providing some evidence to evaluate this MOA. Additionally, several studies were identified that specifically evaluated the role of alpha2u-globulin in *tert*-butanol-induced renal tubule nephropathy and carcinogenicity (Borghoff et al., 2001; Williams and Borghoff, 2001; Takahashi et al., 1993; Hard et al., 2019). Because the evidence reported in these studies is specific to alpha2u-globulin accumulation, it is presented in this section; it was not included in the animal evidence tables in the previous section.

The hypothesized sequence of alpha2u-globulin renal tubule nephropathy, as described by U.S. EPA (1991a), is as follows. Chemicals that induce alpha2u-globulin accumulation do so rapidly. alpha2u-globulin accumulating in hyaline droplets is deposited in the S2 (P2) segment of the proximal tubule within 24 hours of exposure. Hyaline droplets are a normal constitutive feature of the mature male rat kidney; they are particularly evident in the S2 (P2) segment of the proximal tubule and contain alpha2u-globulin (U.S. EPA, 1991a). Abnormal increases in hyaline droplets have more than one etiology and can be associated with the accumulation of different proteins. As hyaline droplet deposition continues, single-cell necrosis occurs in the S2 (P2) segment, which leads to exfoliation of these cells into the tubule lumen within 5 days of chemical exposure. In response to the cell loss, cell proliferation occurs in the S2 (P2) segment after 3 weeks and continues for the duration of the exposure. After 2 or 3 weeks of exposure, the cell debris accumulates in the S3 (P3) segment of the proximal tubule to form granular casts. Continued chemical exposure for 3 to 12 months leads to the formation of calcium hydroxyapatite in the papillae which results in linear mineralization. After 1 or more years of chemical exposure, these lesions can result in the induction of renal tubule adenomas and carcinomas (Figure 1-7).

U.S. EPA (1991a) identified two questions that must be addressed to determine the extent to which alpha2u-globulin -mediated processes induce renal tubule nephropathy and carcinogenicity. First, whether the alpha2u-globulin process occurs in male rats and influences renal tubule tumor development must be determined. Second, whether the renal effects in male rats exposed to *tert*-butanol are due solely to the alpha2u-globulin process must be determined.

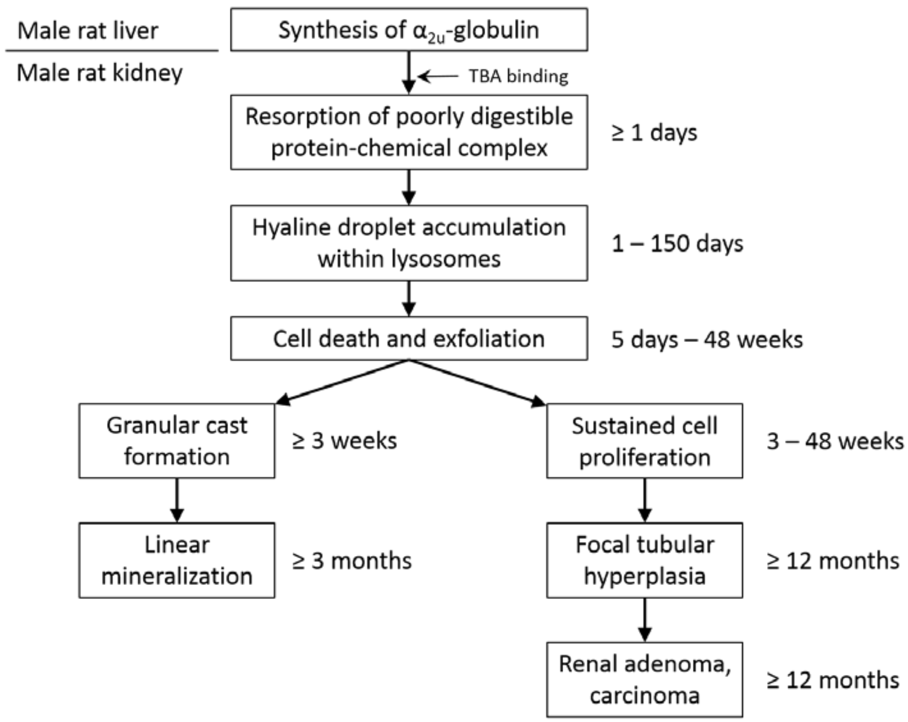
U.S. EPA (1991a) stated the criteria for answering the first question in the affirmative are as follows:

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

2) the protein in the hyaline droplets in treated male rats is alpha2u-globulin (i.e., immunohistochemical evidence), and

3) several (but not necessarily all) additional steps in the pathological sequence appear in treated male rats as a function of time, dose, and progressively increasing severity consistent with the understanding of the underlying biology, as described above, and illustrated in Figure 1-7.

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



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

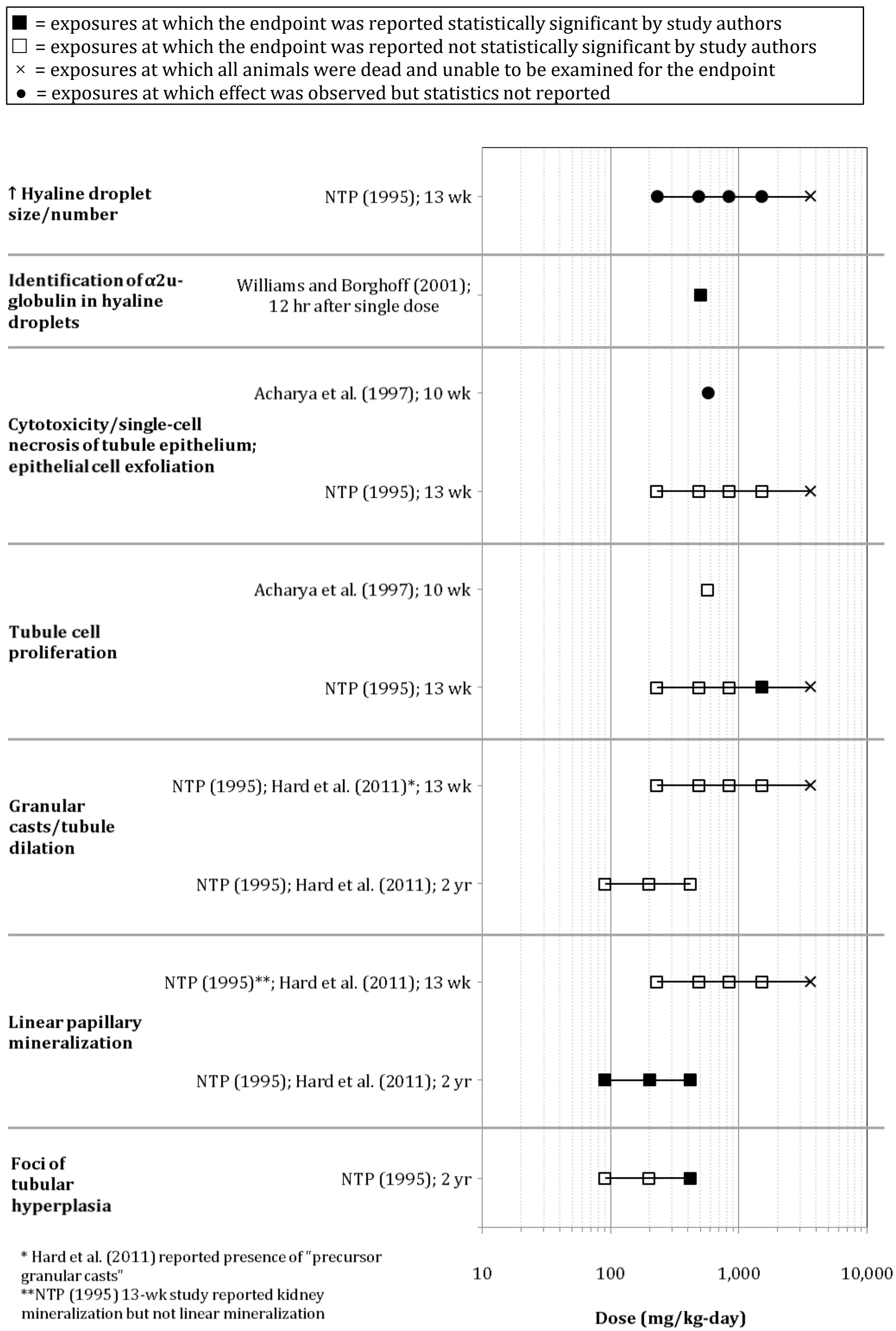
Figure 1-7. Temporal pathogenesis of alpha2u-globulin -associated nephropathy in male rats. alpha2u-globulin synthesized in the livers of male rats is delivered to the kidney, where it can accumulate in hyaline droplets and be retained by epithelial cells lining the S2 (P2) segment of the proximal tubules. Renal pathogenesis following continued *tert*-butanol exposure and increasing droplet accumulation can progress stepwise from increasing epithelial cell damage, death and dysfunction leading to the formation of granular casts in the corticomedullary junction, linear mineralization of the renal papillae, and carcinogenesis of the renal tubular epithelium.

Table 1-9. Summary of data on the alpha2u-globulin process in male rats exposed to *tert*-butanol

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

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

+ = Statistically significant change reported in one or more treated groups.
(+) = Effect was reported in one or more treated groups, but statistics not reported.
– = No statistically significant change reported in any of the treated groups.
[a]NTP (1997) did not observe any effects consistent with alpha2u-globulin nephropathy.
[b]Precursors to granular casts reported.
[c]Reanalysis of hematoxylin and eosin-stained kidney sections from all male control and 1,520-mg/kg-d groups and a representative sample of kidney sections stained with Mallory Heidenhain stain, from the 13-wk study from NTP (1995).
[d]Reanalysis of slides for all males in the control and 420-mg/kg-d dose groups and all animals with renal tubule tumors from 2-yr NTP (1995). Protein casts reported, not granular casts.



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

Figure 1-8. Exposure-response array for effects potentially associated with alpha2u-globulin renal tubule nephropathy and tumors in male rats after oral exposure to *tert*-butanol.

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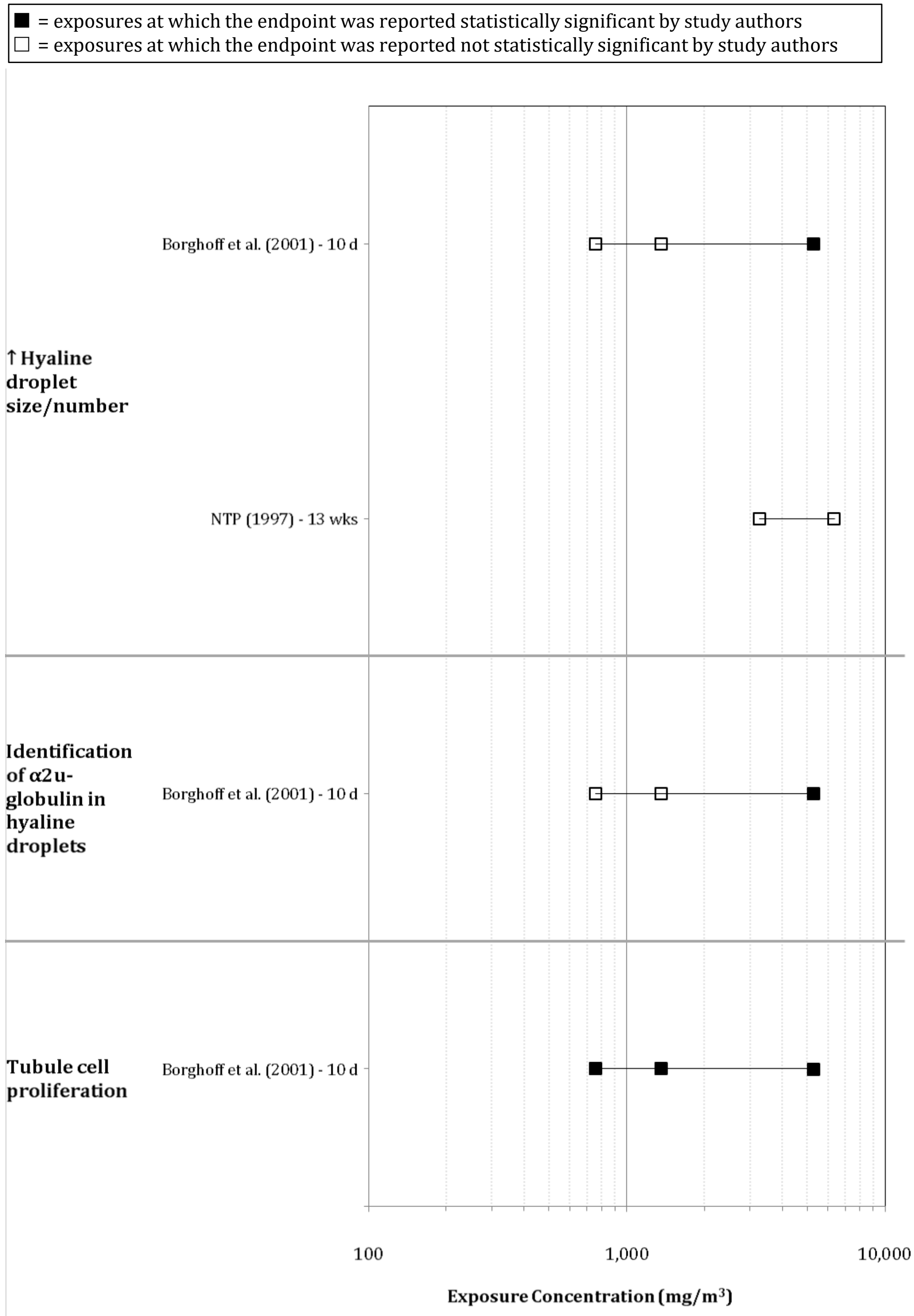

Figure 1-9. Exposure-response array for effects potentially associated with alpha2u-globulin renal tubule nephropathy and tumors in male rats after inhalation exposure to *tert*-butanol.

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Question One: Is the α_{2u}globulin process occurring in male rats exposed to tert-butanol?[9]

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

NTP (1997), however, found no difference between the control and treatment groups stained for hyaline droplet formation in male rats exposed to 0-, 3,273-, or 6,368-mg/m^3 *tert*-butanol via inhalation for 13 weeks; in fact, this study reported no other lesions that could be specifically associated with alpha2u-globulin nephropathy in male rats. These results from NTP (1997), which are inconsistent with the findings of both Borghoff et al. (2001) and NTP (1995), do not appear to be due to differences in dose. Comparison of the oral and inhalation studies on the basis of *tert*-butanol blood concentration (see Supplemental Information) showed that an exposure in the range of the NTP (1995) doses of 490–840 mg/kg-day for 13 weeks leads to the same average blood concentration as inhalation exposures to 3,273–6,368 mg/m^3 for 6hr/day, 5 day/week. The absence of similar histopathological findings in the 13-week inhalation NTP (1997) study compared to those reported in the two oral studies is not understood, but might be indicative of the strength of *tert*-butanol to induce, consistently, alpha2u-globulin nephropathy. The results from the two other studies (Borghoff et al., 2001; NTP, 1995) indicate that hyaline droplets increase in size and number in male rats following *tert*-butanol exposures. Despite the inconsistency, the findings from (Borghoff et al., 2001; NTP, 1995) ,are considered as sufficient evidence to fulfill the first criterion that hyaline droplets are increased in size and number in male rats.

(2) The second criterion to consider is whether the protein in the hyaline droplets in male rats is alpha2u-globulin . Accumulated hyaline droplets with an alpha2u-globulin etiology can be confirmed by using immunohistochemistry to identify the alpha2u-globulin protein. Two short-term studies measured alpha2u-globulin immunoreactivity in the hyaline droplets of the renal

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

proximal tubular epithelium (Borghoff et al., 2001; Williams and Borghoff, 2001). Following 10 days of inhalation exposure, Borghoff et al. (2001) did not observe an exposure-related increase in alpha2u-globulin using immunohistochemical staining. However, when using an enzyme-linked immunosorbent assay (ELISA), a more sensitive method of detecting alpha2u-globulin , a statistically significant positive correlation of alpha2u-globulin concentration with dose of *tert*-butanol was observed. The accumulation of alpha2u-globulin protein was statistically significant by pairwise comparison only in the highest dose group. No positive staining for alpha2u-globulin was observed in exposed female rats. In a follow-up study, Williams and Borghoff (2001) used a single gavage dose of 500 mg/kg [selected on the basis of results by NTP (1995) for induction of hyaline droplet accumulation], and reported a statistically significantly higher renal concentration of alpha2u-globulin (by ELISA) in treated male rats than in controls 12 hours after exposure. Further, equilibrium dialysis methods determined that the binding of *tert*-butanol to alpha2u-globulin was reversible. These data indicate the presence of alpha2u-globulin in *tert*-butanol-treated male rats, although requiring a more sensitive method of detection for alpha2u-globulin than is typically used could indicate that *tert*-butanol is not a strong inducer of alpha2u-globulin accumulation. Therefore, the available data are sufficient to fulfill the second criterion for alpha2u-globulin present in the hyaline droplets but suggest weak induction of alpha2u-globulin by *tert*-butanol.

(3) The third criterion considered is whether several (but not necessarily all) additional events in the histopathological sequence associated with alpha2u-globulin nephropathy appear in male rats in a manner consistent with the understanding of alpha2u-globulin pathogenesis. Evidence of cytotoxicity and single-cell necrosis of the tubule epithelium subsequent to the excessive accumulation of hyaline droplets, with exfoliation of degenerate epithelial cells, should be observable after ~5 days of continuous exposure, peaking at ~19 days [reviewed in U.S. EPA (1991a)]. The formation and accumulation of granular casts from the exfoliated cellular debris would follow, causing tubule dilation at the junction of the S3 (P3) segment of the proximal tubule and the descending thin loop of Henle, and the commencement of compensatory cell proliferation within the S2 (P2) segment, both occurring after 3 weeks of continuous exposure. Following chronic exposures, this regenerative proliferation could result in focal tubular hyperplasia, and eventually progress to renal adenoma and carcinoma (Figure 1-7).

Several of these steps were observed following *tert*-butanol exposure in male rats, most notably linear papillary mineralization and foci of tubular hyperplasia, consistent with the expected disease progression. Some lack of consistency and dose-related concordance, however, was evident across the remaining steps in the histopathological sequence. First, the accumulation of hyaline droplets and the concentrations of alpha2u-globulin in the hyaline droplets at doses that induced significant tumor formation in male rats were not significant. Next, necrosis or cytotoxicity was absent, and only precursors to granular casts at stages well within the expected timeframe of detectability were present. Finally, a 13-week inhalation study found no evidence of alpha2u-globulin nephropathy (NTP, 1997), despite evaluating exposure concentrations predicted to result in similar blood *tert*-butanol levels as for the 13-week oral study (NTP, 1995), which reported increases in droplet accumulation and sustained regenerative tubule cell proliferation. A detailed evaluation and analysis of all the evidence relevant to this criterion follows.

