Tert-Butyl Alcohol

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Acronyms and Abbreviations

Acronyms and Abbreviations	Definition
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike information criterion
ATSDR	Agency for Toxic Substances and Disease Registry
α2u-globulin	alpha 2u-globulin
°C	degrees Celsius
BMC	benchmark concentration
$BMCL_{10}$	BMC 95% lower confidence limit at the 10% response level
BMD	benchmark dose
$BMDL_{10}$	BMD 95% lower confidence limit at the 10% response level
BMR	benchmark response
BrdU	5-bromo-2-deoxyuridine
CNS	central nervous system
CPN	chronic progressive nephropathy
d	day(s)
DSD	development support document
ELISA	enzyme-linked immunosorbent assay
ESL	Effects Screening Level
$^{acute}ESL$	acute health-based Effects Screening Level for chemicals meeting minimum database requirements
$^{acute}ESL_{generic}$	acute health-based Effects Screening Level for chemicals not meeting minimum database requirements
$^{acute}ESL_{odor}$	acute odor-based Effects Screening Level
$^{acute}ESL_{veg}$	acute vegetation-based Effects Screening Level
$^{chronic}ESL_{threshold(c)}$	chronic health-based Effects Screening Level for threshold dose response cancer effect
$^{chronic}ESL_{threshold(nc)}$	chronic health-based Effects Screening Level for threshold dose response noncancer effects

Acronyms and Abbreviations	Definition
$^{chronic}ESL_{nonthreshold(c)}$	chronic health-based Effects Screening Level for nonthreshold dose response cancer effects
$^{chronic}ESL_{nonthreshold(nc)}$	chronic health-based Effects Screening Level for nonthreshold dose response noncancer effects
$^{chronic}ESL_{veg}$	chronic vegetation-based Effects Screening Level
ETBE	ethyl *tert*-butyl ether
F	female(s)
GD	gestation day
GLP	Good Laboratory Practices
h	hour(s)
$H_{b/g}$	blood:gas partition coefficient
$(H_{b/g})_A$	blood:gas partition coefficient, animal
$(H_{b/g})_H$	blood:gas partition coefficient, human
HEC	human equivalent concentration
HQ	hazard quotient
HSDB	Hazardous Substance Data Base
IARC	International Agency for Research on Cancer
IOAEL	inhalation observed adverse effect level
$^{acute}IOAEL$	acute inhalation observed adverse effect level
$^{subacute}IOAEL$	subacute inhalation observed adverse effect level
$^{chronic}IOAEL_{(nc)}$	chronic inhalation observed adverse effect level (noncancer effects)
$^{chronic}IOAEL_{(c)}$	chronic inhalation observed adverse effect level (cancer effects)
IPCS	International Programme on Chemical Safety
IRIS	USEPA Integrated Risk Information System
kg	kilogram
K_{ow}	n-octanol-water partition coefficient
LOAEL	lowest-observed-adverse-effect-level
M	male(s)

Acronyms and Abbreviations	Definition
mmHg	A millimeter of mercury; approximately 1 torr, or 1/760 of standard atmospheric pressure
MTBE	methyl *tert*-butyl ether
MW	molecular weight
µg	microgram
µg/m^3	micrograms per cubic meter of air
mg	milligram
mg/m^3	milligrams per cubic meter of air
min	minute(s)
MOA	mode of action
n	number
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NRC	National Research Council
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
PND	postnatal day
POD	point of departure
POD_{ADJ}	point of departure adjusted for exposure duration
POD_{HEC}	point of departure adjusted for human equivalent concentration
ppb	parts per billion
ppm	parts per million
ReV	reference value
Acute ReV	acute (e.g., 1-hour) health-based reference value for chemicals meeting minimum database requirements
Acute ReV-24hr	acute 24-hour health-based reference value for chemicals meeting minimum database requirements
Chronic $ReV_{threshold(nc)}$	chronic health-based reference value for threshold dose response noncancer effects

Acronyms and Abbreviations	Definition
RfC	reference concentration
RPF	relative potency factor
rpm	revolutions per minute
RGDR	Regional Gas Dose Ratio
TBA	*tert* butyl alcohol
TCEQ	Texas Commission on Environmental Quality
TD	Toxicology, Risk Assessment, and Research Division
UF	uncertainty factor
UF_H	interindividual or intraspecies human uncertainty factor
UF_A	animal to human uncertainty factor
UF_{Sub}	subchronic to chronic exposure uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_D	incomplete database uncertainty factor
USEPA	United States Environmental Protection Agency
VAF+	Virus Antibody Free
wk	week(s)
yr	year(s)

Chapter 1 Summary Tables

Table 1 and Table 2 provide a summary of health- and welfare-based values from an acute and chronic evaluation of *tert*-butyl alcohol (TBA), respectively, for use in air permitting and air monitoring. Please refer to Section 1.6.2 of the *TCEQ Guidelines to Develop Toxicity Factors* (TCEQ 2015a) for an explanation of air monitoring comparison values (AMCVs), reference values (ReVs) and effects screening levels (ESLs) used for review of ambient air monitoring data and air permitting. Table 3 provides summary information and the physical/chemical data of TBA.

Table 1. Acute Health and Welfare-Based Screening Values for *tert*-butyl alcohol (TBA)

Screening Level Type	Duration	Value 1 (µg/m^3)	Value 2 (ppb)	Usage	Flags	Surrogated/ RPF	Critical Effect(s)	Notes
Acute ReV	1 h	15,000	5,000	M	A	---	Ataxia, hyperactivity and hypoactivity in rats	--
Acute ReV-24hr	---	---	---	---	---	---	---	---
acuteESL [a]	1 h	4,500	1,500	P	S,D	---	Same as above	---
acuteIOAEL	1 h	2,700,000	900,000	N	none	---	Same as above	---
subacuteIOAEL	---	---	---	---	---	---	---	---
acuteESL_{odor}	---	14,000	4,600	M	A	---	camphor-like odor	---
acuteESL_{veg}	---	---	---	---	---	---	---	---

Bold values used for air permit reviews

[a] Based on the acute ReV multiplied by 0.3 (i.e., HQ = 0.3) to account for cumulative and aggregate risk during the air permit review.