Detailed evaluation of the available evidence supporting the third criterion

a. Single cell death and exfoliation into the renal tubules might logically be expected to accompany the occurrence of CPN, but this result was inconsistently observed. Single cell death or necrosis was not associated with *tert*-butanol exposure in male rat kidneys after 10 or 13 weeks (Acharya et al., 1997; NTP, 1995). Acharya et al. (1997) reported degeneration of renal tubules, one pathological consequence of single cell necrosis, in male rats exposed to *tert*-butanol in drinking water for 10 weeks. As renal tubule epithelial cell death and epithelial degeneration should occur as early as 5 days post exposure and persist for up to 48 weeks (Swenberg and Lehman-McKeeman, 1999; Short et al., 1989), the lack of consistency in these observations could be the result of both weak induction of alpha2u-globulin and a lack of later examinations.

b. Sustained regenerative cell proliferation also might be logically expected to accompany the occurrence of CPN, but this result was also inconsistently observed. Acharya et al. (1997) did not observe *tert*-butanol-induced proliferation following 10 weeks of oral exposure, but renal tubule proliferation was observed following another chemical exposure (trichloroacetic acid) in the same study. Therefore, the inference is that *tert*-butanol treatment did not induce regenerative tubule cell proliferation in male rats from this study. Borghoff et al. (2001), however, reported a dose-related increase in epithelial cell proliferation within the proximal tubule as measured by BrdU (bromodeoxyuridine) labeling indices in all male rats exposed to *tert*-butanol via inhalation for 10 days. The study did not report cytotoxicity and combined with the early time point makes it unlikely that the cell proliferation was compensatory. NTP (1995) also observed increased cell proliferation in the renal tubule epithelium following 13-week oral exposures in male rats [only male rats were studied in the retrospective analysis by Takahashi et al. (1993) reported in NTP (1995)]. Proliferation was elevated at 840–1,520 mg/kg-day, a range higher than the single 575-mg/kg-day dose that elicited epithelial degeneration (Acharya et al., 1997) which could be consistent with a compensatory proliferative effect. NTP (1995) reported, however, that no necrosis or exfoliation was observed. Altogether, proliferation and necrosis or degeneration were not observed within the same study despite several attempts to measure both effects. Thus, these data provide inadequate evidence to conclude that the proliferation was compensatory.

c. Granular cast formation was not observed, although one study noted precursors to cast formation. NTP (1995) did not observe the formation of granular casts or tubular dilation;

however, Hard et al. (2011) reanalyzed the 13-week oral NTP data from male rats treated with 0 or 1,520 mg/kg-day and identified precursors to granular casts in 5/10 animals in the treated group. The significance of these granular cast precursors, described as sporadic basophilic tubules containing cellular debris, is unknown, because 13 weeks of exposure is within the expected timeframe of frank formation and accumulation of granular casts (≥3 weeks). Granular cast formation, however, might not be significantly elevated with weak inducers of alpha2u-globulin (Short et al., 1986), which is consistent with the reported difficulty in measuring alpha2u-globulin in hyaline droplets associated with *tert*-butanol exposure.

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

e. Renal tubule hyperplasia was observed in the only available 2-year study. Renal tubule hyperplasia is the preneoplastic lesion associated with alpha2u-globulin nephropathy in chronic exposures that leads to renal tubule tumors (U.S. EPA, 1991a). A dose-related increase in renal tubule hyperplasia was observed in male rats following 2-year oral exposures (NTP, 1995). By comparison, renal tubule hyperplasia was observed in only one high-dose female.

The progression of histopathological lesions for alpha2u-globulin nephropathy is predicated on the initial response of excessive hyaline droplet accumulation (containing alpha2u-globulin) leading to cell necrosis and cytotoxicity, which in turn cause the accumulation of granular casts, linear mineralization, and tubular hyperplasia. Therefore, observations of temporal and dose-response concordance for these effects are informative for drawing conclusions on causation.

As mentioned above, most steps in the sequence of alpha2u-globulin nephropathy are observed at the expected time points following exposure to *tert*-butanol. Accumulation of hyaline droplets was observed early, at 12 hours following a single bolus exposure (Williams and Borghoff, 2001) and at 10 days (Borghoff et al., 2001) or 13 weeks (NTP, 1995) following continuous exposure; alpha2u-globulin was identified as the protein in these droplets (Borghoff et al., 2001; Williams and Borghoff, 2001). Lack of necrosis and exfoliation might be due to the weak induction of alpha2u-globulin and a lack of later examinations. Granular cast formation was not reported in any of the available studies, which could also indicate weak alpha2u-globulin induction. Regenerative cell proliferation, which was not observed, is discussed in more detail below. Observations of the subsequent linear mineralization of tubules and focal tubular hyperplasia fall within the expected timeframe of the appearance of these lesions. Overall, no explicit inconsistencies are present in the temporal appearance of the histopathological lesions associated with alpha2u-globulin nephropathy; however, the dataset would be bolstered by measurements at additional time points to lend strength to the MOA evaluation.

Inconsistencies do occur in the dose-response among lesions associated with the alpha2u-globulin nephropathy progression. Hyaline droplets were induced in the proximal tubule of all surviving male rats in the 13-week NTP oral study (NTP, 1995; Takahashi et al., 1993; Lindamood et al., 1992), although the incidence at the lowest dose was minimal, while the incidence at the three higher doses was more prominent. These results are discordant with the tumor results, given that all treated groups of male rats in the NTP 2-year oral bioassay had increased kidney tumor incidence, including the lowest dose of 90 mg/kg-day [according to the reanalysis by Hard et al. (2011)]. This lowest dose was less than the 230 mg/kg-day in the 13-week oral study that had only minimal hyaline droplet formation. Furthermore, although the incidence of renal tubule hyperplasia had a dose-related increase (NTP, 1995), a corresponding dose-related increase in the severity of tubular hyperplasia did not result. Severity of tubule hyperplasia was increased only at the highest dose, which was not consistent with renal tumor incidence.

Although the histopathological sequence has data gaps, such as the lack of observable necrosis or cytotoxicity or granular casts at stages within the timeframe of detectability, overall, a sufficient number of steps (e.g., linear papillary mineralization, foci of tubular hyperplasia) were observed to fulfill the third criterion.

Consideration of additional IARC 1999 Criteria

An alpha2u-globulin framework was published by IARC in 1999 (Capen et al., 1999). See Table 1-10 for criterion laid out in the IARC consensus document.

Table 1-10. International Agency for Research on Cancer (IARC) criteria for an agent causing kidney tumors through an alpha2u-globulin associated response in male rats (Capen et al., 1999)

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

A few minor differences exist between the EPA and IARC criteria. The EPA framework requires the identification of several (but not necessarily all) additional steps in the histopathological sequence associated with alpha2u-globulin nephropathy, whereas IARC requires the "induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory" but doesn't specify which or how many of the additional histopathological changes must be observed to consider this criteria met. In addition, IARC (Capen et al., 1999) has specific criteria pertaining to lack of genotoxicity of parent compound/metabolite and male rat specificity for nephropathy and renal tumorigenicity whereas the EPA framework considers these data as supplemental information (*see Part 4, XVII B. Additional information Useful for Analysis*) Additional criteria required by IARC (Capen et al., 1999) which are not considered essential in the EPA's framework are discussed below.

Lack of genotoxic action

There are a limited number of studies available to assess the genotoxic potential of *tert*-butanol (see Appendix B.2.2. 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 in human HL-60 leukemia cells (Tang et al., 1997). In another study (Sgambato et al., 2009), an initial increase in DNA damage was observed as measured by nuclear fragmentation, but the damage reduced drastically following 4 hours of exposure and entirely disappeared after 12 hours of exposure to *tert*-butanol.

Overall, the evidence base is limited in terms of either the array of genotoxicity tests conducted or the number of studies within the same type of test. In addition, the results are either conflicting or inconsistent. The test strains, solvents, or control for volatility used in certain studies are variable and could influence results. Furthermore, in some studies, the specificity of the methodology used has been challenged. Given the inconsistencies and limitations of the evidence base in terms of the methodology used, number of studies in the overall evidence base, coverage of studies across the genotoxicity battery, and the quality of the studies, the weight-of-evidence analysis is inconclusive.

Male rat specificity for nephropathy and tumors

Kidney tumors were observed only in male rats and were not observed in either female rats or either sex of mice. Because an alpha2u-globulin MOA is specific to male rats, the endpoints would not be expected in female rats or either sex of mice and none were observed (see Table 1-2). No treatment related changes in kidney histopathology following oral or inhalation exposures were observed in male or female mice (NTP, 1997, 1995). No protein droplets, alpha2u-globulin immunostaining, or increases in cell proliferation were observed in the kidneys of female rats, but they were seen in the male rats (Borghoff et al., 2001). Cell proliferation increased in male, but not female rats exposed to *t*-butanol via inhalation exposure for 10 days (Borghoff et al., 2001) and drinking water exposure for 90 days (Lindamood et al., 1992). Increased kidney weights were observed in female rats exposed to 1,364 or 5,304 mg/m^3 *t*-butanol after 10-days (Borghoff et al., 2001), in female F344 rats exposed to 290, 590, 850, 1,560, and 3,620 mg/kg-day *t*-butanol in a 13-week drinking water study (NTP, 1995), in female B6C3F$_1$ mice exposed to 11,620 mg/kg-day *t*-butanol in a 13-week drinking water study (NTP, 1995), and female F344 rats exposed to 6,368 mg/m^3 *t*-butanol in a 13-week inhalation study (NTP, 1997). Kidney transitional epithelial hyperplasia and inflammation were observed in female F344 rats exposed to 850, 1,560, and 3,620 mg/kg-day for 13 weeks, as well as 180, 330, and 650 mg/kg-d for 2 years (NTP, 1995). Female F344 rats exposed to 850, 1,560, and 3,620 mg/kg-day *t*-butanol had a dose-related increase in the incidence of nephropathy and the incidences were greater than that of controls (NTP, 1995). 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 alpha2u-globulin nephropathy. Therefore, the results indicate that there are some kidney effects in female rats and mice, but that the characteristic changes that occur with alpha2u-globulin accumulation are only observed in male rats. This criterion of male rat specificity is met.

Summary and Conclusions for Question One: Is the alpha2u-globulin process occurring in male rats exposed to tert-butanol?

Oral exposure to male F344 rats resulted in an increased incidence of renal tubule tumors in a 2-year oral bioassay (Hard et al., 2011; NTP, 1995). Several histopathological observations in exposed male rats were consistent with an alpha2u-globulin MOA. This evidence includes the increased size and number of hyaline droplets and the accumulated alpha2u-globulin protein in the hyaline droplets. Additionally, several subsequent steps in the histopathological sequence were observed. Overall, available data are sufficient for all three required EPA criteria, suggesting that the alpha2u-globulin process is operative. Furthermore, the available data is sufficient to fulfill the IARC criteria for establishing the role for alpha2u-globulin in male rats, with the exception of genotoxic potential because of a limited genotoxicity evidence base. Although the evidence indicates a role for alpha2u-globulin accumulation in the etiology of kidney tumors induced by exposure to *tert*-butanol in male rats, it is plausible that *tert*-butanol is a weak inducer of alpha2u-globulin considering the available histopathological observations and uncertainty regarding the temporal and dose concordance of the lesions.

Question Two: Are the renal effects in male rats exposed to tert-butanol due solely to the alpha2u-globulin process?

If the alpha2u-globulin process is operative, U.S. EPA (1991a) identifies a second question that must be answered regarding whether the renal effects are solely due to the alpha2u-globulin process, a combination of the alpha2u-globulin process and other carcinogenic processes, or primarily due to other processes. U.S. EPA (1991a) states that additional data can help inform whether the alpha2u-globulin process is the sole contributor to renal tubule tumor development in male rats. These additional data are considered and discussed in detail below.

(a) Hypothesis-testing of the alpha2u-globulin sequence of effects and structure-activity relationships that might suggest the chemical belongs in a different class of suspected carcinogens: No data are available to evaluate these considerations.

(b) Biochemical information regarding binding of the chemical to the alpha2u-globulin protein: Williams and Borghoff (2001) report that *tert*-butanol reversibly and noncovalently binds to alpha2u-globulin in the kidneys of male rats. This provides additional support to the involvement of the alpha2u-globulin process.

(c) Presence of sustained cell replication in the S2 (P2) segment of the renal tubule at doses used in the cancer bioassay and a dose-related increase in hyperplasia of the renal tubule:

Sustained cell division in the proximal tubule of the male rat is consistent with, although not specific to, the alpha2u-globulin process. Cell proliferation was observed in two studies [13-week, NTP (1995) and 10-day, Borghoff et al. (2001)] but whether the proliferation was compensatory is unknown, as cytotoxicity was not observed in these studies. Although the data do not support sustained occurrence of cell division subsequent to cytotoxic cell death, renal tubule hyperplasia in male rats was reported after 2 years of exposure (NTP, 1995). Thus, although some evidence of sustained cell replication is available, it does not specifically support alpha2u-globulin protein accumulation.

(d) Covalent binding to DNA or other macromolecules, suggesting another process leading to tumors and genotoxicity (alpha2u-globulin -inducers are essentially nongenotoxic): One study (Yuan et al., 2007) observed a dose-related increase in *tert*-butanol-DNA adducts in liver, kidney, and lung of mice administered a single low dose of *tert*-butanol (≤1 mg/kg) in saline via gavage (see Appendix B.3 in Supplemental Information for further details). An extremely sensitive method of detection was used (accelerator mass spectrometry), but the DNA adduct species were not identified, and no replication of these results has been identified in the literature. The few studies available to assess the direct genotoxic potential of *tert*-butanol primarily are negative, although a few studies report DNA damage induced by oxidative stress. DNA damage induced by oxidative stress is consistent with the decreased levels of glutathione in male rat kidneys reported by Acharya et al. (1995) after 10 weeks of *tert*-butanol exposure. This type of genetic damage would not necessarily preclude a role for alpha2u-globulin , but not enough information is available to

determine whether oxidative stress could initiate or promote kidney tumors in concert with alpha2u-globulin accumulation in male rat kidneys.

(e) Nephrotoxicity in the male rat not associated with the alpha2u-globulin process or CPN, suggesting the possibility of other processes leading to renal tubule nephrotoxicity and carcinogenicity: Nephropathy reported in the 13-week oral and inhalation and 2-year oral studies was considered CPN and these effects were exacerbated by treatment with *tert*-butanol. At 13 weeks (NTP, 1997, 1995) and 2 years (NTP, 1995), oral and inhalation exposure increased the severity of nephropathy in male rats (NTP, 1995). Similarly, the severity of nephropathy was increased in females at 2 years, but only the incidence of nephropathy was increased in females following a 13-week oral exposure (NTP, 1995).

Increased incidences of suppurative inflammation and kidney transitional epithelial hyperplasia were observed in female rats orally exposed to *tert*-butanol for 2 years. NTP (1995) and Frazier et al. (2012) characterized these endpoints as associated with CPN, and an analysis of the individual animals indicates these endpoints are moderately correlated with CPN. However, most cases of suppurative inflammation and transitional epithelial hyperplasia are spontaneous changes whose cause is unknown and are typically unrelated to CPN or have been noted as secondary changes to CPN (NIEHS, 2019). At 2 years, the male rats also exhibited a dose-related increase in transitional epithelial hyperplasia, and the correlation of this endpoint with CPN was stronger than in female rats.

Kidney weights were increased in male and female rats in the 13-week oral and inhalation evaluations (NTP, 1997, 1995) and 15-month oral evaluation (NTP, 1995). The dose-related increases observed in both male and female rats suggest that the kidney weight changes are indicative of treatment-related molecular processes primarily unrelated to alpha2u-globulin protein accumulation. Given that CPN also was increased at these time points, however, the influence of CPN on kidney weights cannot be ruled out.

Overall, the nephrotoxicity observed in the male rat is difficult to disentangle from CPN and alpha2u-globulin processes. The moderate correlation between CPN severity and renal tumor incidence in male rats (Spearman's rank coefficient = 0.45) and the very weak correlation between renal tubule hyperplasia and renal tumors (Spearman's rank coefficient = 0.16) (Table 1-8) suggests that alpha2u-globulin nephropathy is not solely responsible for the renal tumors. Furthermore, considering that the treatment-related exacerbation of CPN severity in female rats occurs without the subsequent induction of renal tumors, this suggests that other processes besides alpha2u-globulin and CPN in males might be responsible for the renal tubule tumors.

Summary and Conclusions for Question Two:

Although the evidence suggests that *tert*-butanol induces alpha2u-globulin nephropathy, the data indicate that *tert*-butanol is a weak inducer of alpha2u-globulin and that this process is not solely responsible for the renal tubule nephropathy and carcinogenicity observed in male rats. The lack of compensatory cell proliferation in male rats and evidence of nephrotoxicity in female rats suggest that other processes, in addition to the alpha2u-globulin process, are operating. Furthermore, the accumulation of hyaline droplets and the induction of renal tubule hyperplasia were affected at higher doses compared to those inducing renal tubule tumors. Collectively, these data suggest that *tert*-butanol induces the alpha2u-globulin pathway at high doses (>420 mg/kg-day), which results in tumor formation. Other, unknown pathways, however, could be operative at lower doses (<420 mg/kg-day), which may contribute to renal tumor induction.

Chronic Progressive Nephropathy and Renal Carcinogenicity

Scientists disagree about the extent to which CPN can be characterized as a carcinogenic MOA suitable for analysis under the EPA's cancer guidelines (Hard et al., 2013; Melnick et al., 2013; Melnick et al., 2012). The etiology of CPN is unknown and CPN is both a spontaneous and complex disease whose processes are affected by aging and strain specificity (NIEHS, 2019). Therefore, it is difficult to separate the effects of spontaneously occurring CPN from those effects on CPN induced by chemical exposure. Proponents of CPN as an MOA have developed an evolving series of empirical criteria for attributing renal tubule tumors to CPN. Hard and Khan (2004) proposed criteria for concluding that a chemical is associated with renal tubule tumors through an interaction with CPN. Hard et al. (2013) slightly revised and restated their criteria for considering exacerbation of CPN as a MOA for renal tubule tumors in rats. Table 1-11 lists these sets of proposed empirical criteria for attributing renal tubule tumors to CPN.

Table 1-11. Proposed empirical criteria for attributing renal tumors to CPN

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

Hard et al. (2013) maintain that knowing the detailed etiology or underlying mechanism for CPN is unnecessary. Instead, identifying increased CPN with its associated increase in tubule cell proliferation as the key event is adequate. Nonetheless, Hard et al. (2013) also postulated a sequence of key events for renal tumorigenesis involving exacerbation of CPN:

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

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

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

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

Strength, consistency, specificity of association. The relationship between exacerbated CPN and renal tumors is moderate in male rats in the NTP (1995) study. According to the NTP (1995) analysis, the mean CPN grades (same as "severity of nephropathy" reported by NTP) presented on a scale 1–4 for male rats with renal tumors were 3.5, 3.6, 3.7, and 3.4 at doses 0, 1.25, 2.5, and 5 mg/mL. The mean CPN grades for male rats without renal tumors were 2.9, 2.8, 2.8, and 3.2 for the same dose groups. The reanalysis of the NTP data by Hard et al. (2011) yielded similar numbers. Analysis of the individual occurrence of CPN and renal tumors demonstrated a moderately positive correlation (Spearman's rank coefficient $r_s = 0.43$) (Table 1-8). The relationship between CPN and renal tumors, however, is neither consistent nor specific in the NTP (1995) study: No female rats developed renal tumors regardless of the presence of relatively low-grade or relatively high-grade CPN. For example, in female rats surviving more than 700 days, the mean CPN grades were 1.7 and 3.2 at doses of 0 and 10 mg/mL, respectively, but no tumors developed in either group.

Dose-response concordance. The dose-response relationships for CPN, renal tubule hyperplasia, and renal tubule tumors somewhat differ between the two analyses. According to the NTP (1995) analysis, at doses of 0, 1.25, 2.5, and 5 mg/mL, the mean CPN grades for all male rats were 3.0, 3.1, 3.1, and 3.3; the incidences of renal tubule hyperplasia (standard and extended evaluation combined) were 14/50, 20/50, 17/50, and 25/50; and the incidences of renal tubule adenomas or carcinomas were 8/50, 13/50, 19/50, and 13/50 (Table 1-3). The reanalysis by Hard et al. (2011) reported similar tumor incidences (4/50, 13/50, 18/50, and 12/50), except that four fewer rats in the controls and one fewer rat in the group exposed to 2.5 mg/mL had tumors. The lower control incidence (4/50 versus 14/50 in the NTP study) observed in this reanalysis accentuates the differences in these dose-response relationships. For example, the maximal tumor response (4/50 in controls versus 18/50 at the middle dose) does not parallel the marginal change in CPN severity (i.e., group average of 3.0 to 3.1). That a marginal increase in CPN severity would be associated with significant tumor induction seems inconsistent. Furthermore, CPN severity is nearly as great in the female rats, yet no females developed tumors, as noted above.

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

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

Conclusions about the hypothesized CPN-related MOA

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

(a) *Is the hypothesized MOA sufficiently supported in the test animals?* Exacerbated CPN leading to renal tubule tumors in male rats late in life appears to have some support. Consistency is lacking, however, between males and females and in the dose-response relationships between CPN, hyperplasia, and adenomas, Melnick et al. (2013); Melnick et al. (2012) concluded, based on an analysis of 60 NTP studies, no consistent association exists between exacerbated CPN and the incidence of renal tubule tumors in rats. Without a consistent association and an understanding of its key events, they maintain that determining the human relevance of processes that might be occurring in rats is not possible. An earlier analysis of 28 NTP studies (Seely et al., 2002) found a slight but statistically significant increase in CPN severity in animals with renal tubule tumors, without determining that this relationship is causal. They suggested that the number of tumors due to chemically exacerbated CPN would be few.

These inconsistencies make difficult attributing all renal tumors to either CPN or to alpha2u-globulin -related nephropathy (see previous section on alpha2u-globulin), raising the likelihood of another, yet unspecified MOA.

(b) *Is the hypothesized MOA relevant to humans?* CPN is a common and well-established constellation of age-related lesions in the kidney of rats, and no counterpart to CPN in aging humans is known (NIEHS, 2019). Scientists disagree, however, on the relevancy of the CPN MOA to humans. Hard et al. (2013); Hard et al. (2009) cite several differences in pathology between rat CPN and human nephropathies in their arguments that CPN-related renal tumors in rats are not relevant to humans. On the other hand, Melnick et al. (2013); Melnick et al. (2012) argue that the etiology of CPN and the mechanisms for its exacerbation by chemicals are unknown and fail to meet fundamental principles for defining an MOA and for evaluating human relevance. While the morphological spectrum observed in CPN in male rats does not appear to have a human analogue in the aging kidney (NIEHS, 2019), these individual lesions or processes could occur in a human kidney, and their occurrence as a group in the aged rat kidney does not make each one rat-specific if a treatment effect occurs for one or more of them. Given that the etiology of CPN is unknown and the disease process is complex it is plausible that any chemical that causes CPN in rats may have the potential to exacerbate disease processes in the human kidney (NIEHS, 2019). This issue is unresolved.

(c) *Which populations or lifestages can be particularly susceptible to the hypothesized MOA?* It is unknown whether certain human populations or lifestages are especially susceptible to tumors induced through exacerbated CPN.

In summary, the renal tubule tumors are partially attributed to CPN in male rats and not in female rats, considering discrepant patterns in the dose-response relationships for CPN, hyperplasia, and renal tubule tumors; the moderately strong correlation between CPN grades and renal tubule tumors in male rats; and the lack of relationships between CPN severity and renal tumors in female rats together with the lack of a generally accepted MOA for CPN.

This position can be reconciled with that of Melnick et al. (2013); Melnick et al. (2012), who argued against dismissing renal tubule tumors in rats that can be related to exacerbated CPN. It also can be reconciled with Hard et al. (2013), who, while maintaining these tumors are not relevant to humans, also allow there is no generally accepted MOA for CPN akin to that for alpha2u-globulin -related nephropathy. Hard et al. (2013) made this statement after reporting on the collective experience of national and international health agencies worldwide with the use of CPN as an MOA. Of 21 substances that exacerbated CPN and caused renal tumors, most were multisite carcinogens, and other tumor sites contributed to the evaluations. Only two assessments explicitly considered CPN as a renal tumor mechanism. One was the assessment of ethylbenzene by the German Federal Institute for Occupational Safety and Health, in which the agency concluded that the kidney tumors were associated with the high, strain-specific incidence of CPN that is unknown for humans [as discussed in Hard et al. (2013)]. The other was the IRIS assessment of tetrahydrofuran, for which EPA found the evidence insufficient to conclude that the kidney tumors are mediated solely by the hypothesized MOAs (U.S. EPA, 2012d). Hard et al. (2013) attributed these different conclusions to either different data for the two chemicals or the lack of a generally accepted MOA akin to alpha2u-globulin -related nephropathy.

Relevant to this last point, IARC (1999) developed a consensus statement that listed considerations for evaluating alpha2u-globulin -related nephropathy in rats, which was based on the work of 22 scientists, including 3 who were co-authors of Hard et al. (2013) and 2 who were co-authors of Melnick et al. (2013); Melnick et al. (2012). A similar broad-based consensus that defines a sequence of key events for exacerbated CPN, distinguishes it more clearly from alpha2u-globulin - related nephropathy, and evaluates its relevance to humans would be helpful in advancing the understanding of these issues.

Overall Conclusions on MOA for Kidney Effects

tert-Butanol increases alpha2u-globulin deposition and hyaline droplet accumulation in male rat kidneys and several of the subsequent steps in that pathological sequence. These data provide some evidence that the alpha2u-globulin process is operating. This chemical appears to be a weak inducer of alpha2u-globulin nephropathy and this induction is not the sole contributor to renal tubule nephropathy and carcinogenicity. CPN and the exacerbation of CPN (likely due to both alpha2u-globulin and *tert*-butanol) play a role in renal tubule nephropathy. The available evidence indicates that CPN might be involved in the induction of renal tubule tumors in male rats, likely by providing proliferative stimulus in the form of compensatory regeneration following toxicity to the renal tubule epithelium, although these effects were not observed in some studies. Additionally, several endpoints in female rats indicate that renal tubule nephrotoxicity and increased kidney weights related to *tert*-butanol exposure cannot be explained by the alpha2u-globulin process.

Integration of Kidney Effects

Kidney effects (increases in nephropathy, severity of nephropathy, hyaline droplets, linear mineralization, suppurative inflammation, transitional epithelial hyperplasia, mineralization, and kidney weight) were observed, predominantly in male and female rats across the multiple *tert*-butanol studies. The available evidence indicates that multiple processes induce the noncancer kidney effects. Two endpoints in male rats (hyaline droplets, linear mineralization) are components of the alpha2u-globulin process. U.S. EPA (1991a) states that if the alpha2u-globulin process were occurring in male rats, the renal tubule effects associated with this process in male rats would not be relevant to humans for the purposes of hazard identification. In cases such as these, the characterization of human health hazard for noncancer kidney toxicity would rely on effects not specifically associated with the alpha2u-globulin process in male rats.

The group of lesions generally reported as “nephropathy,” is related to CPN. CPN is a common and well-established constellation of age-related lesions in the kidney of rats; for which no known counterpart to CPN exists in aging humans, and the mode of action is unknown (NIEHS, 2019). CPN is not, inherently, a specific diagnosis, however, but an aggregate term describing a spectrum of effects. The individual lesions associated with CPN (tubular degeneration, glomerular sclerosis, etc.) also occur in the human kidney. ((Zoja et al., 2015; Gorriz and Martinez-Castelao, 2012). Thus, it cannot be ruled out that chemicals which exacerbate CPN in rats may have the potential to exacerbate disease processes in the human kidney (NIEHS, 2019).

Because female rats are not affected by alpha2u-globulin nephropathy, lesions associated with CPN in female rats are informative for human hazard characterization. Several other noncancer endpoints resulted from *tert*-butanol exposure and are appropriate for consideration of a kidney hazard, specifically: suppurative inflammation in female rats, transitional epithelial hyperplasia in female rats, severity of nephropathy in female rats, incidence of nephropathy in female rats, and increased kidney weights in rats but not mice. Based on dose-related increases in these noncancer endpoints in rats, kidney effects are a potential human hazard of *tert*-butanol exposure. The hazard and dose-response conclusions regarding these noncancer endpoints associated with *tert*-butanol exposure are discussed further in Section 1.3.1.

The carcinogenic effects observed following *tert*-butanol exposure include increased incidences of renal tubule hyperplasia (considered a preneoplastic effect) and tumors in male rats. EPA concluded that the three criteria were met to indicate that an alpha2u-globulin process is operating. Because renal tubule tumors in male rats did not arise solely due to the alpha2u-globulin and CPN processes and some of the tumors are attributable to other carcinogenic processes, such tumors remain relevant for purposes of hazard identification (U.S. EPA, 1991a).[10] The hazard and dose-response conclusions regarding the renal tubule hyperplasia and tumors associated with *tert*-butanol exposure are further discussed as part of the overall weight of evidence for carcinogenicity in Section 1.3.2.

1.2.2 Thyroid Effects

Synthesis of Effects in Thyroid

The evidence base on thyroid effects following *tert*-butanol exposure contains no human data, two oral subchronic and two oral chronic studies (one of each duration in rats and in mice) (NTP, 1995), and two inhalation subchronic studies (one in rats and one in mice) (NTP, 1997). Studies employing short-term and acute exposures that examined thyroid effects are not included in the evidence table. These studies are discussed, however, in the text if they provide data informative of MOA or hazard identification. No gross thyroid effects were reported in the 13-week evaluations of mice or rats following oral or inhalation exposure (NTP, 1997, 1995), and therefore subchronic studies were not included in the evidence table. The two available chronic studies are arranged in the evidence table by effect and then by species (Table 1-12; Figure 1-10).

[10]When the alpha2u-globulin process is occurring, U.S. EPA (1991a) states that one of the following conclusions will be made: (a) if renal tumors in male rats are attributable solely to the alpha2u-globulin process, such tumors will not be used for human cancer hazard identification or for dose-response extrapolations; (b) if renal tumors in male rats are not linked to the α_{2u} globulin process, such tumors are an appropriate endpoint for human hazard identification and are considered, along with other appropriate endpoints, for quantitative risk estimation; or (c) if some renal tumors in male rats are attributable to the alpha2u-globulin process and some are attributable to other carcinogenic processes, such tumors remain relevant for purposes of hazard identification, but a dose-response estimate based on such tumors in male rats should not be performed unless enough information is available to determine the relative contribution of each process to the overall renal tumor response.

Thyroid effects, specifically follicular cell hyperplasia and adenomas, were observed in mice of both sexes after 2 years of oral exposure via drinking water (NTP, 1995). NTP (1995) noted, "[p]roliferation of thyroid gland follicular cells is generally considered to follow a progression from hyperplasia to adenoma and carcinoma." Both male and female mice exhibited a dose-related increase in the incidence of hyperplasia, and the average severity across all dose groups was minimal to mild with scores ranging from 1.2 to 2.2 (out of 4). Increased incidence of adenomas also was observed in the *tert*-butanol-treated female mice at the high-dose, with the only carcinoma observed in high-dose males. At the highest dose, mean body weights of female mice were 10 to 15% lower than control animals from week 13 to the end of the study with a final average body weight reduction of 12%, and the final average body weight reduction in male mice was 5% to 10%, raising some question that the thyroid tumors were the result of excessive toxicity in mice rather than carcinogenicity of *tert*-butanol. EPA's Cancer Guidelines (U.S. EPA, 2005a) discusses the determination of an "excessively high dose" as compared to an "adequate high dose" and describes the process as one of expert judgment which requires that "...adequate data demonstrate that the effects are solely the result of excessive toxicity rather than the carcinogenicity of the tested agent" (U.S. EPA, 2005a). In the 2-year oral bioassay (NTP, 1995), study authors noted that water consumption by exposed females and males was similar to controls and that no overt toxicity was observed. Furthermore, female mice in the high dose group had higher rates of survival than control animals. No treatment-related thyroid effects were reported in rats of either sex following 2 years of oral exposure (NTP, 1995).

The tumor response in male mice, adjusted for early mortality, showed a statistically significant increasing trend (Cochran-Armitage trend test, $p = 0.041$; analysis performed by EPA). Although the response appeared nonmonotonic, with a slightly lower response at the high-dose level than at the mid-dose level, the increased mortality reported in the high-dose group occurred before tumors appeared; about 40% of the high-dose males died before the first tumor (a carcinoma) appeared in this group at week 83. By comparison, only ~10% of the control group had died by this time, and the single tumor in the control group was observed at study termination. Mortality in the exposed female mice was similar to controls.

Table 1-12. 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	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

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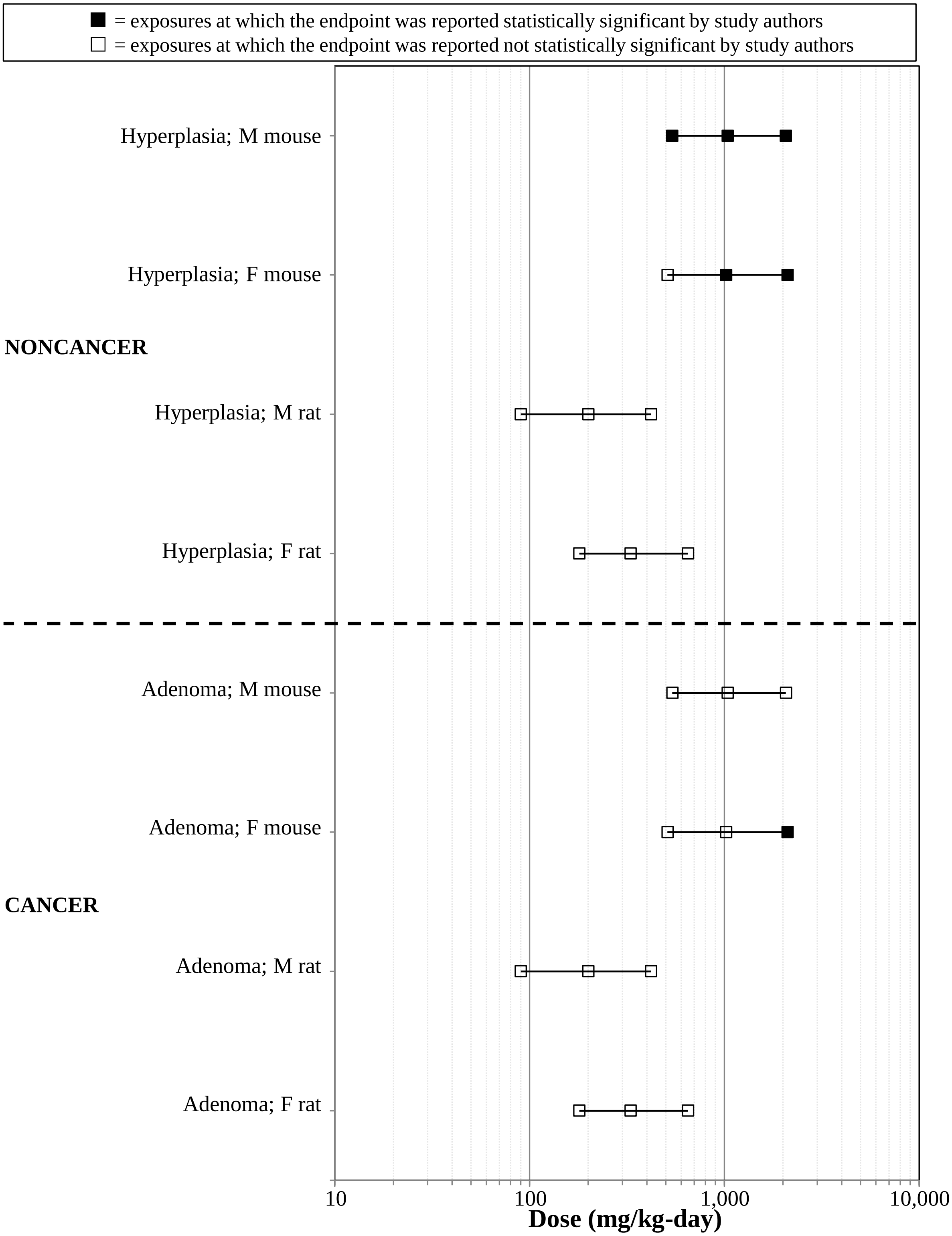
Reference and study design	Results			
F: 0, 180, 330, or 650[a] mg/kg-d 2 years	200	0/50	330	0/50
	420[a]	0/50	650[a]	0/50
NTP (1995) $B6C3F_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	Incidence (severity)			
	Males		**Females**	
	Dose (mg/kg-d)	Follicular cell hyperplasia	Dose (mg/kg-d)	Follicular cell hyperplasia
	0	5/60 (1.2)	0	19/58 (1.8)
	540	18/59* (1.6)	510	28/60 (1.9)
	1,040	15/59* (1.4)	1,020	33/59* (1.7)
	2,070[a]	18/57* (2.1)	2,110	47/59* (2.2)

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

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

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



Source: NTP (1995)

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

Mode of Action Analysis—Thyroid Effects

The MOA responsible for *tert*-butanol-induced thyroid effects has been the subject of little study. One hypothesis is that *tert*-butanol increases liver metabolism of thyroid hormones, triggering a compensatory increase in pituitary thyroid-stimulating hormone (TSH) production. Such sustained increases in TSH could induce elevated thyroid follicular cell proliferation and hyperplasia and lead to follicular cell adenoma and carcinoma. This enhancement of liver metabolism and excretion of thyroid hormones is one of several potential antithyroid MOAs, as identified in EPA's guidance on the assessment of thyroid follicular cell tumors (U.S. EPA, 1998b).

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

<u>1) Increases in cell growth (required)</u>

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

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

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

Evidence of hormonal changes, including decreases in triiodothyronine (T_3) and thyroxine (T_4) and increases in TSH, are required to demonstrate a disruption in the thyroid-pituitary signaling axis (U.S. EPA, 1998b). Blanck et al. (2010) evaluated serum thyroid hormones in mice after 3 or 14 days of exposure to *tert*-butanol. No *tert*-butanol-related effects were observed in T_3, T_4, or TSH levels after 3 days. Although both T_3 and T_4 levels were significantly decreased approximately 10–20% after 14 days of treatment with *tert*-butanol, TSH levels remained unaffected. Similar results were reported with the positive control (phenobarbital). The limited evidence available from this single study suggests that although T_3 and T_4 levels were decreased after 14 days, this perturbation likely did not exceed the range of homeostatic regulation in female B6C3F$_1$ mice and thus was not likely to induce compensatory thyroid follicular cell proliferation. Multiple lines of evidence support this observation: (1) TSH levels were unaffected, indicating that the decrease in T_3 and T_4 levels was not severe enough to stimulate increased TSH secretion by the pituitary in this timeframe; (2) thyroid hyperplasia was not induced in this study, or any others exposing mice to similar or greater concentrations for 2.5–13 weeks, suggesting that thyroid proliferation was either not induced by the hormone fluctuations or that any follicular cell proliferation during this period was too slight to be detected by routine histopathological examination; (3) the maximal decrease in T_3 or T_4 hormone levels induced by *tert*-butanol exposure after 14 days (i.e., ~20%) was well within the range of fluctuation in T_3 and T_4 hormone levels reported to occur between the 3- and 14-day control groups [15–40%; (Blanck et al., 2010)]. Although the lower T_3 and T_4 levels following *tert*-butanol were later attributed by the study authors to an increase in liver metabolism (see next section), alternatively, they could be due to a variety of other possible, yet uninvestigated, molecular interactions of *tert*-butanol. Such interactions might include (1) inhibition of iodide transport into thyroid follicular cells, (2) thyroid peroxidase inhibition, (3) thyroid follicular cell dysfunction leading to inhibition of thyroid hormone production or release, or (4) inhibition of 5'-monodeiondinase (Hurley, 1998; U.S. EPA, 1998b).