Usage:
P = Used in Air Permitting
M = Used to Evaluate Air Monitoring Data
R = Used to Calculate Remediation Cleanup Levels
N = Usage Not Defined

Flags:
A = AMCV report
S = ESL Summary Report
D = ESL Detail Report

Table 2. Chronic Health and Welfare-Based Screening Values for *tert*-butyl alcohol (TBA)

Screening Level Type	Duration	Value 1 (µg/m³)	Value 2 (ppb)	Usage	Flags	Surrogated/ RPF	Critical Effect(s)	Notes
Chronic $ReV_{threshold(nc)}$ [a]	70 yr	8,200	2,700	M	A	---	Increased absolute kidney weights in female rats	Oral route to inhalation route extrapolation
$^{chronic}ESL_{threshold(nc)}$ [b]	70 yr	2,400	810	P	S,D	---	Same as above	---
$^{chronic}IOAEL_{(nc)}$	Annual	450,000	150,000	N	none	---	Same as above	---
$^{chronic}ESL_{threshold(c)}$	---	N/A [c]	N/A	---	---	---	---	---
$^{chronic}ESL_{nonthreshold(c)}$	**---**	**N/A**	**N/A**	**---**	**---**	**---**	**---**	**---**
$^{chronic}IOAEL_{(c)}$	---	N/A	N/A	---	---	---	---	---
$^{chronic}ESL_{veg}$	---	---	---	---	---	---	---	---
$^{chronic}ESL_{animal}$	---	---	---	---	---	---	---	---

Bold values used for air permit reviews

[a] Based on route-to-route extrapolation (i.e., oral-to-inhalation route) using physiologically based pharmacokinetic (PBPK) modeling.

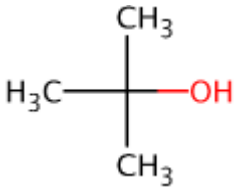
[b] Based on the chronic ReV multiplied by 0.3 (i.e., HQ = 0.3) to account for cumulative and aggregate risk during the air permit review.

[c] Not applicable (N/A)

Usage:
P = Used in Air Permitting
M = Used to Evaluate Air Monitoring Data
R = Used to Calculate Remediation Cleanup Levels
N = Usage Not Defined

Flags:
A = AMCV report
S = ESL Summary Report
D = ESL Detail Report

Table 3. Chemical and Physical Data

Parameter	Value	Reference
Chemical Structure		USEPA 2021a
Molecular Formula	$C_4H_{10}O$	USEPA 2021a
Molecular Weight (g/mol)	74.123	USEPA 2021a
Physical State at 25°C	Solid (liquid >25.7°C)	USEPA 2021a
Color	white crystalline solid ≤25.7°C colorless flammable liquid >25.7°C	USEPA 2021a
Odor	camphor-like	USEPA 2021a
CAS Registry Number	75-65-0	USEPA 2021a
Common Synonym(s)	2-propanol, 2-methyl-, 1,1-dimethylethanol, 2-methylpropan-2-ol, 2-methylpropane-2-ol, 2-metilpropan-2-ol, t-butanol, trimethyl carbinol, trimethylcarbinol, trimethylmethanol, arconol, t-butyl hydroxide, tert-butanol	USEPA 2021a
Solubility in water (mol/L)	13.5	USEPA 2021a
Log K_{ow}	3.5	USEPA 2021a
Vapor Pressure (mmHg at 20°C)	40.7	USEPA 2021a
Conversion Factors	1 ppm = 3.031 mg/m^3 1 mg/m^3 = 0.330 ppm	USEPA 2021a

Chapter 2 Background Information

2.1 Physical/Chemical Properties

Tert-butyl alcohol (TBA) is a white crystalline solid or colorless, highly flammable liquid (above 25.7°C) with a camphor-like odor (NIOSH 2005; IPCS 1987). TBA contains a hydroxyl chemical functional group; is miscible with alcohol, ether, and other organic solvents; and is soluble in water (IPCS 1987). Chemical and physical properties of TBA are presented in Table 3 above (USEPA 2021a).

2.2 Sources and Uses

The following information on sources and uses was taken from USEPA (2021a).

2.2.1 Sources

The Toxics Release Inventory (TRI) program National Analysis Report estimated that more than 1 million pounds of TBA have 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 TBA released into the air, discharged to bodies of water, disposed of at the facility to land, and disposed of in underground injection wells. Total off-site disposal or other releases of TBA amounted to 67,060 pounds (USEPA 2016). The industrial chemical *tert*-butyl acetate also can degrade to form TBA in the environment and as a metabolite in animals post exposure (USEPA 2021a).

2.2.2 Uses

TBA is primarily 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 TBA, including imports, was approximately 4 billion pounds in 2012 (USEPA 2014).

TBA has been used as a fuel oxygenate, an octane booster in unleaded gasoline, and a denaturant for ethanol. From 1997 to 2005, the annual TBA 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 ethyl *tert*-butyl ether (ETBE). MTBE and ETBE are fuel oxygenates that were used in the United States (US) prior to 2007 at levels of more than 2 billion gallons annually. Current use levels of MTBE and ETBE in the US are much lower, but the use in Europe and Asia remains strong.

TBA has been used for a variety of other purposes, including as a dehydrating agent and solvent. It is added to lacquers, paint removers, and nail enamels and polishes. TBA also is used to manufacture methyl methacrylate plastics and flotation devices. It is used in the

manufacture of food flavoring, and, because of its camphor-like aroma, it also is used to create artificial musk, fruit essences, and perfume (HSDB 2007). TBA is used in coatings on metal and paperboard food containers (CalEPA 1999) and industrial cleaning compounds and can be used for chemical extraction in pharmaceutical applications (HSDB 2007).

Chapter 3 Acute Evaluation

3.1 Health-Based Acute ReV and ^{acute}ESL

No relevant data on acute exposures of TBA in humans are available. Limited data on acute exposures in animals are available; in GLP-compliant subacute inhalation studies in mice and rats, all animals exposed to a target concentration of 7000 ppm TBA were sacrificed in moribund condition on Day 2, following a single 6-h exposure on the previous day (NTP 1997). The endpoint of mortality is not appropriate for derivation of an acute ReV and ^{acute}ESL. Therefore, available data on subacute exposures in animals were considered for derivation of the acute ReV and ^{acute}ESL.

3.1.1 Key and Supporting Studies

3.1.1.1 Animal Studies

3.1.1.1.1 NTP (1997) Subacute Inhalation Toxicity Study in Rats – Key Study

A subacute GLP-compliant inhalation study in rats was selected as the key study. In this study, five F344/N rats/sex/group (6 weeks old, Taconic Laboratory and Animal Services, Germantown, NY) were exposed by inhalation to target concentrations of 0, 450, 900, 1750, 3500 and 7000 ppm TBA for 6 h/day, 5 days/week for 12 exposure days. The average concentrations of TBA were 457, 910, 1750, 3523, and 6989 ppm. Animals were observed twice daily for morbidity and mortality. Clinical observations were performed twice daily (before and after exposure) and body weights were obtained 5 days prior to exposure, on Day 8 and at the end of the study. Animals were necropsied and organs (brain, heart, right kidney, liver, lung, right testis, and thymus) were weighed. Complete histopathologic evaluation was performed on all mice in the 0, 3500 and 7000 ppm groups, and gross lesions were evaluated in the remaining groups.