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

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

The thyroid and liver are two of several potential sites of antithyroid action, with the liver the most common, where increased microsomal enzyme activity could enhance thyroid hormone metabolism and removal (U.S. EPA, 1998b). Rats are thought to be more sensitive than mice to this aspect of antithyroid activity (Roques et al., 2013; Qatanani et al., 2005; U.S. EPA, 1998b); however, rats exposed to *tert*-butanol for 2 years exhibited no treatment-related thyroid effects, while mice did. Typically, chronic induction of liver microsomal enzyme activity resulting from repeated chemical exposure would manifest some manner of liver histopathology, such as hepatocellular hypertrophy or hyperplasia (U.S. EPA, 1998b; NTP, 1995). In a 14-day mechanistic investigation, *tert*-butanol had no effect on liver weight when compared to the control group, but centrilobular hepatocellular hypertrophy was reported in 2/5 livers from high-dose mice versus 0/6 in control and 0/5 in low-dose mice (Blanck et al., 2010). Relative liver weights increased in male and female mice after 13 weeks of oral exposure (NTP, 1995) to higher doses than those evaluated by Blanck et al. (2010), although absolute liver weight measurements in treated animals showed little change from controls suggesting that the relative measures could have been related to decreases in body weight rather than specific liver effects. Relative (and absolute) liver weights were increased in female mice (only) after 13 weeks of inhalation exposure at the two highest concentrations (NTP, 1997); liver weight was not reported in mice orally exposed for 2 years (NTP, 1995). No increase in mouse hepatocellular hypertrophic or hyperplastic histopathology was reported following 2.5 weeks to 2 years of exposure (NTP, 1997, 1995). In fact, the only liver pathology associated with *tert*-butanol exposure in either rats or mice from these studies was an increase in fatty liver in male mice in the high-dose group after 2 years of oral exposure (NTP, 1995). Although increased fatty liver could indicate some nonspecific metabolic alteration, the absence of a similar treatment-related effect in livers from female mice, which were sensitive to both thyroid follicular cell hyperplasia and tumor induction, suggests that it might not be related to the thyroid tumorigenesis.

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

4) Dose correlation (required)

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

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

5) Reversibility (required)

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

In summary, the available evidence base sufficiently supports only (1) increases in thyroid cell growth. The existing data are inadequate to evaluate (2) thyroid and pituitary hormone changes consistent with the antithyroid MOA, (3) site(s) of the antithyroid action, or (5) reversibility of effects in the early stages of disruption. Although these inadequacies also limit the evaluation of (4) dose correlation among the various effects, the available evidence suggests that little correlation exists among reported thyroid, pituitary, and liver endpoints. An additional consideration is the evaluation of genotoxic potential. As summarized in Appendix B, there is limited evidence to suggest that thyroid tumors following *tert*-butanol exposure are due to mutagenic changes. Together, the evidence base is inadequate to determine if an antithyroid MOA is operating in mice. In the absence of information to indicate otherwise, the thyroid tumors observed in mice are considered relevant to humans.

Integration of Thyroid Effects

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

1.2.3 Developmental Effects

Synthesis of Effects Related to Development

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

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

In these studies, fetal effects are generally observed at high doses that cause toxicity in the dams as measured by clinical signs (e.g., decreased [~7–36%] body weight gain and food consumption and reported ataxia and lethargy) (Table 1-13; Figure 1-11; Figure 1-12). As stated in the *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991b), "an integrated evaluation must be performed considering all maternal and developmental endpoints." "[W]hen adverse developmental effects are produced only at doses that cause minimal maternal toxicity; in these cases, the developmental effects are still considered to represent developmental toxicity and should not be discounted." Although, at doses of "excessive maternal toxicity…information on developmental effects may be difficult to interpret and of limited value." In considering the observed fetal and maternal toxicity data following *tert*-butanol exposure and the severity of the maternal effects, the role of maternal toxicity in the developmental effects observed at the doses used remains unclear. Specifically, discerning from the available data whether the fetal effects are directly related to *tert*-butanol treatment or are secondary to maternal toxicity is not possible.

Table 1-13. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Huntingdon Life Sciences (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 PND 21
F1 males and females: 7 weeks (throughout gestation and lactation; 1 male and 1 female from each litter were dosed directly from PND 21–28)</td>
<td>Response relative to control

Maternal effects

Percent change compared to control:
<table>
<tr><th>Dose (mg/kg-d)</th><th>Body weight gain GD 0–20</th><th>Food consumption GD 0–20</th><th>Body weight gain PND 1–21</th><th>Food consumption LD 1–14</th><th>Live pups/litter response</th></tr>
<tr><td>0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>64</td><td>−3</td><td>0</td><td>3</td><td>−2</td><td>−9</td></tr>
<tr><td>160</td><td>−4</td><td>0</td><td>−10</td><td>−6</td><td>−11</td></tr>
<tr><td>400</td><td>0</td><td>4</td><td>3</td><td>0</td><td>−7</td></tr>
<tr><td>1000</td><td>−16*</td><td>0</td><td>100*[a]</td><td>−16</td><td>−33*</td></tr>
</table>
Dams dosed with 400 or 1000 mg/kg-d showed CNS effects (e.g., ataxia, lethargy) that were undetectable by 4 weeks of exposure in animals exposed to 400 mg/kg-d but not those in the higher dose group. [a] Large body weight gain from PND 1-21 potentially due to make up from gestational losses and smaller litter sizes during lactation.
F1 effects
<table>
<tr><th>Dose (mg/kg-d)</th><th>Viability index (pup survival to PND 4)</th><th>Lactation index (pup survival to PND 21)</th><th>Sex ratio (% males)</th><th>Pup weight/litter PND 1 relative to control (%)</th><th colspan="2">Pup weight PND 28 relative to control (%)</th></tr>
<tr><th></th><th></th><th></th><th></th><th></th><th>Male</th><th>Female</th></tr>
<tr><td>0</td><td>96.4</td><td>100</td><td>54.4</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>64</td><td>98.7</td><td>100</td><td>52.3</td><td>6</td><td>2</td><td>0</td></tr>
<tr><td>160</td><td>98.2</td><td>100</td><td>50.9</td><td>4</td><td>0</td><td>−4</td></tr>
<tr><td>400</td><td>99.4</td><td>99.2</td><td>53.5</td><td>7</td><td>0</td><td>−2</td></tr>
<tr><td>1000</td><td>74.1*</td><td>98.8</td><td>52.1</td><td>−10</td><td>−12*</td><td>−8</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Daniel and Evans (1982)
Swiss Webster (Cox) mouse; 15 pregnant dams/treatment
Liquid diet (0, 0.5, 0.75, 1.0%, w/v)
0 (isocaloric amounts of maltose/dextrin), 3,324, 4,879, 6,677 mg/kg-d
GD 6–20</td><td>No statistical analysis was conducted on any of these data.
Maternal
Percent change compared to control:
<table>
<tr><th>Dose (mg/kg-d)</th><th>Food consumption (mean g/animal/day)</th><th>Body weight gain</th><th>Number of litters (% pregnant dams)</th></tr>
<tr><td>0</td><td>-</td><td>-</td><td>11 (77%)</td></tr>
<tr><td>3,324</td><td>2</td><td>−3</td><td>12 (80%)</td></tr>
<tr><td>4,879</td><td>−3</td><td>−19</td><td>8 (53%)</td></tr>
<tr><td>6,677</td><td>−4</td><td>−20</td><td>7 (47%)</td></tr>
</table>
Authors note that lower food consumption in higher tert-butanol dose groups reflects problems with pair feeding and maternal sedation.
Fetal
Percent change compared to control:
<table>
<tr><th>Dose (mg/kg-d)</th><th>Number of neonates/litter</th><th>Fetal body weight on PND 2</th></tr>
<tr><td>0</td><td>-</td><td>-</td></tr>
<tr><td>3,324</td><td>−1</td><td>−7</td></tr>
<tr><td>4,879</td><td>−29</td><td>−19</td></tr>
<tr><td>6,677</td><td>−49</td><td>−38</td></tr>
</table>
Number of stillborn also increased with dose (3, 6, 14, and 20, respectively), but the number of stillborn per litter was not provided. The high dose also caused a delay in eye opening and a lag in weight gain during PND 2–10 (information was provided only in text or figures)</td></tr>
<tr><td>Faulkner et al. (1989)
CBA/J mouse; 7 pregnant females in control, 12 pregnant females in treated
Gavage (10.5 mmoles/kg twice a day);
0 (tap water), 1,556 mg/kg-d
GD 6–18</td><td>Maternal results not reported.
Fetal
<table>
<tr><th></th><th colspan="3">Percent change compared to control:</th><th colspan="2">Incidence:</th></tr>
<tr><th>Dose (mg/kg-d)</th><th>Resorptions/litter</th><th>Live fetuses/ litter</th><th>Fetal weight</th><th>Sternebral variations</th><th>Skull variations</th></tr>
<tr><td>0</td><td>-</td><td>-</td><td>-</td><td>4/28</td><td>1/28</td></tr>
<tr><td>1,556</td><td>118*</td><td>−41*</td><td>−4</td><td>7/30</td><td>3/30</td></tr>
</table>
Sternebral variations: misaligned or unossified sternebrae
Skull variations: moderate reduction in ossification of supraoccipital bone
Number of total resorptions (10 resorptions/66 implants in controls, 37/94 implants in treated) increased ($p < 0.05$)</td></tr>
</table>

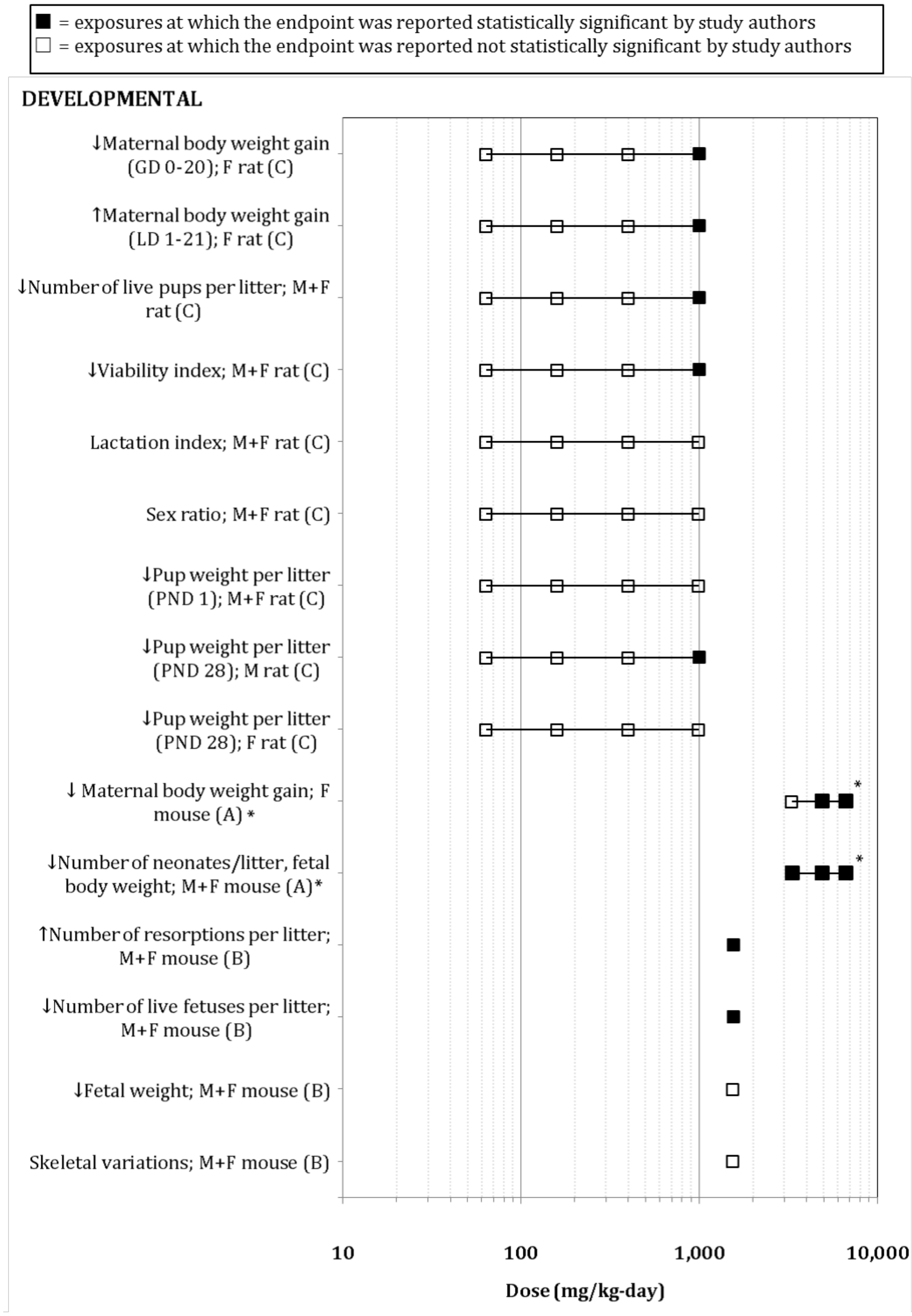
<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td>Faulkner et al. (1989)
C57BL/6J mouse; 5 pregnant females in controls, 9 pregnant females treated
Gavage (10.5 mmoles/kg twice a day)
0 (tap water), 1,556 mg/kg-d
GD 6–18</td>
<td>Maternal results not reported.

Fetal
<table>
<tr><th></th><th colspan="3">Percent change compared to control:</th><th colspan="2">Incidence:</th></tr>
<tr><th>Dose (mg/kg-d)</th><th>Resorptions/litter</th><th>Live fetuses/ litter</th><th>Fetal weight</th><th>Sternebral variations</th><th>Skull variations</th></tr>
<tr><td>0</td><td>-</td><td>-</td><td>-</td><td>5/21</td><td>1/21</td></tr>
<tr><td>1,556</td><td>428*</td><td>−58*</td><td>−4</td><td>9/16</td><td>7/16</td></tr>
</table>
Sternebral variations: misaligned or unossified sternebrae
Skull variations: moderate reduction in ossification of supraoccipital bone
Number of total resorptions (4 resorptions/44 implants in controls, 38/68 implants in treated) increased (p < 0.05)</td></tr>
<tr><td>Nelson et al. (1989)
Sprague-Dawley rat; 15 pregnant dams/treatment
Inhalation analytical concentration: 0, 2,200, 3,510, 5,030 ppm (0, 6,669, 10,640, 15,248 mg/m³), dynamic whole-body chamber
7 hr/d
GD 1–19</td>
<td>Maternal: Unsteady gait (no statistical tests reported), dose-dependent ↓ in body weight gain (results presented in figure only), dose-dependent ↓ in food consumption ranging from 7 to 36%, depending on dose and time

Fetal Percent change compared to control (mean ± standard error):
<table>
<tr><th>Dose (mg/m³)</th><th>Number of live fetuses/litter</th><th>Resorptions per litter</th></tr>
<tr><td>0</td><td>−(13 ± 2)</td><td>−(1.1 ± 1.2)</td></tr>
<tr><td>6,669</td><td>0 (13 ± 4)</td><td>9 (1.2 ± 1.1)</td></tr>
<tr><td>10,640</td><td>15 (15 ± 2)</td><td>−18 (0.9 ± 1.0)</td></tr>
<tr><td>15,248</td><td>8 (14 ± 2)</td><td>0 (1.1 ± 0.9)</td></tr>
</table>
<table>
<tr><th></th><th colspan="2">Percent change compared to control:</th><th colspan="2">Incidence:</th></tr>
<tr><th>Dose (mg/m³)</th><th>Fetal weight (males)</th><th>Fetal weight (females)</th><th>Skeletal variation by litter</th><th>Skeletal variation by fetus</th></tr>
<tr><td>0</td><td>-</td><td>-</td><td>10/15</td><td>18/96</td></tr>
<tr><td>6,669</td><td>−9*</td><td>−9*</td><td>14/17</td><td>35/104</td></tr>
<tr><td>10,640</td><td>−12*</td><td>−13*</td><td>14/14</td><td>53/103*</td></tr>
<tr><td>15,248</td><td>−32*</td><td>−31*</td><td>12/12</td><td>76/83*</td></tr>
</table>
Skeletal variation by litter refers to the number of variations observed in the number of litters examined. Skeletal variation by fetus refers to the number of variations observed in the total number of fetuses examined. Fetuses are not categorized by litter.</td></tr>
</table>

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

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



*Study authors did not conduct statistical analysis on these endpoints, but results are determined by EPA to be biologically significant.

Sources: (A) Daniel and Evans (1982); (B) Faulkner et al. (1989); (C) Huntingdon Life Sciences (2004)

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

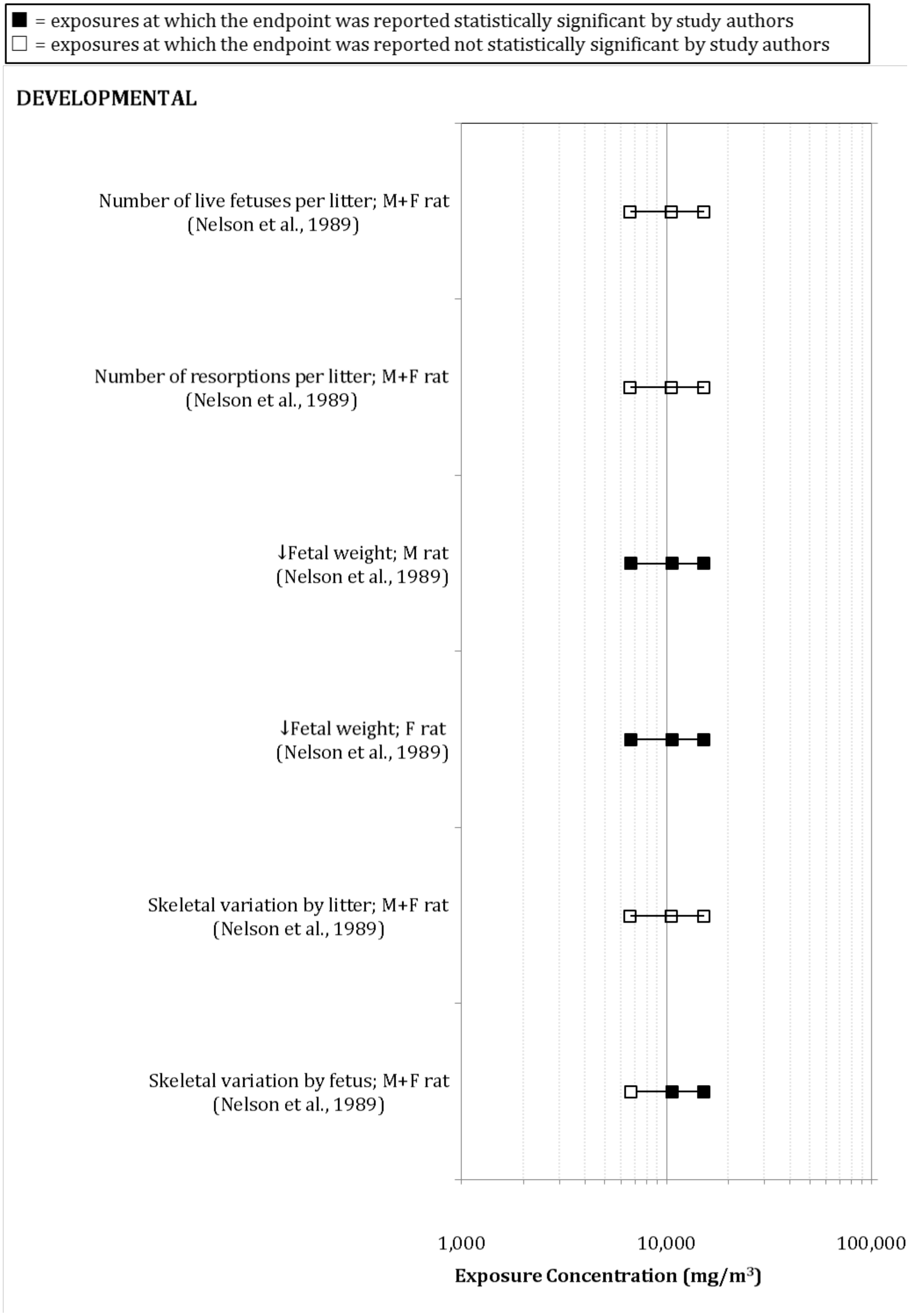


Figure 1-12. Exposure-response array of developmental effects following inhalation exposure to *tert*-butanol.

Mechanistic Evidence

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

Integration of Developmental Effects

Although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify selective developmental effects as a potential human health hazard of *tert*-butanol exposure. Exposure to *tert*-butanol during gestation resulted in increased fetal loss, decreased fetal body weight, and increases in skeletal variations in exposed offspring. Dams, however, had body weight losses or gains (or both), decreased food consumption, and clinical signs of intoxication at the same doses of *tert*-butanol causing fetal effects. Therefore, determining whether *tert*-butanol exposure results in specific developmental toxicity or the fetal effects are due to maternal toxicity is difficult, if not impossible, from the available data. Selective developmental toxicity of *tert*-butanol at the higher doses examined, however, cannot be ruled out. Furthermore, no adverse effects were reported in one- and two-generation reproductive/developmental studies on ETBE (Gaoua, 2004a, 2004b), further supporting the lack of evidence for developmental effects as possible human hazards following *tert*-butanol exposure.

1.2.4 Neurodevelopmental Effects

Synthesis of Effects Related to Neurodevelopment

Three studies evaluated neurodevelopmental effects (Nelson et al., 1991; Daniel and Evans, 1982)[one in female mice; one in male rats; one in female rats] following *tert*-butanol exposure via liquid diet (maltose/dextrin) or inhalation. No epidemiological studies on neurodevelopment are available. The animal studies evaluating neurodevelopmental effects of *tert*-butanol contain study design limitations. Daniel and Evans (1982) had few animals per treatment group, lacked comparison of treatment-related effects to controls for all endpoints investigated, and performed no long-term neurodevelopmental testing. Further, animals in this study had decreased dietary intake compared to ad libitum control animals. The authors addressed this issue with a pair-fed experimental design, but a slight decrease in maternal dietary intake remained. This decrease was likely due to difficulties in the pair feeding or increased maternal sedation Daniel and Evans (1982). The two studies by Nelson et al. (1991) evaluated neurodevelopmental effects after either paternal or maternal exposure but did not run the exposures concurrently. The studies are arranged in the evidence tables by species and sex.

Various neurodevelopmental effects have been observed in the available studies. Effects include changes in rotarod performance following oral or inhalation exposures, decreases in open field behavior and cliff avoidance following oral exposure, and reduced time hanging on wire after inhalation exposure during gestation (Table 1-14).

Rotarod performance

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

Neurochemical measurements

Biochemical or physiological changes in the brain of offspring exposed during gestation or early in the postnatal period were examined in one study. In this study, Nelson et al. (1991) reported statistically significant changes in neurochemical measurements in the brain in offspring of both dams exposed via inhalation during gestation and treated adult males mated with untreated dams. The strength of these results is compromised, however, because the two concentrations tested (in both experiments) were not run concurrently, and only data on statistically significant effects were reported. Therefore, comparison across doses or trend analysis for the effects is not feasible.

Physiological and psychomotor development

Daniel and Evans (1982) cross-fostered half the mouse pups born to treated mothers with untreated surrogate females to test the effects of maternal nutrition and behavioral factors on pup physiological and psychomotor development. Results indicated that pups fostered to control dams performed significantly better than those maintained with treated dams (Table 1-13)(Daniel and Evans, 1982). These data suggest that neurodevelopmental effects were not solely due to in utero exposure to *tert*-butanol (Daniel and Evans, 1982). Interpretation of these results is limited, however, as the neurodevelopmental data were presented only in figures and could not be compared with controls.

Table 1-14. Evidence pertaining to neurodevelopmental effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Daniel and Evans (1982) Swiss Webster (Cox) mouse; 15 pregnant dams/treatment (3 or 4 dams/treatment group for neurodevelopmental endpoints) Liquid diet (0, 0.5, 0.75, or 1.0%, w/v); GD 6–20; after birth, half the pups were nursed with their treated dams and the other half were fostered by untreated dams who recently gave birth 0 (isocaloric amounts of maltose/dextrin), 3,324, 4,879, or 6,677 mg/kg-d	• a dose-dependent increase in righting reflex time, with more time needed in animals maintained with maternal dams • a dose-dependent decrease in open field behavior, with less activity in pups maintained with maternal dams • a dose-dependent decrease in rotarod performance with the pups from maternal dams having lower performances • a dose-dependent decrease in the amount of time the pups were able to avoid a cliff, with animals maintained with their maternal dams having less avoidance time
Nelson et al. (1991) Sprague-Dawley rat; 15 pregnant dams/treatment (no. of litters born not reported) Inhalation analytical concentration: 0, 6,000, or 12,000 mg/m^3; dynamic whole body chamber 7 hr/d GD 1–19	Data were not presented specifically by dose nor were any tables or figures of the data provided Maternal toxicity was noted by decreased food consumption and body weight gains Results in offspring • increase in rotarod performance in high-dose group (16 versus 26 revolutions/min for controls and 12,000 mg/m^3 animals, respectively) • decreased time held on wire in the performance ascent test in the low-dose group (16 sec versus 10 sec for controls and 1,750 mg/m^3 animals, respectively) • for the high-dose group, no effects were noted for ascent on a wire mesh screen, open field activity, automated motor activity, avoidance conditioning, operant conditioning • for the low-dose group, no effects were observed on rotarod, open field activity, automated motor activity, avoidance conditioning, operant conditioning 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 • no effects were observed for other neurotransmitter levels (acetylcholine, dopamine, substance P) at both low and high doses

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

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

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

Mechanistic Evidence

No mechanistic evidence for neurodevelopmental effects was identified by the literature search. The available mechanistic information for *tert*-butanol is limited to three studies examining muscarinic acetylcholine receptor function, and what, if any, relationship these effects might have pertaining to developmental neurotoxicity effects remains unclear (Bale and Lee, 2016).

Integration of Neurodevelopmental Effects

Neurodevelopmental effects, including decreased brain weight, changes in brain biochemistry, and changes in behavioral performances, have been observed. Each study evaluating neurodevelopmental effects, however, had limitations in study design, reporting, or both. In addition, results were not always consistent between studies or across dose. Although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify neurodevelopmental effects as a potential human health hazard of *tert*-butanol exposure.

1.2.5 Reproductive Effects

Synthesis of Effects Related to Reproduction

Several studies evaluated reproductive effects [a one-generation, oral reproductive study

(Huntingdon Life Sciences, 2004) and subchronic effects in rats and mice following oral and inhalation exposure (NTP, 1997, 1995)] in animals exposed to *tert*-butanol via oral gavage, drinking water, or inhalation for ≥63 days. The studies are arranged in the evidence tables by sex, route of exposure, duration of exposure, and species. The collection of studies evaluating reproductive effects of *tert*-butanol is limited by the absence of two-generation reproductive oral or inhalation studies and by lack of human studies on reproduction. The design, conduct, and reporting of each study were reviewed, and each study was considered adequate to provide information pertinent to this assessment.

Reproductive endpoints, such as reproductive organ weights, estrous cycle length, and sperm effects were examined following either oral or inhalation exposure (Huntingdon Life Sciences, 2004; NTP, 1997, 1995) (Table 1-15;Figure 1-13; Figure 1-14). In males, the only significant effect observed was a slight decrease in sperm motility for F0 males treated with 1000 mg/kg-day *tert*-butanol (Huntingdon Life Sciences, 2004). No significant changes in sperm motility were reported following oral exposure in other rat studies or via inhalation exposure in mice or rats. In addition, the reduced motility in treated animals falls within the range of historical control data, and, therefore, its biological significance is uncertain. In female B6C3F$_1$ mice, estrous cycle length was increased 28% following oral exposure to 11,620 mg/kg-day (NTP, 1995). No significant changes in estrous cycle length were observed following oral exposure in rats or inhalation exposure in mice or rats. However, there was some evidence of increased numbers of animals with long, unclear, or absent cycles in *tert*-butanol exposed mice (oral/inhalation) and rats (oral) (Table 1-14). It is noteworthy that these effects were limited to the highest doses tested with some doses accompanied by body weight loss or lethality.

Table 1-15. Evidence pertaining to reproductive effects in animals following exposure to *tert*-butanol

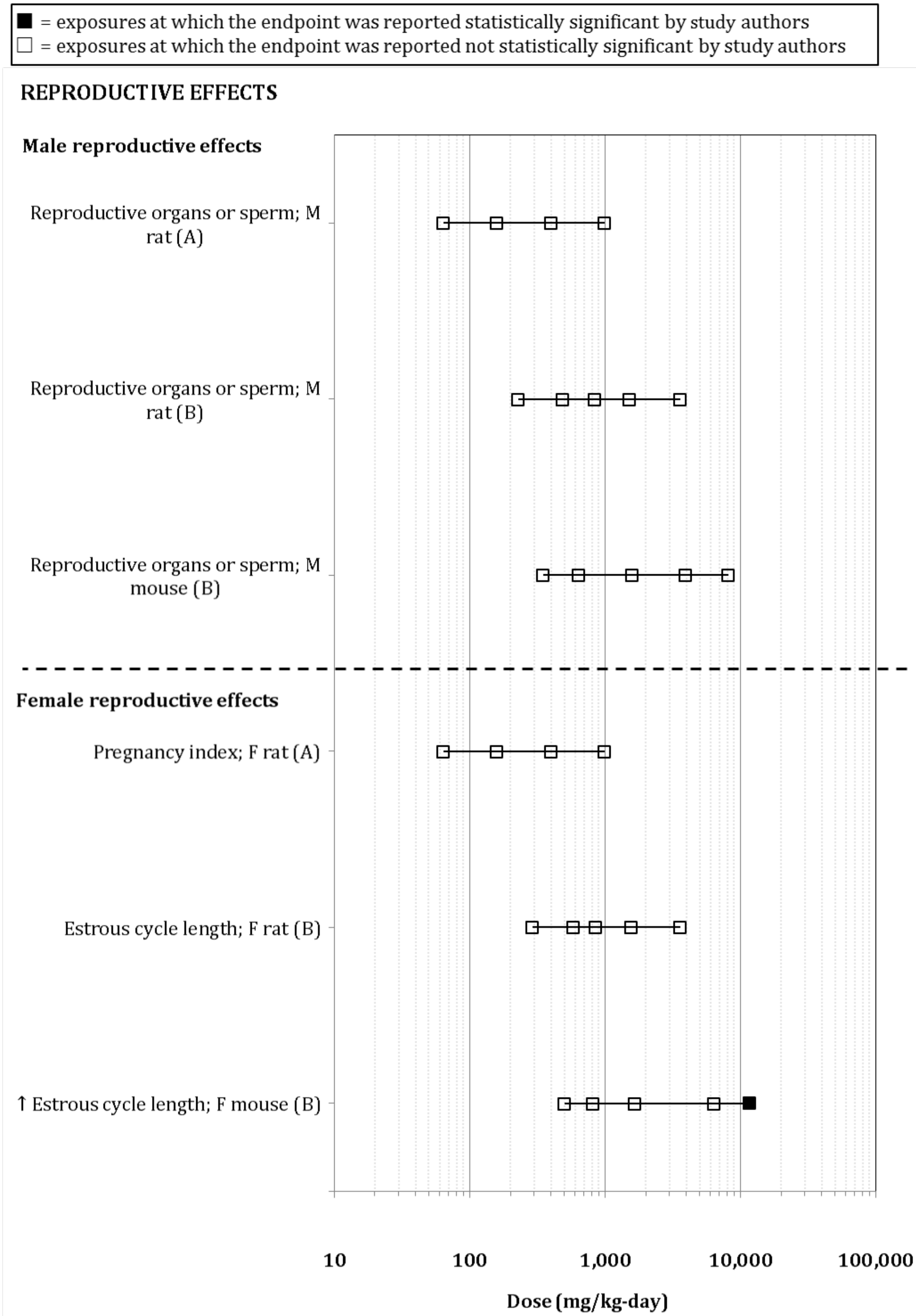
Reference and study design	Results
Male reproductive effects	
Huntingdon Life Sciences (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d F0 males: 9 weeks beginning 4 weeks prior to mating PND 21	F0 reproductive effects Sperm motility (only control and high-dose groups examined) 0: 94% 1000: 91%* No other significant effect on weights of male reproductive organs or sperm observed
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d 13 weeks	No significant effect on weights of male reproductive organs or sperm observed

Reference and study design	Results
NTP (1995) $B6C3F_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d 13 weeks	No significant effect on weights of male reproductive organs or sperm observed Note: NTP results unclear in regard to testis weight – Table F3 shows a significant decrease in testis weight at 8,210 mg/kg-day (0.115 mg to 0.096 mg) but Table H2 shows the same dose decreasing testis weight non-significantly from 0.115 mg to 0.101 mg.
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3), dynamic whole body chamber 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on weights of male reproductive organs or sperm observed Evaluations were performed only for concentrations ≥542 ppm (1,643 mg/m^3)
NTP (1997) $B6C3F_1$ mouse; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3), dynamic whole body chamber 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on weights of male reproductive organs or sperm observed Evaluations were performed only for concentrations ≥542 ppm (1,643 mg/m^3)
Female reproductive effects	
Huntingdon Life Sciences (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d F0 females: 4 weeks prior to mating through PND 21	Pregnancy index 91.7% 91.7% 100% 100% 91.7%
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	No significant effect on female estrous cycle length (0, −2, −4, 0, 8% change relative to control) Note: Number of animals that had > 7-day cycle length, unclear cycles, or no cycles 0: 0 1,560: 2/10 3,620: 4/4**
NTP (1995) $B6C3F_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	↑ length of estrous cycle *Response relative to control*: 0, 5, 5, 5, 6, 28*% Note: Animals with > 7-day cycle length, unclear cycles, or no cycles 0: 0/10 500: 0/9 820:1/10 1,660: 1/10 6,430: 1/9 11,620: 4/6***

Reference and study design	Results
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3), dynamic whole body chamber 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on female estrous cycle length (0, −4, 2, 4% change relative to control) or the number of animals cycling Evaluations were performed only for concentrations ≥542 ppm (1,643 mg/m^3)
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3), dynamic whole body chamber 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No significant effect on female estrous cycle length (0, −3, −9, −5% change relative to control) Evaluations were only performed for concentrations ≥542 ppm (1,643 mg/m^3) Note: Number of animals with > 7-day cycle length, unclear cycles, or no cycles 0:0/10 542: 2/10 1,080: 1/10 2,101: 3/10

*Statistically significant $p \leq 0.05$, as determined by the study authors.
**Statistically significant $p \leq 0.01$, as determined by EPA.
***Statistically significant $p \leq 0.05$, as determined by EPA.

Notes: 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.
Percent change compared to control = (treated value – control value) ÷ control value × 100



Sources: (A) Huntingdon Life Sciences (2004); (B) NTP (1995).

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

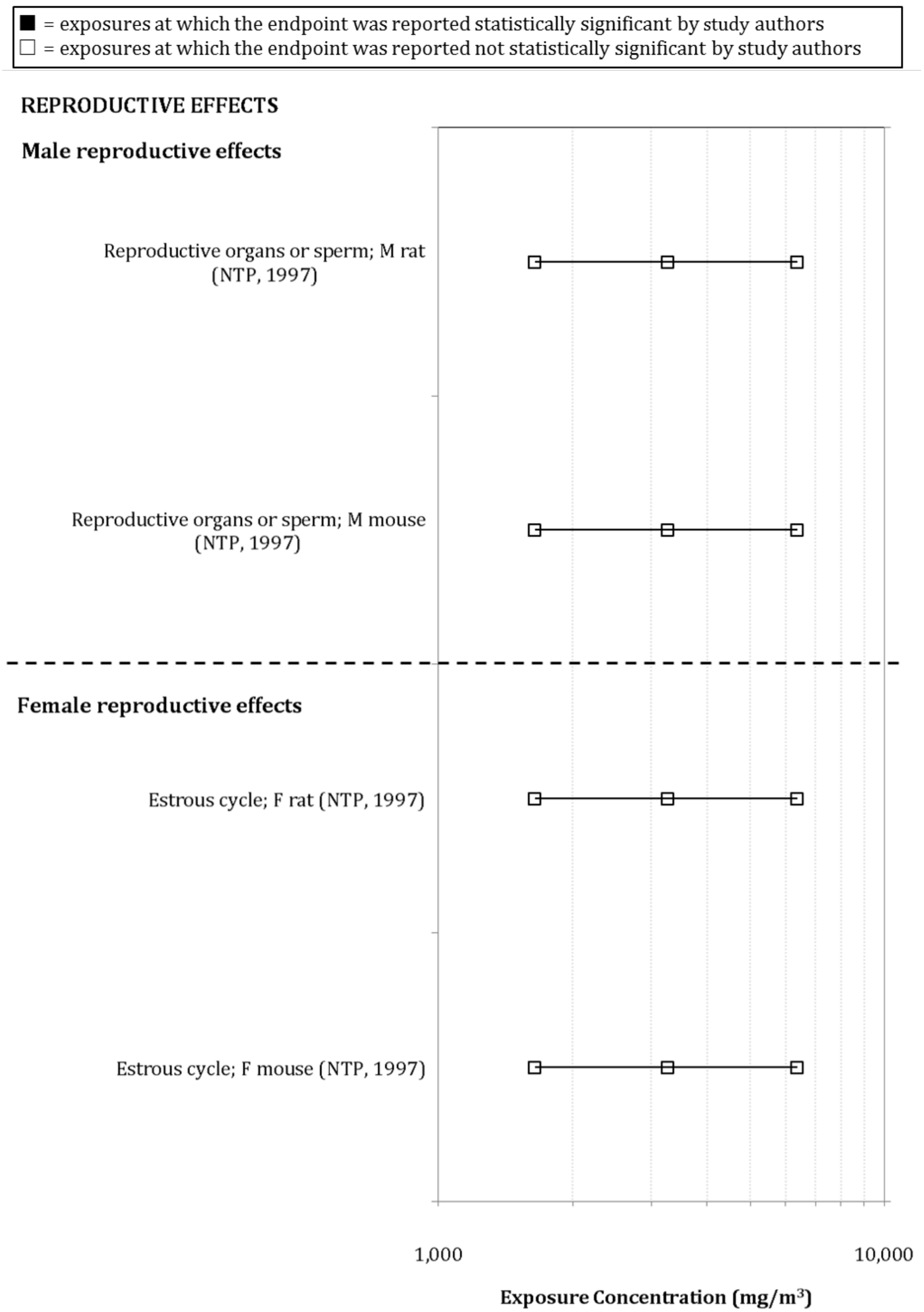


Figure 1-14. Exposure-response array of reproductive effects following inhalation exposure to *tert*-butanol.