All males and females exposed to 7000 ppm were sacrificed in moribund condition on Day 2, following a single 6-h exposure on the previous day. All other rats survived to the end of the study. In the 3500 ppm group, males and females had statistically significantly lower mean body weights and the end of the study, due to reductions in body weight gain in comparison to the control group. At 3500 ppm, mean male body weight and body weight gain were 14% and 27% lower, respectively, than control males at the end of the study. At 3500 ppm, mean female body weight and body weight gain were 13% and 34% lower, respectively, than control females

at the end of the study. In males and females exposed to ≥ 900 ppm TBA, clinical observations seen included ataxia, hyperactivity, and hypoactivity. Absolute and relative weights of thymus of males and females exposed to 3500 ppm TBA were statistically significantly lower than controls. At 3500 ppm, male absolute and relative thymus weights were 35% and 24% lower than control, and female absolute and relative thymus weights were 32% and 22% lower than control. However, there were no TBA-related gross or microscopic findings in rats sacrificed on Day 2 and those that survived to the end of the study.

Based on clinical observations of ataxia, hyperactivity and hypoactivity at ≥ 900 ppm TBA, the LOAEL was 900 ppm, and the NOAEL was 450 ppm.

3.1.1.1.2 Borghoff et al. (2001) Subacute Inhalation Toxicity Study in Rats

Male and female F-344 rats (10 weeks old, Charles River, Raleigh, NC) were exposed for 6 h/day for 10 days to target concentrations of 0, 250, 450 or 1750 ppm TBA. Target concentrations were based on a subacute inhalation study in rats conducted by NTP (NTP 1997, Section 3.1.1.1.1 NTP (1997) Subacute Inhalation Toxicity Study in Rats – Key Study). Average exposure concentrations were within 10% of the target concentration. A total of 10 rats/sex/group was evaluated for toxicity; each group included 5 rats/sex/group designated for histopathology of kidney and 5 rats/sex/group were designated for biochemical analysis of α2u-globulin in kidney tissue homogenates, and measurement of liver and kidney weights. At 3.5 days prior to euthanasia, animals designated for histopathology were subcutaneously implanted with an osmotic pump for delivery of 5-bromo-2-deoxyruridine (BrdU). One day after the final exposure, these rats were euthanized, and kidneys were perfused and collected for histopathologic examination. The histologic endpoints evaluated were renal lesions in hematoxylin and eosin-stained sections, protein droplet accumulation in Mallory's Heidenhain-stained sections, quantification of the labeling index by immunohistochemical staining for BrdU, and immunohistochemical staining for α2u-globulin. Protein droplet accumulation and severity of renal findings were graded on a 4-point scale (0-4). The labeling index was defined as the percent of positively stained epithelial cells within the proximal tubule; a total of 2000 tubular epithelial cells in the renal cortex (1000 cells per kidney) were counted.

An additional 180 rats (3/sex/timepoint in each TBA group for a total of 60 rats/TBA group) were evaluated for concentrations of TBA in blood, liver and kidney after a single 6 h exposure and after 8 days of exposure. The timepoints were 2, 4, 6, 8 and 16 h following these exposures.

The focus of this study was to evaluate the potential for α2u-globulin nephropathy, a male rat-specific finding which is not relevant to humans (USEPA 1991, Capen et al. 1999). Therefore α2u-globulin nephropathy is not an appropriate endpoint for derivation of a ReV. In male rats in this study, a dose-related increase in protein droplet accumulation and formation of large coalescing globules of protein and rare crystalloid protein structures were seen in the proximal

tubules of the kidney. Also, in male rats, positive staining for α2u-globulin was observed, and renal cytosolic concentrations of α2u-globulin as measured by an enzyme-linked immunosorbent assay (ELISA) were statistically significantly increased at 1750 ppm. A dose-related increase in the labeling index in kidney sections of male rats was observed; additionally, there was an exposure-related correlation between the labeling index and the renal cytosolic α2u-globulin concentration. Protein droplets were not observed in kidney sections of female rats, and there was no evidence of immunohistochemical staining for α2u-globulin. Also, there were no statistically significant differences in the labeling index in females. Therefore, renal cytosolic concentrations of α2u-globulin were not measured for females. Overall, the findings of α2u-globulin, protein droplets and increased cell proliferation observed in the kidney of male rats only were consistent with α2u-globulin nephropathy, a male rat-specific finding that is not relevant to humans (USEPA 1991, Capen et al. 1999).

TBA liver:blood ratios did not change in males and females at all exposure concentrations when concentrations were compared following one day and eight days of exposure. However, in males, but not females, kidney:blood ratios increased following 8 days of exposure when compared to a single exposure.

There were no statistically significant differences in terminal body weights. At 1750 ppm, there were no differences in absolute kidney weights, but relative kidney weights were slightly higher (~7%) than those of controls in males and females. Decreased absolute (9.5%) and relative (14%) liver weights were observed in males at 1750 ppm. A NOAEL in males was not identified, as α2u-globulin nephropathy was observed at all concentrations of TBA. Because α2u-globulin nephropathy is a male rat-specific finding that is not relevant to humans, the NOAEL of 450 ppm in males based on reductions in liver weights may be relevant for derivation of a ReV. Additionally, the associated LOAEL (1750 ppm) is higher than that described above for hyperactivity and hypoactivity in rats (900 ppm, NTP 1997 See Section 3.1.1.1.1 NTP (1997) Subacute Inhalation Toxicity Study in Rats – Key Study) and USEPA cites a lack of consistency in liver effects with inadequate available information to draw conclusions regarding liver toxicity at this time (Section 1.2.6 of USEPA 2021a). The NOAEL in females was 1750 ppm, the highest concentration tested, as no adverse findings were seen in females.

3.1.1.1.3 NTP (1997) Subacute Inhalation Toxicity Study in Mice

In this GLP-compliant study, five B6C3F1 mice/sex/group (6 weeks old, Taconic Laboratory and Animal Services, Germantown, NY) were exposed by inhalation to target concentrations of 0, 450, 900, 1750, 3500 or 7000 ppm TBA for 6 h/day, 5 days/week for 12 exposure days. Average concentrations of TBA were 457, 910, 1751, 3524 and 7024 ppm. Animals were observed twice daily for morbidity and mortality. Clinical observations were performed twice daily (before and after exposure) and body weights were obtained 5 days prior to exposure, on Day 8 and at the end of the study. Animals were necropsied and organs (brain, heart, right kidney, liver, lung,

right testis, and thymus) were weighed. Complete histopathologic evaluation was performed on all mice in the 0, 3500 and 7000 ppm groups, and gross lesions were evaluated in the remaining groups.

All mice exposed to 7000 ppm were sacrificed in moribund condition on Day 2, following a single 6-h exposure on the previous day. One male in the 3500-ppm group died on Day 3. At 3500 ppm, animals were prostrate following the first exposure through Day 3 of the study. Thereafter, clinical signs in the 3500-ppm group were seen predominantly post-exposure and included hypoactivity, ataxia and rapid respiration. In mice exposed to 1750 ppm TBA, hypoactivity, hyperactivity, ataxia and urogenital wetness occurred at lower incidences. In comparison to controls, statistically significantly increased absolute and/or relative liver weights were seen in males and females in the 3500-ppm group. Absolute and relative liver weights were increased in males (16% and 15%, respectively relative to control) and females (28% and 24%, respectively, relative to control) in the 3500-ppm group. Also, at 3500 ppm, absolute and relative thymus weights were statistically significantly lower in females (26% and 29%, respectively, relative to control). There were no TBA-related differences in body weight or body weight gain, or gross or microscopic findings.

Based on clinical findings of hypoactivity, hyperactivity, ataxia and urogenital wetness in male and female mice at 1750 ppm, the LOAEL was 1750 ppm, and the NOAEL was 900 ppm.

3.1.1.3 Reproductive and Developmental Studies

Reproductive and developmental data via the inhalation route are limited.

3.1.1.3.1 Reproductive Studies in Mice and Rats

The potential for reproductive effects was evaluated in GLP-compliant 13-week inhalation toxicity studies conducted in mice and rats (NTP 1997). Ten B6C3F1 mice and 10 F344/N rats/sex/group (7 weeks old, Taconic Laboratory and Animal Services, Germantown, NY) were exposed by inhalation to nominal concentrations of 0, 135, 270, 540, 1080 or 2100 ppm TBA for 6 h/day, 5 days/week for 13 weeks. After the last exposure, animals were necropsied and the right epididymis, right cauda and right testis were weighed. Complete histopathologic evaluation, including prostate gland, testis (with epididymis and seminal vesicle), and uterus was performed on all mice and rats in the 0 and 2100 ppm groups, and gross lesions were evaluated in the remaining groups. Sperm evaluations (density, morphology and motility) and vaginal cytology evaluation (estrous cycle length, duration in various estrous stages) were performed on all mice and rats in the 0, 540, 1080 and 2100 ppm groups at the end of the study. There were no TBA-related effects on reproductive endpoints evaluated in male and female mice and rats. Therefore, the NOAEL for effects on the reproductive tract was 2100 ppm for male and female mice and rats.

3.1.1.3.2 Developmental Studies in Rats

One embryo/fetal development study and one postnatal development study via the inhalation route are available (Nelson et al. 1989, Nelson et al. 1991). In the embryo/fetal development study, 13-18 pregnant Sprague-Dawley rats/group (Charles River, Wilmington, MA) were exposed 7 h/day from Gestation Day (GD) 1 to 19 to target concentrations of 0, 2000, 3500 or 5000 ppm TBA (Nelson et al. 1989). Concentrations were selected based on a pilot phase in which 6 nonpregnant rats were exposed for 7 h to 10,000 ppm; this resulted in severe narcosis in all rats, and death in 5 out of 6 rats. In the definitive study, the high concentration of 5000 ppm was selected to be maternally toxic. On GD 20, females were euthanized and the numbers of corpora lutea, resorptions (classified as early, middle or late) and live fetuses were counted. Fetuses were examined for external malformations, weighed and sexed. One half were processed for examination of skeletal malformations and variations, and the other half were fixed for examination of visceral malformations and variations.

All maternal animals experienced narcosis at 5000 ppm. At all exposure concentrations, unsteady gait was observed, and locomotor activity was impaired at 3500 and 5000 ppm. At 5000 ppm, animals experienced initially a body weight loss and then a body weight gain, which correlated with a statistically significantly reduced food consumption for the first 2 weeks. Mean food consumption values in 5000 ppm-exposed rats were 36% and 28% lower, respectively, than controls. At 5000 ppm maternal body weights were statistically significantly lower than control values throughout the study. When compared to the control group, there were no statistically significant differences in the numbers of corpora lutea/litter, resorptions/litter and live fetuses/litter and %females/litter in any TBA group. In all TBA groups, fetal weights of males and females were statistically significantly lower than controls. Skeletal variations were statistically significantly increased in the 3500 and 5000 ppm groups. In comparison to controls, there were no statistically significant differences in skeletal and visceral malformations and in visceral variations. NOAELs for maternal and fetal toxicity were not identified in this study. The LOAEL for maternal animals was 2000 ppm based on clinical observations of unsteady gait, and the LOAEL for fetal toxicity was 2000 ppm based on reduced body weights of males and females, which may be due to maternal toxicity rather than a direct effect of TBA.

In a postnatal development study in rats, 15 pregnant Sprague-Dawley rats/group (VAF+, Charles River Laboratories, Wilmington, DE) were exposed to 0, 2000 or 4000 ppm TBA 7 hours/day on GD 1-20 (Nelson et al. 1991). In addition, 18 male Sprague-Dawley rats/group were exposed to 2000 or 4000 ppm TBA for 7 hours/day for 6 weeks; these males were subsequently mated to non-exposed females. The authors note that the exposures to the two concentrations of TBA were run at different times (separated by approximately three months); therefore, data were compared against the concurrent control group and comparisons between the two concentrations of TBA are not appropriate. On the day of birth, litters were culled to

4 (± 1) pups/sex and fostered to untreated controls. Animals were weighed each week through 5 weeks of age; body weight data of the pups were not included in the publication. On postnatal day (PND) 10, 1 male and 1 female per litter were randomly assigned to one of four groups and tested: ascent on wire mesh screen on PND 10, 12, 14; activity in an open field and a photoelectrically-monitored activity device on PND 16-18, 30-32, 44-46, 58-60; running wheel activity on PND 32 and 33; avoidance conditioning (separate groups tested beginning on PND 34 and 60); operant conditioning (starting on PND 40). Also, brains from 10 pups/group at 21 days of age (1 male and 1 female/litter) were collected and dissected into cerebrum, cerebellum, brainstem and midbrain. Brain regions were analyzed for protein and the neurotransmitters acetylcholine, dopamine, norepinephrine, serotonin, metenkephalin, ß-endorphin, and substance P.

Decreased body weights were seen in maternally exposed females at 4000 ppm; mean body weights were 7.6% to 9.7% lower than controls during gestation. The decreased maternal body weights correlated with a 39% decrease in mean food consumption during the first week of gestation. During the third week of gestation, mean water consumption of pregnant females in the 4000 ppm was 53% greater than that of controls. At 2000 ppm, there were no effects on body weight, food consumption and water intake in pregnant rats. The authors state that males in both TBA groups did gain weight over the 6-week exposure period.

For the behavioral data, few differences were seen between the control group and the maternally-exposed or paternally-exposed groups (data not shown). At a maternal exposure of 2000 ppm (but not 4000 ppm), a significant effect on the ascent test was seen; the duration the animals held the wire was not significant but the distance the animals climbed (data not shown) was statistically significantly different from control. The authors then state that there were no differences in the ascent test in the paternally-exposed groups, but then go on to state that the mean time the animals held the wire for the maternally exposed 2000 ppm group (10 seconds) was statistically significantly less than the mean time of 16 seconds for the control group.

At 4000 ppm, there was a statistically significant effect on rotarod performance. The maternal group mean of 26 revolutions per minute (rpm) and the paternal group mean of 20 rpm were statistically significantly higher than the control mean of 16 rpm. For open field testing, the latency to reach the outer circles of the field was statistically significantly lower at 4000 ppm for the paternally exposed group (115 seconds) in comparison to the controls (210 seconds).