Mechanistic Evidence

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

Integration of Reproductive Effects

Although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify reproductive effects as a potential human health hazard of *tert*-butanol exposure. The evidence base is limited to a one-generation study (Huntingdon Life Sciences, 2004; NTP, 1995). No two-generation reproductive studies are available that evaluate oral or inhalation exposure. In males, the only observed effect was a slight decrease in sperm motility for F0 males in the highest dose group of rats treated with *tert*-butanol. This effect was not observed, however, in other studies with orally treated rats and mice or in rats exposed via inhalation. In females, NTP (1995) reported an increased length of the estrous cycle in the highest dose group of orally exposed mice. This effect was not observed in similarly treated rats or in mice and rats exposed via inhalation. In addition, there was limited evidence of increased numbers of animals with long, unclear, or absent cycles in exposed rats and mice. However, these effects were limited to the highest doses tested (some with accompanying body weight loss or lethality) and were not consistent across species or route of exposure. Furthermore, no adverse effects were reported in one- and two-generation reproductive/developmental studies on ETBE (Gaoua, 2004a, 2004b), providing additional support for the lack of evidence supporting reproductive effects as possible human hazards following *tert*-butanol exposure.

1.2.6 Other Toxicological Effects

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

Additionally, central nervous system (CNS) effects similar to those caused by ethanol (animals appearing intoxicated and having withdrawal symptoms after cessation of oral or inhalation exposure) were observed. Due to study quality concerns (e.g., lack of data reporting, small number of animals per treatment group), however, adequate information to assess CNS toxicity is unavailable at this time. For more information on these other toxicological effects, see Appendix B.3.

1.3 INTEGRATION AND EVALUATION

1.3.1 Effects Other Than Cancer

Kidney effects were identified as a potential human hazard of *tert*-butanol exposure based on several endpoints in female rats, including suppurative inflammation, transitional epithelial hyperplasia, severity and incidence of nephropathy, and increased kidney weights. These effects are similar to the kidney effects observed with ETBE exposure (e.g., CPN and urothelial hyperplasia) and MTBE (e.g., CPN and mineralization) (ATSDR, 1996).

Based on mechanistic evidence indicating that an alpha2u-globulin -related process is operating in male rats (Hard et al., 2011; Cirvello et al., 1995; NTP, 1995; Lindamood et al., 1992), any kidney effects associated with alpha2u-globulin nephropathy are not considered relevant for human hazard identification. Because alpha2u-globulin nephropathy contributes to CPN, CPN and CPN-associated lesions in male rats were not considered for human hazard identification. Furthermore, mineralization in male rats was not considered clinically important to rats or relevant to human health and was not considered for dose-response analysis.

CPN played a role in the renal tubule nephropathy observed following *tert*-butanol exposure in female rats. Because female rats were not affected by alpha2u-globulin nephropathy and the individual lesions associated with the spectrum of toxicities collectively described as CPN can occur in the human kidney (NIEHS, 2019), exacerbation of one or more of these lesions might reflect a type of injury relevant to the human kidney. Effects associated with such nephropathy are considered relevant for human hazard identification and suitable for derivation of reference values. Overall, the female rat kidney effects (suppurative inflammation, transitional epithelial hyperplasia, increased severity of CPN, and increased kidney weights) are considered the result of *tert*-butanol exposure and relevant to human hazard characterization. These effects therefore are suitable for consideration for dose-response analysis and derivation of reference values, in Section 2.

Although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify developmental effects as a potential human health hazard of *tert*-butanol exposure. Increased fetal loss, decreased fetal body weight, and increases in skeletal variations in exposed offspring were observed following exposure to relatively high doses of *tert*-butanol during gestation. These effects are similar to the developmental effects observed with MTBE exposure (e.g., decreased fetal body weight and increases in skeletal variations) (ATSDR, 1996). Dams had body weight losses or gains (or both), decreased food consumption, and clinical signs of intoxication, however, at the same doses of *tert*-butanol causing fetal effects. Therefore, determining whether *tert*-butanol exposure results in specific developmental toxicity or the fetal effects are due to maternal toxicity is difficult, if not impossible, from the available data. Nevertheless, selective developmental toxicity of *tert*-butanol at the higher doses examined cannot be ruled out.

No mechanistic evidence is available for developmental effects of *tert*-butanol. There is inadequate evidence of selective developmental toxicity, due to the uncertainty regarding whether fetal effects were due to direct effects of *tert*-butanol or indirect effects of maternal toxicity and the lack of consistency across some endpoints.

Although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify neurodevelopmental effects as a potential human health hazard of *tert*-butanol exposure. While neurodevelopmental effects have been observed, the studies had limitations in design or reporting, or both, and results were inconsistent between across exposure routes and species, and the limited available mechanistic information is unclear. Therefore, neurodevelopmental effects were not considered further for dose-response analysis and derivation of reference values.

Although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify reproductive effects as a potential human health hazard of *tert*-butanol exposure. The only reproductive effect observed due to *tert*-butanol exposure was increased length of estrous cycle (NTP, 1995) in the highest dose group of orally exposed mice, and this effect was not observed in orally exposed rats or in mice and rats exposed via inhalation. Further, the evidence base was limited and contained only two oral exposure studies and one subchronic inhalation study. No mechanistic or MOA information is available for reproductive effects of *tert*-butanol. These effects were not considered further for dose-response analysis and derivation of reference values.

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

1.3.2 Carcinogenicity

Summary of Evidence

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

In F344/N rats, administration of *tert*-butanol in drinking water increased the incidence of renal tubule tumors, mostly adenomas, in males; no renal tumors in females were reported (Hard et al., 2011; NTP, 1995). As discussed in Section 1.2.1, some of these tumors might be associated with alpha2u-globulin nephropathy, an MOA considered specific to the male rat (U.S. EPA, 1991a). Evidence in support of this hypothesized MOA includes the accumulation of hyaline droplets in renal tubule cells, the presence of alpha2u-globulin in the hyaline droplets, and additional aspects associated with alpha2u-globulin nephropathy, including linear papillary mineralization and foci of tubular hyperplasia. Other evidence, however, is not supportive: The accumulation of hyaline droplets was minimal; concentrations of alpha2u-globulin were low at doses that induced tumors; and no significant necrosis or cytotoxicity was associated with compensatory regenerative proliferation or induction of granular casts observed within a timeframe consistent with alpha2u-globulin -mediated nephropathy. Renal tumors also are associated with chronic progressive nephropathy, but the data on CPN are not coherent: Dose-response relationships for CPN, renal tubule hyperplasia, and renal tubule tumors differed; in addition, CPN was nearly as severe in female rats as in male rats, yet no female rats developed renal tumors. Thus, some renal tumors might be attributable to alpha2u-globulin nephropathy augmented by CPN, and some to other, yet unspecified, processes. Taken together, and according to EPA's guidance on renal tumors in male rats (U.S. EPA, 1991a), renal tumors induced by *tert*-butanol are relevant for human hazard identification.

In addition, as mentioned in Section 1.1.4, *tert*-butanol is a primary metabolite of MTBE and of ETBE, two compounds tested in rats and mice that could provide supplementary information on the carcinogenicity of *tert*-butanol. For MTBE, the most recent cancer evaluation by a national or international health agency is from IARC (1999). IARC reported that oral gavage exposure in Sprague-Dawley rats resulted in testicular tumors in males and lymphomas and leukemias (combined) in females; inhalation exposure in male and female F344 rats resulted in renal tubule adenomas in males; and inhalation exposure in male and female CD-1 mice resulted in hepatocellular adenomas in females (IARC, 1999). For ETBE, the IRIS assessment developed concurrently with this assessment reports that inhalation exposure in male and female F344 rats resulted in hepatocellular tumors, primarily adenomas, in males; no significant tumor increases, including kidney tumors, were reported for 2-year studies by drinking water exposure in male and female F344 rats or by oral gavage in male and female Sprague-Dawley rats (U.S. EPA, 2017).

Integration of evidence

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

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

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

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

Biological considerations for dose–response analysis

Regarding hazards to bring forward to Section 2 for dose-response analysis, EPA's guidance on thyroid tumors (U.S. EPA, 1998b) advise that, for thyroid tumors resulting from thyroid-pituitary disruption, dose-response analysis should use nonlinear extrapolation, in the absence of MOA information to indicate otherwise. As discussed in Section 1.2.2, increases in thyroid follicular cell hyperplasia in male and female mice provide partial support for thyroid-pituitary disruption. Other necessary data on *tert*-butanol, however, are not adequate or are not supportive. There is little correlation among thyroid, pituitary, and liver effects in female mice, and no data are available to evaluate the potential for antithyroid effects in male mice. Data are not adequate to conclude that thyroid hormone changes exceed the range of homeostatic regulation or to evaluate effects on extrahepatic sites involved in thyroid-pituitary disruption. Also, no data are available to evaluate reversibility of effects upon cessation of exposure. Thus, according to EPA's thyroid tumor guidance, concluding that the thyroid tumors result from thyroid-pituitary disruption is premature, and dose-response analysis should use linear extrapolation.

As discussed in Section 1.2.2, the available data do not demonstrate that the thyroid tumors are the result of excessive toxicity in female mice rather than the carcinogenicity of *tert*-butanol. The final average body weight reduction in female mice was 12% (NTP, 1995), but water consumption by exposed females was similar to controls and no overt toxicity was observed. Furthermore, female mice in the high dose group had higher rates of survival than control animals. EPA's Cancer Guidelines (U.S. EPA, 2005a) also states that when there is suggestive evidence of carcinogenicity and when the evidence includes a well-conducted study, "quantitative analysis may be useful for some purposes, for example, providing a sense of magnitude and uncertainty of potential risk, ranking potential hazards, or setting research priorities." Given that the data are well suited to dose-response analysis, coming from an NTP study that tested multiple dose levels, and because quantitative analysis may be useful in providing a sense of the magnitude and uncertainty of potential risks from *tert*-butanol exposure, including worker or consumer exposures, an analysis of thyroid tumors is presented in Section 2.

EPA's guidance on renal tumors in male rats (U.S. EPA, 1991a) advises that, unless the relative contribution of alpha2u-globulin nephropathy and other process can be determined, dose-response analysis should not be performed. As discussed in Section 1.2.1, the available data do not allow such determination, and so an analysis of kidney tumors does not appear in Section 2.

1.3.3 Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

No chemical-specific data that would allow for the identification of populations with increased susceptibility to *tert*-butanol exposure are available. In vitro studies have implicated the liver microsomal mixed function oxidase (MFO) system, namely CYP450 (Cederbaum et al., 1983; Cederbaum and Cohen, 1980), as playing a role in the metabolism of *tert*-butanol. One study evaluated liver enzyme expression and found a dose-responsive induction of CYP2B10 following 14 days of *tert*-butanol exposure in female mice, with much smaller increases in the expression of CYP2B9, and the thyroid hormone-metabolizing enzyme, sulfotransferase 1A1 [(SULT1A1; Blanck et al. (2010)]. No studies, however, have identified the specific CYPs responsible for the biotransformation of *tert*-butanol. Pharmacokinetic differences among the fetus, newborns, children, and the aged might alter responses to chemicals compared to adults, resulting in differences in health effects. In the presence of environmental chemicals, metabolic homeostasis is maintained by the liver's ability to detoxify and eliminate xenobiotics. This process is accomplished, in part, by the expression of xenobiotic metabolizing enzymes and transporters (XMETs), which metabolize and transport xenobiotics and determine whether exposure will result in altered responses. XMETs, including various CYPs, have been found to be underexpressed in the mouse fetus and neonate (Lee et al., 2011) and decreased in older mice (Lee et al., 2011) and rats (Lee et al., 2008). Decreased ability to detoxify and transport *tert*-butanol out of the body could result in increased susceptibility to *tert*-butanol in the young and old.

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

Collectively, evidence on *tert*-butanol is minimal for identifying susceptible populations or lifestages.

2 DOSE-RESPONSE ANALYSIS

2.1 ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

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

2.1.1 Identification of Studies and Effects for Dose-Response Analysis

EPA identified kidney effects as a potential human hazard of *tert*-butanol exposure (see Section 1.2.1). Studies within this effect category were evaluated using general study quality characteristics [as discussed in Section 4 of the Preamble; see also U.S. EPA (2002a)] to help inform the selection of studies from which to derive toxicity values. No other hazards were identified for further consideration in the derivation of reference values.

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

Animal studies were evaluated to determine which studies provided the most relevant routes and durations of exposure, and multiple exposure levels covering a broad range to provide information about the shape of the dose-response curve. The evidence base for *tert*-butanol includes both chronic and subchronic studies showing effects in the kidney that are suitable for deriving reference values.

Kidney Toxicity

EPA identified kidney effects as a potential human hazard of *tert*-butanol-induced toxicity based on findings in female rats (summarized in Section 1.3.1). Kidney toxicity was observed across multiple chronic, subchronic, and short-term studies following oral and inhalation exposure. Kidney effects observed after chronic exposure, such as suppurative inflammation and transitional epithelial hyperplasia, could influence the ability of the kidney to filter waste. Exacerbated nephropathy also would affect kidney function. Observed changes in kidney weight also could indicate toxic effects in the kidney. For the oral *tert*-butanol evidence base, several studies that evaluated these kidney effects are available. Huntingdon Life Sciences (2004) conducted a reproductive study in Sprague-Dawley rats that was of shorter duration, and reported changes in kidney weight but did not examine changes in histopathology. NTP conducted a 2-year drinking water study (NTP, 1995) in F344 rats that evaluated multiple doses in both males and females, and reported on all three endpoints highlighted above. NTP (1995) was identified as most suitable for dose-response assessment considering the study duration, comprehensive reporting of outcomes, and multiple doses tested.

In the NTP (1995) 2-year drinking water study, female F344 rats were exposed to approximate doses of 0, 180, 330, or 650 mg/kg-day. Reduced body weights and survival were observed and reflected in some of the effects. Kidney effects, including changes in organ weight, histopathology, or both, were observed in both sexes of rats after 13 weeks, 15 months, and 2 years of treatment (NTP, 1995). Because the kidney effects in male rats are complicated by alpha2u-globulin , male kidney effects are not considered. Specific endpoints in female rats chosen for dose-response analysis were absolute kidney weight, kidney suppurative inflammation, kidney transitional epithelial hyperplasia, and increases in severity of nephropathy. For absolute kidney weight, data from 15-month duration were selected as described in Section 1.2.1; for the other endpoints, data at the longest duration of 2 years were selected.

2.1.2 Methods of Analysis

No biologically based dose-response models are available for *tert*-butanol. In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. The models in EPA's Benchmark Dose Software (BMDS) were applied. Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the BMD and the BMDL are estimated using a benchmark response (BMR) to represent a minimal, biologically significant level of change. In the absence of information regarding the level of change considered biologically significant, a BMR of 1 standard deviation from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data is used to estimate the BMD and BMDL and to facilitate a consistent basis of comparison across endpoints, studies, and assessments. Endpoint-specific BMRs, where feasible, are described further below. When modeling was feasible, the estimated BMDLs were used as points of departure (PODs); the PODs are summarized in Table 2-1. Details including the modeling output and graphical results for the model selected for each endpoint are presented in Appendix C of the Supplemental Information to this Toxicological Review. When modeling was not feasible, the study NOAEL or LOAEL was used as the POD.

Kidney weights were analyzed as absolute weights rather than weights relative to body weight. In general, both absolute and relative kidney weight data are considered appropriate endpoints for analysis (Bailey et al., 2004). In the NTP (1995) 2-year drinking water study, body weight in exposed animals noticeably decreased relative to controls at the 15-month interim sacrifice, but this decrease in body weight disproportionately influenced the measure of relative kidney weight, resulting in exaggerated kidney weight changes. Because there was greater confidence in the absolute kidney weight measure, it was considered more appropriate for dose-response analysis, and changes in relative kidney weights were not analyzed. A 10% relative change from control was used as a BMR for absolute kidney weight, analogous to a 10% change in body weight as an indicator of toxicity. A BMR of 10% extra risk was considered appropriate for the quantal data on incidences of kidney suppurative inflammation and kidney transitional epithelial hyperplasia. Dose-response modeling was not conducted on the increases in severity of nephropathy because the data was not amenable to modeling.

Human equivalent doses (HEDs) for oral exposures were derived from the PODs according to the hierarchy of approaches outlined in EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011a). The preferred approach is physiologically based pharmacokinetic (PBPK) modeling. Other approaches include using chemical-specific information in the absence of a complete PBPK model. As discussed in Appendix B of the Supplemental Information, human PBPK models for inhalation of ETBE or inhalation and dermal exposure to MTBE have been published, which include *tert*-butanol submodels. A validated human PBPK model for *tert*-butanol, however, is not available for extrapolating doses from animals to humans. In lieu of either chemical-specific models or data to inform the derivation of human equivalent oral exposures, body weight scaling to the ¾ power ($BW^{3/4}$) is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans for the purpose of deriving an oral RfD.

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

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

where

BW_a = animal body weight

BW_h = human body weight

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

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

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

Table 2-1. Summary of derivations of points of departure following oral exposure for up to 2 years

Endpoint and reference	Species/ sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{ADJ}[b] (mg/kg-d)	POD_{HED}[c] (mg/kg-d)
Kidney							
Increased absolute kidney weight at 15 months NTP (1995)	Rat/F	Exponential (M4) (constant variance)	10%	164	91	91	22
Kidney inflammation (suppurative) NTP (1995)	Rat/F	Log-probit	10%	254	200	200	48
Kidney transitional epithelial hyperplasia NTP (1995)	Rat/F	Multistage, 3-degree	10%	412	339	339	81.4
Increases in severity of nephropathy NTP (1995)	Rat/F	NA	NA	NA	NA	180[d]	43.2

[a]For modeling details, see Appendix C in Supplemental Information.
[b]For studies in which animals were not dosed daily, EPA would adjust administered doses to calculate the time-weighted average daily doses prior to BMD modeling. This adjustment was not required for the NTP (1995) study.

[c]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011a).
[d]POD calculated from the LOAEL (lowest dose tested had a significant increase in severity).