For the biochemical evaluations, a few statistically significant differences from control were noted. The values were expressed relative to protein content. At 2000 ppm, serotonin in the midbrain was lower in the maternally and paternally exposed groups. Additionally at 2000 ppm, lower met-enkephalin in the cerebrum was noted in the maternally and paternally exposed groups. At 4000 ppm, norepinephrine and ß-endorphin were lower in the cerebellum in both the maternally exposed and paternally exposed groups. Also at 4000 ppm, met-

enkephalin in the cerebrum was lower in the maternally exposed and paternally exposed groups. The authors noted that the reductions in met-enkephalin seen at 2000 and 4000 ppm TBA were not dose-related.

The authors concluded that "the small number of statistically significant behavioral and neurochemical effects did not provide evidence of a dose-effect relationship or discernible pattern of effects" and that "the few effects were likely of little or no biological significance". The authors suggest that more investigations be conducted.

Based on reductions in body weight and food consumption, the LOAEL for pregnant rats was 4000 ppm, and the NOAEL was 2000 ppm. It is not known what the NOAEL or LOAEL was for males exposed for 6 weeks because there was no mention of comparison to control males. Based primarily on the behavioral effects, and possibly also reductions in neurochemicals in brain, the TCEQ considers ≥ 2000 ppm a tentative LOAEL in pups (maternally and/or paternally exposed) and a NOAEL in pups cannot be confidently identified.

Overall, the embryo/fetal and postnatal development studies conducted in rats via the inhalation route were of low quality and poor study design. The embryo/fetal development study did not identify a NOAEL for maternal and fetal toxicity; therefore, it is not clear if the decreased body weights of the pups was due to maternal toxicity and/or a direct effect of TBA. In the postnatal development study exposures to the two concentrations of TBA were run at different times (separated by approximately three months); therefore, data were compared against the concurrent control group and comparisons between the two concentrations of TBA are not appropriate.

In conclusion, USEPA (2021a) indicated that although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify developmental, including neurodevelopmental, effects or reproductive effects as a potential human health hazard of TBA exposure (see Sections 1.2.3, 1.2.4, and 1.2.5 of USEPA 2021a).

3.1.2 Metabolism and Mode of Action (MOA) Analysis

TBA is rapidly absorbed and distributed throughout the body following inhalation exposure (USEPA 2021a). TBA is a poor substrate for alcohol dehydrogenase and is metabolized by cytochrome P450 enzymes in rats and humans (USEPA 2021a). MOA data are not available for the clinical observations of ataxia, hyperactivity and hypoactivity observed in rats in the key study. However, based on the studies conducted in animals, the effects of TBA are consistent with central nervous system (CNS) depression observed with alcohols (NTP 1997).

3.1.3 Health-Based Acute 1-h ReV and ESL

3.1.3.1 Selection of the Key Study, Point of Departure (POD), and Critical Effect

Table 4 provides a summary of the subacute inhalation toxicity studies conducted with TBA.

Table 4. Summary of Subacute Inhalation Toxicity Studies of *Tert*-butyl Alcohol in Animals

Species	Duration and Concentration	NOAEL	LOAEL	Effects at the LOAEL	Reference
B6C3F1 mice (10/sex/group)	0, 450, 900, 1750, 3500 and 7000 ppm for 6 h/day, 5 days/week for 12 exposure days	900 ppm	1750 ppm	Hypoactivity, hyperactivity, ataxia and urogenital wetness in males and females	NTP 1997
F-344 rats (10/sex/group)	0, 250, 450 or 1750 ppm for 6 h/day for 10 days	450 ppm (males) 1750 ppm (females)	1750 ppm (males) Not identified (females)	Decreases in absolute (9.5%) and relative (14%) liver weights in males No adverse findings in females	Borghoff et al. 2001
F344/N rats (10/sex/group)	0, 450, 900, 1750, 3500, and 7000 ppm for 6 h/day, 5 days/week for 12 exposure days	450 ppm	900 ppm	Ataxia, hyperactivity and hypoactivity in males and females	NTP 1997

The NTP (1997) subacute inhalation toxicity study in rats, which used several exposure concentrations and was GLP-compliant, was selected as the key study. This subacute inhalation toxicity study in rats identified a NOAEL of 450 ppm and a LOAEL of 900 ppm, based on clinical findings of ataxia, hyperactivity and hypoactivity in males and females at exposure concentrations of ≥ 900 ppm. A subacute inhalation toxicity study in mice (NTP 1997) was conducted with the same duration and target concentrations resulting in similar clinical observations observed at the LOAEL in rats, but the NOAEL (900 ppm) and LOAEL (1750 ppm) observed in mice were greater than those observed in rats. In a 10-day inhalation toxicity study in rats (Borghoff et al. 2001), the NOAEL in males (450 ppm) based on decreases in absolute

(9.5%) and relative (14%) liver weights relative to control is similar to the NOAEL in the key study. In summary, the NOAEL of 450 ppm based on ataxia, hyperactivity and hypoactivity in rats was selected as the point-of-departure (POD) to derive the 1-h ReV. The nominal concentration of 450 ppm was used; this nominal concentration is within 2% of the average measured concentrations in the key study (457 ppm, NTP 1997) and the supporting 10-day inhalation toxicity study (442 ppm, Borghoff et al. 2001).

3.1.3.2 MOA and Dose Metric for Critical Effect

MOA data are not available for the clinical observations of ataxia, hyperactivity and hypoactivity observed in rats in the key study. However, based on the studies conducted in animals, the effects of TBA are consistent with CNS depressions observed with alcohols (NTP 1997), and is a threshold effect. In the key study (NTP 1997), exposure concentration data are available. The exposure concentration of TBA was used as the default dose metric for the key study.

3.1.3.3 Adjustments to the POD

3.1.3.3.1 Benchmark Concentration (BMC) Modeling

The incidences of clinical findings at each exposure concentration were not included in the key study publication (NTP 1997); therefore, benchmark concentration modeling was not performed. In the supporting 10-day inhalation toxicity study (Borghoff et al. 2001), the approximate 10% response in absolute liver weight decrease in males occurred at the LOAEL of 1750 ppm, which is higher than the LOAEL of 900 ppm in the key study (NTP 1997). Therefore, the NOAEL of 450 ppm based on clinical observations consistent with CNS depression was used as the POD.

3.1.3.3.2 Default Exposure Duration Adjustments

The 6 h/day NOAEL POD of 450 ppm was not adjusted to a 1-h duration, because the CNS depression observed with alcohols may be more dependent on exposure concentration than duration (section 3.8.1 of the *TCEQ Guidelines to Develop Toxicity Factors* [TCEQ 2015a]). Moreover, the CNS effects were seen during the course of the subacute study, not just at the end of 12 exposure days. Therefore, the POD_{ADJ} is the NOAEL POD of 450 ppm.

3.1.3.3.3 Default Dosimetry Adjustments from Animal-to-Human Exposure

TBA is rapidly absorbed and distributed throughout the body following inhalation exposure (USEPA 2021a). Exposure to TBA results in CNS depression, which is a systemic effect. Therefore, TBA is considered a Category 3 gas (USEPA 2021a). For Category 3 gases, the default dosimetric adjustment from an animal concentration to a POD_{HEC} is calculated using the following equation:

$POD_{HEC} = POD_{ADJ}$ x regional gas dose ratio (RGDR)

where: RGDR =[$(H_{b/g})_A$ / $(H_{b/g})_H$]

$H_{b/g}$ = ratio of the blood:gas partition coefficient

A = animal

H = human

The measured blood/air partition coefficients in human ($(H_{b/g})_H$) and in the rat ($(H_{b/g})_A$) for TBA are 481 and 462, respectively, resulting in a RGDR of 1.04 (Borghoff et al. 1996, Nihlén et al. 1995). Because the ratio of the animal-to-human blood:gas partition coefficients (481/462 = 1.04) is greater than one, a default value of one is used as the regional gas dose ratio as recommended by TCEQ (see section 3.9.1 of the *TCEQ Guidelines to Develop Toxicity Factors* [TCEQ 2015a]). The resulting POD_{HEC} from the POD_{ADJ} of 450 ppm is 450 ppm for TBA.

3.1.3.4 Adjustments to the POD_{HEC}

The following uncertainty factors (UFs) were applied to the POD_{HEC} of 450 ppm:

- A UF_H of 10 was used to account for variation in susceptibility among members of the human population to the CNS depressive effects of TBA;
- A UF_A of 3 for interspecies uncertainty because a default dosimetric adjustment was conducted to account for toxicokinetic differences between animals and humans; and
- A UF_D of 3 for database uncertainty. GLP-compliant subacute inhalation toxicity studies with several exposure concentrations were conducted in rats and mice. One prenatal developmental toxicity study and one postnatal developmental toxicity study via the inhalation route in rats only were available and were of low quality. In the prenatal developmental toxicity study NOAELs for maternal toxicity and fetal toxicity were not identified; therefore, it is unclear if the fetal toxicity observed (decreased body weights) may be due to maternal toxicity and/or a direct effect of TBA. However, minimal effects were observed at doses that cause toxicity in other organ systems (USEPA 2021a), and the associated LOAELs (≥ 2000 ppm) from these prenatal and postnatal developmental studies are higher than that associated with the critical CNS depressive effects identified (900 ppm), as is the NOAEL (2100 ppm) for reproductive toxicity.

acute ReV = POD_{HEC} / (UF_H x UF_A x UF_D)

= 450 ppm / (10 x 3 x 3)
= 450 ppm / 90
= 5.0 ppm
= 5.0 ppm or 5,000 ppb (rounded to two significant figures)

3.1.3.5 Health-Based 1-h Acute ReV and ^{acute}ESL

The resulting 1-h acute ReV was rounded to two significant figures at the end of all calculations. The rounded acute ReV was then used to calculate the ^{acute}ESL at the target hazard quotient (HQ) of 0.3 (Table 5).

Table 5. Derivation of the 1-h Acute ReV and ^{acute}ESL

Parameter	Summary
Study	NTP (1997) subacute study (GLP-compliant)
Study Population	F344/N rats (10/sex/group)
Study Quality	High
Exposure Method	Inhalation exposure to *tert*-butyl alcohol at 0, 450, 900, 1750, 3500 and 7000 ppm
Exposure Duration	6 h/day, 5 days/week for a total of 12 exposure days
Critical Effects	ataxia, hyperactivity and hypoactivity in male and female rats
NOAEL	450 ppm
LOAEL	900 ppm
POD_{ADJ}	450 ppm
POD_{HEC}	450 ppm
Total uncertainty factors (UFs)	90
Interspecies UF	3
Intraspecies UF	10
LOAEL-to-NOAEL UF	N/A
Incomplete Database UF *Database Quality*	3 Medium-high
Acute ReV [1 h] (HQ = 1)	**15,000 µg/m^3 (5,000 ppb)**
^{acute}ESL [1 h] (HQ = 0.3)	**4,500 µg/m^3 (1,500 ppb)**

3.1.3.6 Acute Inhalation Observed Adverse Effect Level (IOAEL)

Risk assessors, and the general public, often ask to have information on the levels in air where health effects would be expected to occur. So, when possible, the TCEQ provides chemical-specific observed adverse effects levels in DSDs (TCEQ 2015a). As the basis for development of

IOAELs is limited to available data, future studies could possibly identify a lower POD for this purpose. The acute IOAEL is provided for informational purposes only (TCEQ 2015a).

Note that no relevant inhalation data of TBA in humans are available. The critical effect observed following inhalation exposure to animals are consistent with CNS depression. The 6 h/day LOAEL of 900 ppm observed in the subacute inhalation study in rats was not adjusted to a 1-h duration because duration adjustments are not conducted for acute IOAELs (TCEQ 2015a). Additionally, the CNS depression observed with alcohols may be more dependent on exposure concentration than duration (section 3.8.1 of the *TCEQ Guidelines to Develop Toxicity Factors* [TCEQ 2015a]). To calculate the $LOAEL_{HEC}$, a RGDR of 1 was used ($LOAEL_{HEC}$ = LOAEL x RGDR); thus, the $LOAEL_{HEC}$ is 900 ppm. The $LOAEL_{HEC}$ of 900 ppm (900,000 ppb; 2,700,000 µg/m^3) is then used as the ^{acute}IOAEL.

The margin of exposure between the estimated ^{acute}IOAEL (900,000 ppb) and the acute 1-h ReV (5,000 ppb) for TBA is a factor of 180.

3.1.4 Health-Based Acute 24-h ReV

TBA is not one of the chemicals evaluated in the TCEQ ambient air monitoring network; therefore, the TD did not derive a 24-h ReV.

3.2 Welfare-Based Acute Evaluation

3.2.1 Odor Perception

TBA has a camphor-like odor (USEPA 2021). The acute odor-based ESL ($^{acute}ESL_{odor}$) for TBA, using an evidence-integration approach as described in the *Approaches to Derive Odor-Based Values* (TCEQ 2015b), is 14,000 µg/m^3 (4,600 ppb).

3.2.2 Vegetation Effects

There are no relevant data on the potential adverse acute effects of TBA on plants or grain, so an acute vegetation-based ESL ($^{acute}ESL_{veg}$) was not derived.

3.3 Summary of the Acute Values

The acute evaluation resulted in the derivation of the following values:

- Acute 1-h ReV = 5,000 ppb (15,000 µg/m^3)
- ^{acute}ESL [1 h] = 1,500 ppb (4,500 µg/m^3)
- $^{acute}ESL_{odor}$ = 4,600 ppb (14,000 µg/m^3)

Although TBA is not currently analyzed for in the TCEQ ambient air monitoring network, the $^{acute}ESL_{odor}$ and acute 1-h ReV will be used to evaluate any 1-h monitoring data for TBA in the future. The health-based $^{acute}ESL$ will be used as the 1-h ESL for air permitting (Table 1).