NA= not applicable

2.1.3 Derivation of Candidate Values

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

An intraspecies uncertainty factor, UF_H, of 10 was applied to all PODs to account for potential differences in toxicokinetics and toxicodynamics in the absence of information on the variability of response in the human population following oral exposure to *tert*-butanol (U.S. EPA, 2002a).

An interspecies uncertainty factor, UF_A, of 3 ($10^{0.5}$ = 3.16, rounded to 3) was applied to all PODs because $BW^{3/4}$ scaling was used to extrapolate oral doses from laboratory animals to humans. Although $BW^{3/4}$ scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes, some residual uncertainty in the extrapolation remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's $BW^{3/4}$ guidance (U.S. EPA, 2011a) recommends use of an uncertainty factor of 3.

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

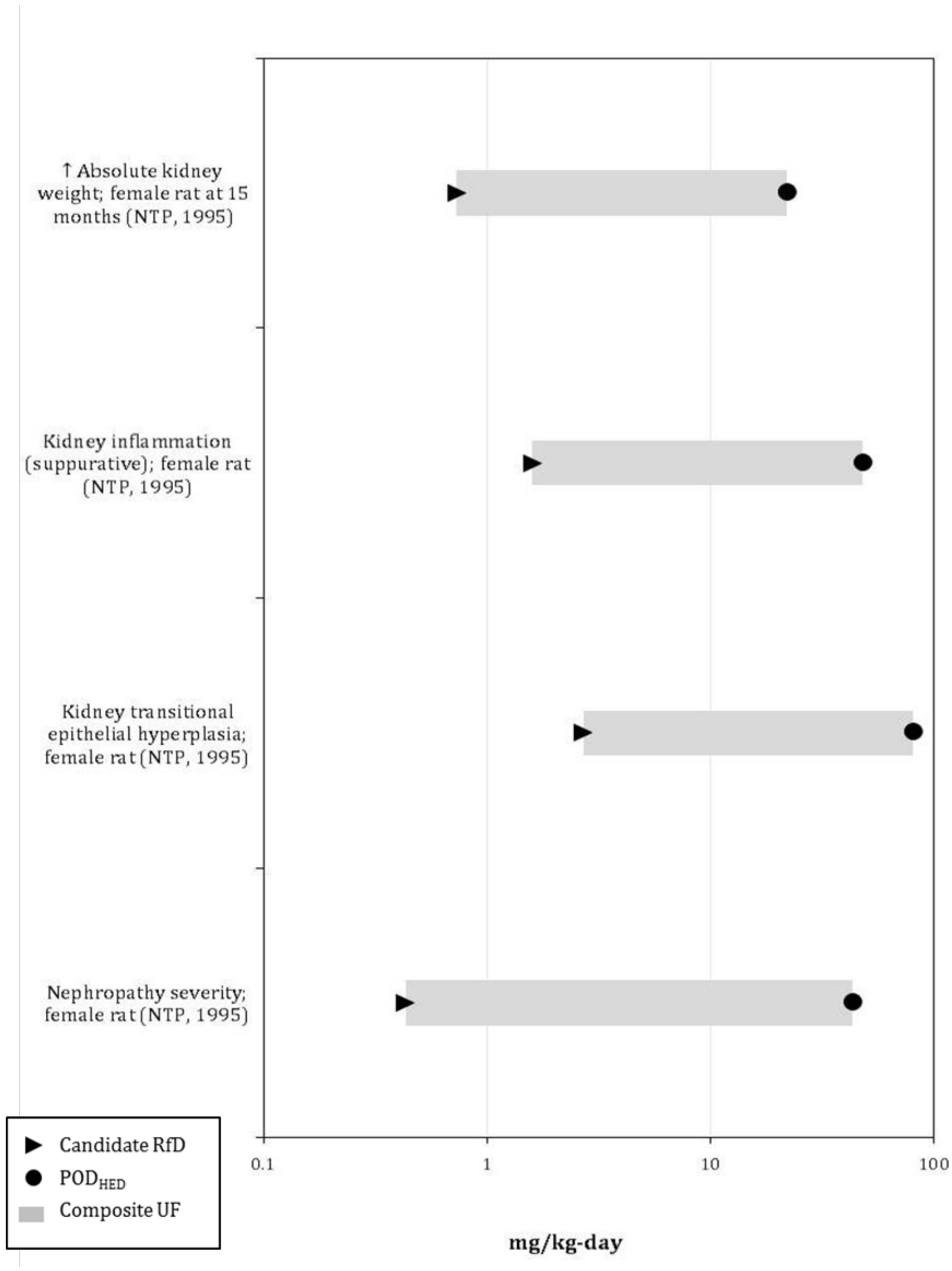
A LOAEL-to-NOAEL uncertainty factor, UF_L, of 1 was applied to most PODs derived because the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, BMRs of a 10% relative change in absolute kidney weight, a 10% extra risk of kidney suppurative inflammation, and a 10% extra risk of transitional cell hyperplasia were selected, assuming they represent minimal biologically significant response levels. A LOAEL-to-NOAEL uncertainty factor of 3 was applied to the increases in severity of nephropathy. Although a LOAEL was used to derive the POD, the severity of 1.9 was only slightly higher than the control value of 1.6, indicating that the LOAEL was close to the result in controls.

A database uncertainty factor, UF, of 1 was applied to all PODs. The *tert*-butanol oral toxicity evidence base includes chronic and subchronic toxicity studies in rats and mice (Acharya et al., 1997; Acharya et al., 1995; NTP, 1995) and developmental toxicity studies in rats and mice (Huntingdon Life Sciences, 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 any selective developmental toxicity is not as sensitive an endpoint as kidney effects. No immunotoxicity or multigenerational reproductive studies are available for *tert*-butanol. Studies on ETBE, which is rapidly metabolized to systemically available *tert*-butanol, are informative for consideration of the gaps in the *tert*-butanol oral evidence base. The evidence base for ETBE does not indicate immunotoxicity (Banton et al., 2011; Li et al., 2011), suggesting immune system effects would not be a sensitive target for *tert*-butanol. No adverse effects were reported in one- and two-generation reproductive/developmental studies on ETBE (Gaoua, 2004a, 2004b), indicating that reproductive/developmental effects would not be a sensitive target for *tert*-butanol. Additionally, a one-generation, reproductive toxicity study in rats from a Toxic Substances Control Act submission (Huntingdon Life Sciences, 2004) is available for *tert*-butanol. This study did not observe reproductive effects. Although the oral toxicity evidence base for *tert*-butanol has some gaps, the available data on *tert*-butanol, informed by the data on ETBE, do not suggest that additional studies would lead to identification of a more sensitive endpoint or a lower POD. Therefore, a database UF_D of 1 was applied.

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

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

Endpoint and reference	POD_{HED} (mg/kg-d)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Kidney									
Increased absolute kidney weight; female rat at 15 months NTP (1995)	22	$BMDL_{10\%}$	3	10	1	1	1	30	7×10^{-1}
Kidney inflammation (suppurative); female rat at 2 years NTP (1995)	48	$BMDL_{10\%}$	3	10	1	1	1	30	2×10^{0}
Kidney transitional epithelial hyperplasia; female rat at 2 years NTP (1995)	81	$BMDL_{10\%}$	3	10	1	1	1	30	3×10^{0}
Increases in severity of nephropathy; female rat at 2 years NTP (1995)	43.2	LOAEL	3	10	3	1	1	100	4×10^{-1}



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

2.1.4 Derivation of Organ/System-Specific Reference Doses

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

Kidney Toxicity

For *tert*-butanol, candidate values were for several different kidney effects in female rats, spanning a range from 4×10^{-1} to 3×10^{0} mg/kg-day, for an overall 7.5-fold range. To estimate an exposure level below which kidney toxicity from *tert*-butanol exposure is not expected to occur, the RfD for greater increases in severity of nephropathy in female rats (**4×10^{-1} mg/kg-day**) was selected as the kidney-specific reference dose for *tert*-butanol. This indicator of kidney toxicity is more specific than the relatively nonspecific endpoint of absolute kidney weight changes, and more sensitive than the endpoints of inflammation and transitional epithelial hyperplasia. Confidence in this kidney-specific RfD is medium in part due to the scientific uncertainty surrounding human relevance of CPN which remains unresolved. . The POD for increases in severity of nephropathy is based on a LOAEL, and the candidate values are derived from a well-conducted long-term study, involving a sufficient number of animals per group, including both sexes, and assessing a wide range of kidney endpoints.

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

Effect	Basis	RfD (mg/kg-day)	Study exposure description	Confidence
Kidney	Increases in severity of nephropathy (NTP, 1995)	4×10^{-1}	Chronic	Medium
Overall RfD	**Kidney**	**4×10^{-1}**	**Chronic**	**Medium**

2.1.5 Selection of the Overall Reference Dose

For *tert*-butanol, only kidney effects were identified as a hazard and carried forward for dose-response analysis; thus only one organ-/system-specific reference dose was derived. Therefore, the kidney specific RfD of **4×10^{-1} mg/kg-day** is the overall RfD for *tert*-butanol. This value is based on greater increases in severity of nephropathy in female rats exposed to *tert*-butanol.

The overall reference dose is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole, including potentially susceptible subgroups (U.S. EPA, 2002a). Decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD. In the case of *tert*-butanol, potential exists for early lifestage susceptibility to *tert*-butanol exposure, as discussed in Section 1.3.3.

2.1.6 Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall evidence base, and the RfD, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994a). Confidence in the principal study (NTP, 1995) is high. This study was well conducted, complied with Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations, involved 50 animals per dose group (including both sexes), and assessed a wide range of tissues and endpoints. The toxicity evidence base for *tert*-butanol has some gaps such as a lack of human studies and limited reproductive/development toxicity data, despite the inclusion of data on ETBE, a parent compound of *tert*-butanol. Therefore, the confidence in the evidence base is medium. Reflecting high confidence in the principal study and medium confidence in the evidence base, confidence in the RfD is medium.

2.1.7 Previous IRIS Assessment

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

2.2 INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

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

2.2.1 Identification of Studies and Effects for Dose-Response Analysis

As for oral exposure, EPA identified kidney effects as a potential human hazard of *tert*-butanol inhalation exposure (summarized in Section 1.3.1). No chronic inhalation study for *tert*-butanol is available; only one 13-week study in rats and mice is available (NTP, 1997). A rat PBPK model was available for both oral and inhalation exposure, which was suitable for a route-to-route extrapolation (Borghoff et al., 2016). As a result, rat studies from both routes of exposure were considered for dose-response analysis.

The evidence base for *tert*-butanol includes oral and inhalation studies and data sets that are potentially suitable for use in deriving inhalation reference values. Specifically, effects associated with *tert*-butanol exposure in animals include observations of organ weight and histological changes in the kidney in chronic and subchronic studies in female rats.

Kidney Toxicity

EPA identified kidney effects as a potential human hazard of *tert*-butanol exposure based on findings of organ weight changes and histopathology primarily in male rats; however, the kidney effects in male rats are complicated by the presence of alpha2u-globulin . Therefore, kidney effects in male rats are not considered. The kidney findings were observed across multiple chronic, subchronic, and short-term studies following oral and inhalation exposure. The subchronic NTP (1997) inhalation study is the only route-specific study available, and was carried forward for further analysis. For oral studies considered for route-to-route extrapolation, see Section 2.1.1 for a summary of considerations for selecting oral studies for dose-response analysis. Overall, the NTP 2-year drinking water study NTP (1995) was identified as the study most suitable for dose-response assessment, given the study duration, comprehensive reporting of outcomes, use of multiple species tested, multiple doses tested, and availability of a PBPK model for route-to-route extrapolation. This study was discussed previously in Section 2.1.1 as part of the derivation of the oral reference dose, so is not reviewed here again. The NTP (1997) subchronic inhalation study shares many strengths with the 2-year drinking water study (NTP, 1995) and is described in more detail below.

NTP (1997) was a well-designed subchronic study that evaluated the effect of *tert*-butanol exposure on multiple species at multiple inhalation doses. Relative kidney weights were elevated in females at 6,368 mg/m^3. Few endpoints were available for consideration in the subchronic inhalation study, but changes in kidney weights also were observed in the oral studies, such as the NTP (1995) 2-year drinking water study.

2.2.2 Methods of Analysis

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

PODs from Inhalation Studies

Because the RfC is applicable to a continuous lifetime human exposure but derived from animal studies featuring intermittent exposure, EPA guidance (U.S. EPA, 1994a) 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 (BMCL) 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:

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

The RfC methodology provides a mechanism for deriving an HEC from the duration-adjusted POD ($BMCL_{ADJ}$) determined from the animal data. The approach takes into account the extra-respiratory nature of the toxicological responses and accommodates species differences by considering blood:air partition coefficients for *tert*-butanol in the laboratory animal (rat or mouse) and humans. According to the RfC guidelines (U.S. EPA, 1994a), *tert*-butanol is a Category 3 gas because extrarespiratory effects were observed. Kaneko et al. (2000) measured a blood:gas partition coefficient [$(H_{b/g})_A$] of 531 ± 102 for *tert*-butanol in the male Wistar rat, while Borghoff et al. (1996) measured a value of 481 ± 29 in male F344 rats. A blood:gas partition coefficient [$(H_{b/g})_H$] of 462 was reported for *tert*-butanol in humans (Nihlén et al., 1995). The calculation, $(H_{b/g})_A \div (H_{b/g})_H$, was used to calculate a blood:gas partition coefficient ratio to apply to the delivered concentration. Because F344 rats were used in the study, the blood:gas partition coefficient for F344 rats was used. Thus, the calculation was 481 ÷ 462 = 1.04. Therefore, a ratio of 1.04 was used to calculate the HEC. This allowed a $BMCL_{HEC}$ to be derived as follows:

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

Table 2-4 summarizes the sequence of calculations leading to the derivation of a human-equivalent POD for each inhalation data set discussed above.

Table 2-4. Summary of derivation of PODs following inhalation exposure

Endpoint and reference	Species/ Sex	Model[a]	BMR	BMC[b] (mg/m³)	BMCL[b] (mg/m³)	POD_{ADJ}[b] (mg/m³)	POD_{HEC}[c] (mg/m³)
Kidney							
Increased absolute kidney weight at 13 weeks NTP (1997)	Female F344 rats	No model selected[d]	10%	--	--	1137	1137

[a]For modeling details, see Appendix C in Supplemental Information.
[b]BMCs, BMCLs, and PODs were adjusted for continuous daily exposure by multiplying by (hours exposed per day / 24 hr) × (days exposed per week / 7 days).
[c]POD_{HEC} calculated by adjusting the POD_{ADJ} by the DAF (= 1.0, rounded from 1.04) for a Category 3 gas (U.S. EPA, 1994a).
[d]BMD modeling failed to calculate a BMD value successfully (see Appendix C); POD calculated from NOAEL of 6368 mg/m³.

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

A PBPK model for *tert*-butanol in rats has been modified, as described in Appendix B of the Supplemental Information. A critical decision in the route-to-route extrapolation is selection of the internal dose metric that establishes “equivalent” oral and inhalation exposures. For *tert*-butanol-induced kidney effects, the two options are the concentration of *tert*-butanol in blood and the rate of *tert*-butanol metabolism. Note that using the kidney concentration of *tert*-butanol will lead to the same route-to-route extrapolation relationship as *tert*-butanol in blood because the distribution from blood to kidney is independent of route. Data are not available that suggest that metabolites of *tert*-butanol mediate its renal toxicity. Without evidence that suggests otherwise, *tert*-butanol is assumed the active toxicological agent. Moreover, since extrapolation is within the same species, use of the rate of metabolism as the metric (the alternate possibility) will only result in a different value to the extent that there is nonlinearity (saturation) in the metabolism vs. concentration, and this differs for oral vs. inhalation exposure. For example, for the internal dose of 61.9 mg/L average blood concentration (Cavg, associated with a BMDL of 200 mg/kg/d oral exposure), the corresponding average rate of metabolism is 0.83 mg/h (Mavg). If using Cavg as the metric, the corresponding continuous inhalation concentration for the rat is 523.7 mg/m³. If using Mavg as the metric, the corresponding continuous rat inhalation concentration is 439.9 mg/m, only 16% lower. Hence from a practical standpoint the choice between these two possible metrics has little impact. On the other hand, the use of metabolic rate has a higher degree of qualitative uncertainty in that there isn’t a sub-model for the key metabolite(s) which can be used to estimate its (their) internal concentration under different scenarios. The rate of metabolism is only inferred by observing the rate of *tert*-butanol clearance, *tert*-butanol blood concentrations after various exposures have been measured directly. Therefore, the concentration of *tert*-butanol in blood was selected as the dose metric.

Using the PBPK model, route-to-route extrapolation of the oral BMDLs or LOAEL to derive inhalation PODs was therefore performed as follows. First, the internal dose in the rat at each oral BMDL or LOAEL (assuming oral exposure by a circadian drinking water pattern) was estimated using the PBPK model, to derive an "internal dose BMDL or LOAEL." More specifically, for non-continuous exposures the PBPK model was run for a number of days or weeks such that the predicted time course of *tert*-butanol in blood did not change with further days or weeks simulated (e.g., until blood concentration during the 2nd-to-last day of exposure was predicted to be the same as the last day of exposure). This state is referred to as "periodicity." The average blood concentration of *tert*-butanol was calculated during the final periodic exposure for oral exposure at the BMDL for a given endpoint. For uniformity, all model scripts calculated the average from episodic exposures on the basis of the final week of exposure, regardless of whether exposure is daily or 5 times per week, since either exposure profile will be fully captured by averaging a 1-week time period.

For continuous inhalation exposures (24 hours/day, 7 days/week), the steady-state blood concentration at the end of a simulation is equal to the average blood concentration for the last week. Therefore, the continuous inhalation exposure equivalent to an oral BMDL was identified by using the PBPK model to identify the inhalation concentration for which the final (steady-state) blood concentration was equal to the average blood concentration for the last week of oral exposure at the oral BMDL. The resulting POD then was converted to a human equivalent concentration POD (POD_{HEC}) using the methodology previously described in the section, *PODs from inhalation studies*:

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

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

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

Endpoint and reference	Species/sex	BMR	BMDL (mg/kg-d)	Internal dose[a] (mg/L)	Equivalent POD[b] (mg/m^3)	Equivalent POD_{HEC}[c] (mg/m^3)
Kidney						

Endpoint and reference	Species/sex	BMR	BMDL (mg/kg-d)	Internal dose[a] (mg/L)	Equivalent POD[b] (mg/m^3)	Equivalent POD_{HEC}[c] (mg/m^3)
Mean absolute kidney weight at 15 months NTP (1995)	Rat/F	10%	91	21.5	238.9	248
Kidney inflammation (suppurative) at 2 years NTP (1995)	Rat/F	10%	200	61.9	523.7	545
Kidney transitional epithelial hyperplasia at 2 years NTP (1995)	Rat/F	10%	339	127	883.9	919
	Species/sex	POD (LOAEL; mg/kg-d)		Internal dose[a] (mg/L)	Equivalent POD[b] (mg/m^3)	Equivalent POD_{HEC}[c] (mg/m^3)
Increases in severity of nephropathy at 2 years NTP (1995)	Rat/F	180		53.6	471.8	491

[a]Average rodent blood concentration of *tert*-butanol under circadian drinking water ingestion at the BMDL.
[b]Continuous inhalation equivalent concentration that leads to the same average blood concentration of *tert*-butanol as circadian drinking water ingestion at the BMDL in the rat.
[c]Continuous inhalation human equivalent concentration that leads to the same average blood concentration of *tert*-butanol as continuous oral exposure at the BMDL. Calculated as the rodent POD x 1.04.