The $^{acute}ESL$ (HQ = 0.3) is not used to evaluate ambient air monitoring data and will be used in air permitting applications.

Chapter 4 Chronic Evaluation

4.1 Noncarcinogenic Potential

Per TCEQ guidelines (Section 5.1, TCEQ 2015), when a toxicity factor or guideline air level is identified in the scientific literature or databases, it is reviewed to determine whether the approaches used to develop the toxicity factor or guideline level are the same or similar to the procedures that would be used by the TCEQ for the given chemical dose-response assessment. The TCEQ's scientific literature search identified USEPA (2021a,b) as having a recent noncarcinogenic dose-response assessment for TBA for consideration under TCEQ guidelines (TCEQ 2015a). Consequently, the TCEQ reviewed the noncarcinogenic dose-response assessment in USEPA (2021a,b) for potential adoption under TCEQ guidelines (TCEQ 2015a). Ultimately, the TD decided to use the USEPA's POD_{HEC} values and adopt the candidate RfC associated with the lowest POD_{HEC} (i.e., BMD_{HEC} or $LOAEL_{HEC}$) as the chronic ReV as per TCEQ guidelines (TCEQ 2015a). As noted in Section 3.10 of the *TCEQ Guidelines to Develop Toxicity Factors* (TCEQ 2015a), dose-response assessments for each potential critical health effect are performed with selection of the critical effect associated with the lowest human equivalent concentration (HEC) or dose (HED). Note that the chronic ReV adopted by TCEQ differs from the RfC adopted by USEPA because USEPA did not utilize the lowest POD_{HEC} when deriving their final RfC. The TCEQ considers selection of the critical effect as that associated with the lowest POD_{HEC} (i.e., BMD_{HEC} or $LOAEL_{HEC}$) as more consistent with the critical effect as the first adverse effect that occurs as the dose rate increases, as opposed to allowing the greater uncertainty associated with a given candidate critical effect to override the dose-response data (and relevant toxicokinetic adjustments) that indicate which effect may be expected to occur first in humans as the dose rate increases. In the sections that follow, the TCEQ provides the information relevant to documentation of the derivation of the chronic ReV for TBA (e.g., key and supporting studies, candidate critical endpoints and PODs, human relevance considerations, oral route-to- inhalation route extrapolations, application of UFs) based on adoption of the candidate RfC associated with the lowest POD_{HEC} (i.e., BMD_{HEC} or $LOAEL_{HEC}$) in USEPA (2021a).

4.1.1 Key and Supporting Studies

4.1.1.1 Human Studies

No chronic inhalation studies in humans were available.

4.1.1.2 Animal Studies

No chronic inhalation studies were identified for derivation of the RfC. Subchronic inhalation studies were conducted in mice and rats (NTP 1997), and chronic oral studies of TBA offered in drinking water were conducted in mice and rats (NTP 1995). As mentioned above, the following provides documentation primarily based on the RfC derived in USEPA (2021a) utilizing rat study results and adopted by the TCEQ here. Specifically, the USEPA (2021a) identified the kidney findings in female rats as the critical effect.

4.1.1.2.1 NTP (1997) Subchronic Inhalation Toxicity Study in Rats

In a GLP-compliant subchronic study, 10 F344/N rats/sex/group (7 weeks old, Taconic Laboratory and Animal Services, Germantown, NY) were exposed by inhalation to nominal concentrations of 0, 135, 270, 540, 1080 or 2100 ppm TBA for 6 h/day, 5 days/week for 13 weeks. The average TBA concentrations were 134, 272, 542, 1080 and 2101 ppm. Animals were observed twice daily for morbidity and mortality. Clinical observations were recorded weekly and animals were weighed 6 days prior to exposure, weekly thereafter, and at the end of the study. Blood was collected for hematology and clinical chemistry evaluations on Day 22 and at the end of the study. Urinalysis (volume, pH, specific gravity and microscopic examination of sediment) was conducted on samples collected for a 12-h period on Day 21 and during Week 12. Animals were necropsied and organs (brain, right epididymis, heart, right kidney, liver, lung, right testis, and thymus) were weighed. Complete histopathologic evaluation was performed on all mice in the 0 and 2100 ppm groups, and gross lesions were evaluated in the remaining groups. Kidneys of all male rats in the remaining exposure groups also were microscopically examined.

There were no TBA-related deaths; one male in the 135 ppm group died accidentally during blood collection on Day 22. There were no TBA-related differences in body weights and body weight gains. There were single observations of emaciation and hypoactivity in females in the 2100 ppm group. Minimally decreased erythrocytic parameters (erythrocytes, hemoglobin and/or hematocrit) were seen in males in all TBA groups. In comparison to concurrent control values, the magnitude of the differences in erythrocytic parameters was no more than 5.7%. On Days 22 and/or Week 13, minimal or mild decreases in serum alkaline phosphatase activity were noted in males exposed to ≥ 540 ppm. At Week 13, urine pH was minimally decreased in females in the 1080 ppm group and in males and females in the 2100 ppm group. Absolute and relative right kidney weights were increased (up to 11% in comparison to controls) in males at ≥ 1080 ppm. Minimal or mild chronic progressive nephropathy (CPN) was seen in most or all

males in all groups, including the control group. The increased kidney weights in TBA-exposed males correlated with a greater severity of CPN, and the mean severity of CPN increased in an exposure-related manner. In females exposed to 2100 ppm, the relative right kidney weight was increased (8.8%) relative to controls. Relative liver weights of females at ≥ 1080 ppm were slightly greater (up to 8.8%) than controls. There were no TBA-related gross findings in males and females, and no TBA-related microscopic findings in females.

The hematologic, clinical chemistry and urinalysis findings observed were minimal or mild and did not have histopathologic correlates. In females the minimal statistically significant differences in relative liver weight at ≥ 1080 ppm and of relative kidney weight at 2100 ppm had no histopathologic correlates, and there were no statistically significant differences in the absolute weight of these organs in females.

The increased mean severity of CPN observed in TBA-exposed males may be considered adverse. Consequently, based on a slight increase in severity of CPN in males at all exposure concentrations, a NOAEL was not identified in males and the LOAEL in males was the lowest exposure concentration of 135 ppm. However, there is conflicting evidence regarding the relevance of CPN to humans, which is further discussed in Appendix 2 TCEQ Position Paper on Human Relevance of Chronic Progressive Nephropathy Observed in Rats. Moreover, in most studies male rats also had histopathologic findings of α2u-globulin nephropathy, a finding which is limited to male rats and is not relevant to humans. Therefore, USEPA did not use male rat kidney findings to derive reference values (USEPA 2021a). See Section 4.1.2 Selection of the Key Study and Critical Effect for further discussion.

USEPA considered increased absolute kidney weight, but not increased relative kidney weight, in female rats an adverse finding. The NOAEL for increased absolute kidney weight in females was the highest exposure concentration of 2100 ppm in this subchronic inhalation study. As stated in (USEPA 2021a), 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, although the potential impact of both should be evaluated. However, for TBA, body weight in exposed rats was 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 (refer to USEPA 2021a Section 1.2.1).