To our knowledge, a meta-analysis of the accuracy of route-to-route (RTR) extrapolation using PBPK models has not been conducted. Ideally one would evaluate results for multiple chemicals for which a PBPK model and both oral and inhalation toxicity studies have been conducted, to determine the accuracy of RTR extrapolation by comparing a predicted point of departure (e.g., BMD) with actual data for the alternate route. For chloroform use of a PBPK model has been shown to be successful at correlating response with internal dose irrespective of exposure route, including combined inhalation and oral exposures (https://heronet.epa.gov/heronet/index.cfm/reference/details/reference_id/1936108). On the other hand, prenatal exposure of rats to inhaled ethanol did not result in the degree of teratological effects expected even though the internal dose achieved (blood ethanol concentration, BEC) was in the range associated with those effects when ethanol is orally ingested (https://heronet.epa.gov/heronet/index.cfm/reference/details/reference_id/2634309). In the latter case, although a PBPK model could successfully predict the inhalation concentration of ethanol that yields a similar BEC to oral exposure, the RTR extrapolation effectively failed because the same level of effect did not occur. Thus, even for a well-studied compound like ethanol, the internal dose metric presumed to be correct may in fact be inadequate for accurate extrapolation. Together these results indicate both promise and uncertainty in RTR extrapolation.

Despite the uncertainty, a chemical must enter the body and be distributed through the blood to have an effect on internal tissues. Therefore, the toxicological activity must be related to blood concentration, although it may not be exactly predicted by a particular metric. In the case of *tert*-butanol, there is toxicological uncertainty due to the fact that a chronic inhalation bioassay has not been conducted. Thus, one must make a judgment as to whether it is more uncertain to extrapolate from a sub-chronic inhalation bioassay or a chronic oral bioassay. Because a quantitative analysis of RTR extrapolation across chemicals has not been conducted, it is not possible to quantitatively compare the uncertainty of these two options. The U.S. EPA has assumed in this assessment that extrapolation across study duration is more uncertain than extrapolation across exposure routes, given that toxicity must be related to the concentration of *tert*-butanol in the blood.

2.2.3 Derivation of Candidate Values

In EPA's *A Review of the Reference Dose and Reference Concentration Processes* [(U.S. EPA, 2002a); Section 4.4.5], also described in the Preamble, five possible areas of uncertainty and variability were considered. Several PODs for the candidate inhalation values were derived using a route-to-route extrapolation from the PODs estimated from the chronic oral toxicity study in rats (NTP, 1995) in the derivation of the oral RfD (Section 2.1). With the exception of the subchronic inhalation (NTP, 1997) study, the UF values selected and applied to PODs derived from the chronic oral (NTP, 1995) study for route-to-route extrapolation are the same as those for the RfD for *tert*-butanol (see Section 2.1.3). The model used to perform this route-to-route extrapolation is a well-characterized model considered appropriate for the purposes of this assessment.

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

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

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

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

Endpoint (sex and species) and reference	POD_{HEC}[a] (mg/m^3)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/m^3)
Kidney									
Increased absolute kidney weight at 13 weeks; female rat	1137	NOAEL	3	10	1	10	1	300	4×10^0

This document is a draft for review purposes only and does not constitute Agency policy.

Endpoint (sex and species) and reference	POD_{HEC}[a] (mg/m³)	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/m³)
NTP (1997)									
Increased absolute kidney weight at 15 months; female rat NTP (1995)	248	$BMCL_{10\%}$	3	10	1	1	1	30	8×10^0 *
Kidney inflammation (suppurative); female rat at 2 years NTP (1995)	546	$BMCL_{10\%}$	3	10	1	1	1	30	2×10^1 *
Kidney transitional epithelial hyperplasia; female rat at 2 years NTP (1995)	920	$BMCL_{10\%}$	3	10	1	1	1	30	3×10^1 *
Increases in severity of nephropathy; female rat at 2 years NTP (1995)	491	LOAEL	3	10	3	1	1	100	5×10^0 *

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

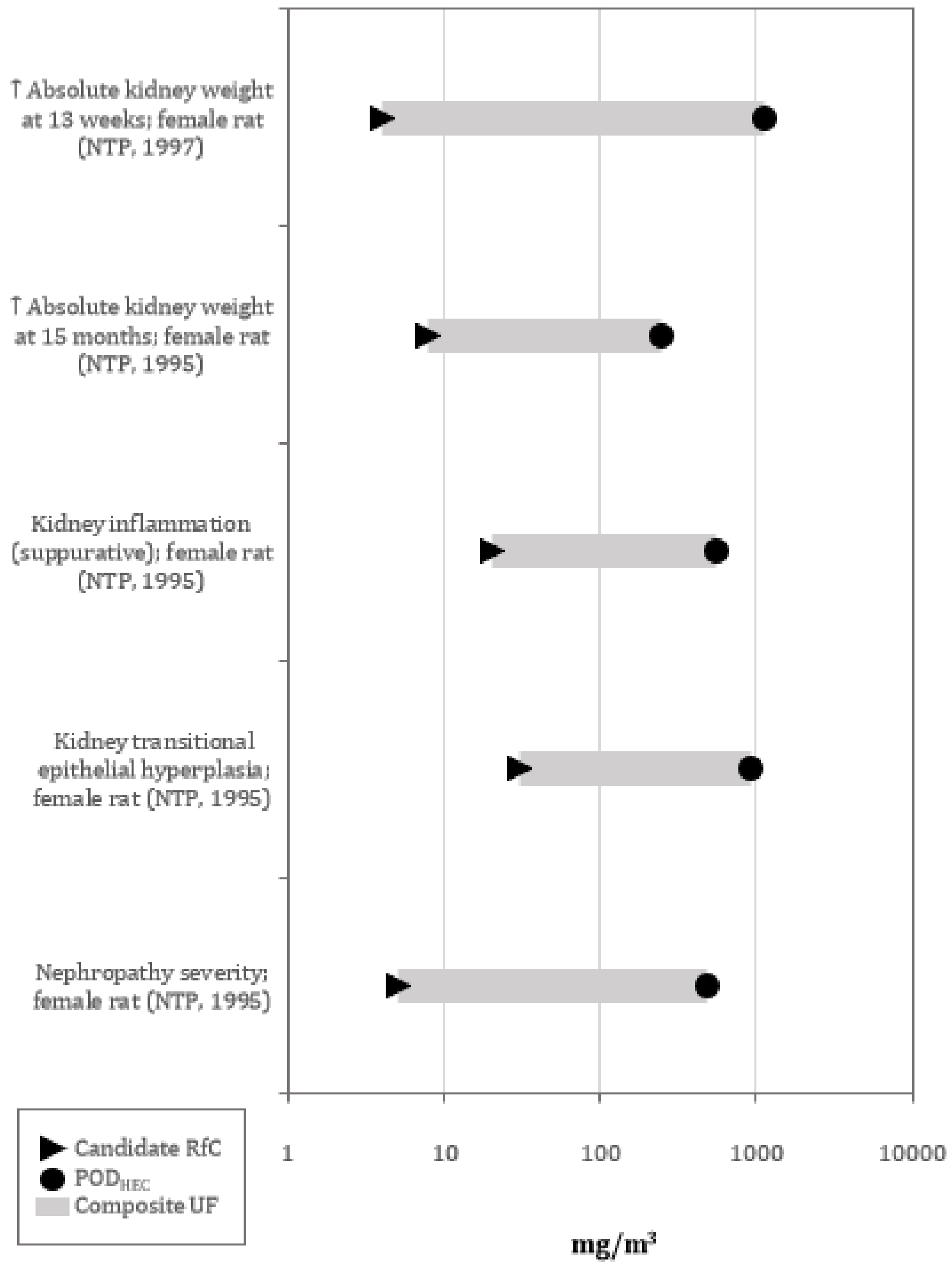


Figure 2-2. Candidate RfC values with corresponding POD and composite UF.

2.2.4 Derivation of Organ/System-Specific Reference Concentrations

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

Kidney Toxicity

For the derivation of candidate values, whether PODs from the subchronic inhalation study of NTP (1997) would provide a better basis than the route-to-route extrapolated PODs based on the chronic oral study of NTP (1995) must be considered. Candidate values were derived for increased kidney weight observed in the subchronic inhalation study (NTP, 1997) and several kidney effects observed in the chronic oral study (NTP, 1995) in female rat, spanning a range from 4×10^0 to 3×10^1 mg/m^3, for an overall 7.5-fold range. To estimate an exposure level below which kidney toxicity from *tert*-butanol exposure is not expected to occur, the RfC for increased increases in severity of nephropathy in female rats (**5×10^0 mg/m^3**) was selected as the kidney-specific RfC for *tert*-butanol, consistent with the selection of the kidney-specific RfD (see Section 2.1.4). This endpoint is based on a longer (chronic) duration and a more specific and sensitive indicator of kidney toxicity than the relatively nonspecific endpoint of kidney weight change and less sensitive endpoints of inflammation and hyperplasia. Confidence in this kidney-specific RfC is medium. The POD for increases in severity of nephropathy is based on a LOAEL, and the candidate values are derived from a well-conducted long-term study, involving a sufficient number of animals per group, including both sexes, and assessing a wide range of kidney endpoints, and availability of a PBPK model for route-to-route extrapolation.

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

Effect	Basis	RfC (mg/m^3)*	Study exposure description	Confidence
Kidney	Increases in severity of nephropathy (NTP, 1995)	5×10^0	Chronic	Medium
Overall RfC	**Kidney**	**5×10^0**	**Chronic**	**Medium**

*Derived from oral study, by route-to-route extrapolation.

2.2.5 Selection of the Overall Reference Concentration

For *tert*-butanol, kidney effects were identified as the primary hazard; thus, a single organ-/system-specific RfC was derived. The kidney-specific RfC of **5×10^0 mg/m^3** is selected as the overall RfC, representing an estimated exposure level below which deleterious effects from *tert*-butanol exposure are not expected to occur.

The overall RfC is derived to be protective of all types of effects for a given duration of exposure and is intended to protect the population as a whole, including potentially susceptible subgroups (U.S. EPA, 2002a). Decisions concerning averaging exposures over time for comparison with the RfC should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could lead to an 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, the potential exists for early lifestage susceptibility to *tert*-butanol exposure, as discussed in Section 1.3.3.

2.2.6 Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall evidence base, 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, 1994a). A PBPK model was used to perform a route-to-route extrapolation to determine a POD for the derivation of the RfC from the NTP (1995) oral study and corresponding critical effect. Confidence in the principal study (NTP, 1995) is high. This study was well conducted, complied with FDA GLP regulations, involved 50 animals per group (including both sexes), and assessed a wide range of tissues and endpoints. Although the toxicity evidence base for *tert*-butanol contains some gaps, these areas are partially informed by the data on ETBE, a parent compound of *tert*-butanol. Therefore, the confidence in the evidence base is medium. Reflecting high confidence in the principal study, medium confidence in the evidence base, and minimal uncertainty surrounding the application of the modified PBPK model for the purposes of a route-to-route extrapolation, the overall confidence in the RfC for *tert*-butanol is medium.

2.2.7 Previous IRIS Assessment

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

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

The following discussion identifies uncertainties associated with the RfD and RfC for *tert*-butanol. To derive the RfD, the UF approach (U.S. EPA, 2000, 1994b) was applied to a POD based on kidney toxicity in rats treated chronically. UF values were applied to the POD to account for extrapolating from an animal bioassay to human exposure, and the likely existence of a diverse human population of varying susceptibilities. These extrapolations are carried out with default approaches, given the lack of data to inform individual steps. To derive the RfC, this same approach was applied, but a PBPK model was used to extrapolate from oral to inhalation exposure.

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

Although the evidence base is adequate for reference value derivation, uncertainty is 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. Effects were not always consistent across exposure routes, however, and maternal toxicity was present whenever developmental effects were observed.

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

Another uncertainty to consider relates to the MOA analysis conducted for the kidney effects. The assessment concluded that *tert*-butanol is a weak inducer of alpha2u-globulin , which is operative in male kidney tumors; therefore, noncancer effects related to alpha2u-globulin were considered not relevant for hazard identification and, therefore, not suitable for dose response consideration. If this conclusion was incorrect and the noncancer effects characterized in this assessment as being related to alpha2u-globulin were relevant to humans, the RfD and RfC values could underestimate toxicity. The assessment also used noncancer effects related to CPN in derivation of the reference values. If noncancer effects characterized in this assessment as being related to CPN were not relevant to humans, the RfD value (0.4 mg/kg-day) could slightly overestimate toxicity compared with an alternative endpoint, increased absolute kidney weight (0.7 mg/kg-day), while the RfC value would be similar (5 mg/m^3 compared with 4 mg/m^3).

2.3 ORAL SLOPE FACTOR FOR CANCER

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

2.3.1 Analysis of Carcinogenicity Data

As noted in Section 1.3.2, 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. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates.

No human data relevant to an evaluation of the carcinogenicity of *tert*-butanol were available. The cancer descriptor was based on the 2-year drinking water study in rats and mice by (NTP, 1995), which reported renal tumors in male rats and thyroid tumors in both male and female mice. This study was considered suitable for dose-response analysis. It was conducted in accordance with FDA GLP regulations, and all aspects were subjected to retrospective quality assurance audits. The study included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting of methods and results. Additionally, the renal tumors were reexamined by a Pathology Working Group (Hard et al., 2011).

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 and thyroid follicular adenomas and carcinoma in male 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 a mode of action analysis, the alpha2u-globulin process was concluded to be at least partially responsible for the male rat renal tumors, in addition to other, unknown, processes. Because the relative contribution of each process to tumor formation cannot be determined (U.S. EPA, 1991a), the male rat renal tumors are not considered suitable for quantitative analysis. Conversely, the mouse thyroid tumors are suitable for dose-response analysis and unit risk estimation, as described in Section 1.3.2.. The available data do not demonstrate that the thyroid tumors are the result of excessive toxicity in female mice rather than the carcinogenicity of *tert*-butanol. The final average body weight reduction in female mice at the highest dose was 12% (NTP, 1995), but water consumption by exposed females was similar to controls and no overt toxicity was observed. Furthermore, female mice in the high dose group had higher rates of survival than control animals. The final average body weight reduction in male mice at the highest dose was 5% to 10% (NTP, 1995) and water consumption by exposed males was similar to controls, but survival was reduced at the highest dose and the tumor response in male mice was adjusted for early mortality. Considering these data, along with the uncertainty associated with the suggestive nature of the weight of evidence, quantitative analysis of the tumor data may be useful for providing a sense of the magnitude of potential carcinogenic risk from *tert*-butanol exposure, including worker and consumer exposures. While this assessment determined that the female mouse thyroid data set is suitable for dose response modeling and calculation of a quantitative risk estimate there is increased uncertainty in this risk estimate due to the suggestive nature of the tumorigenic response (U.S. EPA, 2005a).

2.3.2 Dose-Response Analysis—Adjustments and Extrapolations Methods

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

The second step, extrapolation to lower exposures from the POD, considers what is known about the modes of action for each effect. As above, a biologically based model is preferred (U.S. EPA, 2005a). Otherwise, linear low-dose extrapolation is recommended if the MOA of carcinogenicity is mutagenic or has not been established (U.S. EPA, 2005a). For *tert*-butanol, the mode(s) of carcinogenic action for thyroid follicular cell tumors has not been established (see Section 1.3.2). Therefore, linear low-dose extrapolation was used to estimate human carcinogenic risk.

The dose-response modeling used administered dose because a PBPK model to characterize internal dosimetry in mice was not available. For the analysis of male mice thyroid tumors, the incidence data were adjusted to account for the increased mortality in high-dose male mice, relative to the other groups, that reduced the number of mice at risk for developing tumors. The Poly-3 method (Bailer and Portier, 1988) was used to estimate the number at risk of developing tumors, by weighting the length of time each animal was on study (details in Appendix C of the Supplemental Information). This method was not applied to the female mice data because a difference in survival with increasing exposure was not appreciable and only one tumor, in the high-dose group, occurred before study termination.

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

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

$$= \quad (\text{BMDL in mg/kg-day}) \times 0.14$$

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

2.3.3 Derivation of the Oral Slope Factor

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

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

The recommended slope factor[11] for lifetime oral exposure to *tert*-butanol is **5×10^{-4} per mg/kg-day**, based on the thyroid follicular cell adenoma or carcinoma response in male or female $B6C3F_1$ mice. This slope factor should not be used with exposures exceeding 1,400 mg/kg-day, the highest POD from the two data sets, because above this level the cancer risk might not increase linearly with exposure. The slope of the linear extrapolation from the central estimate BMD_{10HED} derived from the female mouse data set is $0.1/[0.14 \times (2002 \text{ mg/kg-day})] = 4 \times 10^{-4}$ per mg/kg-day.

[11] This value is uncertain because it is based on a determination of *suggestive evidence of carcinogenic potential*; however, the value may be useful for some decision purposes such as providing a sense of the magnitude of potential risks or ranking potential hazards (U.S. EPA, 2005a). The uncertainties in the data leading to this suggestive weight of evidence determination for carcinogenicity are detailed in Section 2.3.4. below.

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

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

[a]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011a).
[b]Human equivalent slope factor = $0.1/BMDL_{10HED}$; see Appendix C of the Supplemental Information for details of modeling results. These values are uncertain because it is based on a determination of *suggestive evidence of carcinogenic potential*; however, the slope factors may be useful for some decision purposes such as providing a sense of the magnitude of potential risks or ranking potential hazards (U.S. EPA, 2005a). The uncertainties in the data leading to this suggestive weight of evidence determination for carcinogenicity are detailed in Section 2.3.4. below.
[c]Because the observed responses were <10%, a BMR of 5% was used to represent the observed response range for low-dose extrapolation; human equivalent slope factor = $0.05/BMDL_{5HED}$.

2.3.4 Uncertainties in the Derivation of the Oral Slope Factor

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

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

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

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

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

2.3.5 Previous IRIS Assessment: Oral Slope Factor

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

2.4 INHALATION UNIT RISK FOR CANCER

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

No chronic inhalation exposure studies to *tert*-butanol are available. Lifetime oral exposure has been associated with increased renal tubule adenomas and carcinoma in male F344 rats, increased thyroid follicular cell adenomas in female $B6C3F_1$ mice, and increased thyroid follicular cell adenomas and carcinomas in male $B6C3F_1$ mice. Because only a rat PBPK model exists, however, route-to-route extrapolation cannot be performed for thyroid tumors in mice at this time. The NTP (1995) drinking water study in rats and mice was the only chronic bioassay available for dose-response analysis. Still, the rat PBPK model and kidney tumors from the NTP (1995) drinking water study were not used for route-to-route extrapolation because enough information to determine the relative contribution of alpha2u-globulin nephropathy and other processes to the overall renal tumor response (U.S. EPA, 1991a) is not available.

2.4.1 Previous IRIS Assessment: Inhalation Unit Risk

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

2.5 APPLICATION OF AGE-DEPENDENT ADJUSTMENT FACTORS

As discussed in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005c), either default or chemical-specific age-dependent adjustment factors (ADAFs) are recommended to account for early-life exposure to carcinogens that act through a mutagenic MOA. Because chemical-specific lifestage susceptibility data for cancer are not available, and because the MOA for *tert*-butanol carcinogenicity is not known (see Section 1.3.2), application of ADAFs is not recommended.

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