4.1.1.2.2 NTP (1995) Chronic Oral (Drinking Water) Toxicity Study in Rats

In a chronic study, 60 male F344/N rats/group were offered 0, 1.25, 2.5 or 5 mg/mL TBA in drinking water and 60 female F344/N rats/group were offered 0, 2.5, 5 or 10 mg/mL TBA in drinking water for 2 years (103 weeks). Rats were obtained from Taconic Laboratory Animals and Services (Germantown, NY), and were 7 weeks old at initiation of dosing. Periodic analysis

(approximately every 8 weeks) of the dose formulations showed that the formulations were within 10% of target concentrations. In the TBA groups, the average daily doses for males were 90, 200 or 420 mg/kg-day and for females were 180, 330 or 650 mg/kg-day. Animals were observed twice daily for morbidity and mortality. Clinical findings were recorded weekly for the first 13 weeks, then every 4 weeks, at 15 months and at the end of the study. Water consumption was recorded every 4 weeks. Ten rats/sex/group were necropsied after 15 months of dosing; prior to the interim sacrifice these rats also had hematologic and urinalysis (volume, specific gravity, pH, microscopic examination of sediment) evaluations performed. At the 15-month interim sacrifice, brain, right kidney and liver were weighed. A complete necropsy and microscopic examination were performed on all rats. Additional kidney sections (step sections) were evaluated.

Survival was significantly lower in males and females in the 5 and 10 mg/mL groups, respectively. At Week 20, body weight gain of males in the 5 mg/mL group was lower than controls; final mean body weight was 24% lower than controls. After Week 29, mean body weight gain of females in the 10 mg/mL group was lower than controls; final mean body weight was 21% lower than controls. Although not mentioned in the NTP report, mean body weights of males in the 1.25 and 5 mg/mL groups were 15% and 18% lower, respectively, than controls at the end of the study. During the second year of the study a dose-related increase in water consumption occurred in males, and a dose-related decrease in water consumption occurred in females. There was an increased incidence of hyperactivity in females in the 10 mg/mL group. At the 15-month interim evaluation, there were no hematology findings in males and females, and no urinalysis findings in males. However, at 15 months females in the 5 and 10 mg/mL groups had decreased urine volumes and increased specific gravity, which was consistent with decreased water consumption.

At the 15-month interim sacrifice, increased absolute and/or relative kidney weights were seen in females in all TBA groups, and in males in the 2.5 and 5 mg/mL groups. More specifically, in female rats the absolute and relative kidney weights were statistically significantly increased at all TBA doses, and the dose-responses were monotonic as well (see Table F2 of NTP 1995). Mineralization, a component of CPN, was increased in males in the 2.5 and 5 mg/mL groups. CPN was seen in all rats, including controls.

At the end of the study, the incidence of focal renal tubule hyperplasia was statistically significantly increased in males in the 5 mg/mL group. In males only, increased incidences of linear papillary mineralization, a lesion associated with α2u-globulin nephropathy, was seen in all TBA groups. Additional examinations of kidney section slides were performed by pathology working groups (a blinded review described in Hard et al. 2011, and an unblinded review reported in Hard et al. 2019) and confirmed that the finding of linear papillary mineralization was consistent with α2u-globulin nephropathy. Additionally, findings of hyaline droplet

accumulation, granular casts, and increased mitoses in the renal cortex, which are consistent with earlier stages of α2u-globulin nephropathy, were observed in males only in a subchronic oral (drinking water) study in rats (NTP 1995, Hard et al. 2011, Hard et al. 2019). Alpha2u-globulin nephropathy is a male rat-specific finding and is not relevant to humans (USEPA 1991, Capen et al. 1999). Therefore, findings consistent with α2u-globulin nephropathy in male rats are not appropriate for derivation of a RfC or chronic ReV and were not utilized by USEPA (2021a) and TCEQ.

The incidence of CPN was similar across all groups, but the mean severity was statistically significantly increased in males at 5 mg/mL and females in all TBA groups. Incidences of lesions associated with nephropathy (mineralization in males at 5 mg/mL, transitional epithelial hyperplasia in males at 2.5 and 5 mg/mL and in females at 10 mg/mL, suppurative inflammation in females at 5 and 10 mg/mL) were statistically significantly increased. There is conflicting evidence regarding the human relevance of exacerbation of CPN observed in rats. Again, this is further discussed in Appendix 2 TCEQ Position Paper on Human Relevance of Chronic Progressive Nephropathy Observed in Rats.

A NOAEL was not identified based on the increased severity of CPN in females. Therefore, the LOAEL in females was the low dose of 180 mg/kg-day. Findings consistent with α2u-globulin nephropathy were seen in males at all doses and are adverse but not relevant to humans (USEPA 1991, Capen et al. 1999). However, adverse decreases in mean body weights were observed at all doses in males (≥ 90 mg/kg-day) at the end of the study, along with increased incidences of findings consistent with CPN at doses of 200 and 420 mg/kg-day.

4.1.1.3 Reproductive and Developmental Studies

A summary of the available reproductive and developmental inhalation studies is provided in Section 3.1.1.3 Reproductive and Developmental Studies. In GLP-compliant 13-week inhalation studies (NTP 1997), there were no TBA-related effects on reproductive endpoints in male and female mice and rats. Reproductive endpoints evaluated were weights of the right epididymis, right cauda and right testis; histopathologic evaluation of the prostate gland, testis (with epididymis and seminal vesicle), and uterus; sperm evaluations (density, morphology and motility); and vaginal cytology evaluation (estrous cycle length, duration in various estrous stages). The NOAEL for effects on the reproductive tract was 2100 ppm for male and female mice and rats.

One embryo/fetal development study and one postnatal development study via the inhalation route are available (Nelson et al. 1989, Nelson et al. 1991). NOAELs for maternal and fetal toxicity were not identified in the embryo/fetal development study (Nelson et al. 1989). The LOAEL for maternal animals was 2000 ppm based on clinical observations of unsteady gait, and the LOAEL for fetal toxicity was 2000 ppm based on reduced body weights of males and

females, which may be due to maternal toxicity rather than a direct effect of TBA. In the postnatal development study (Nelson et al. 1991) the authors concluded that "the small number of statistically significant behavioral and neurochemical effects did not provide evidence of a dose-effect relationship or discernible pattern of effects" and that "the few effects were likely of little or no biological significance". Moreover, the exposures to the two concentrations of TBA evaluated (2000 and 4000 ppm) were not run concurrently thereby making comparisons between the exposure concentrations inappropriate, a dose response was not consistently evident for the findings, and a NOAEL was not identified for postnatal development.

The LOAELs from the prenatal and postnatal developmental studies discussed above (≥ 2000 ppm) are higher than those from the subchronic inhalation and chronic oral studies in rats discussed in Section 4.1.1.2.1 NTP (1997) Subchronic Inhalation Toxicity Study in Rats and Section 4.1.1.2.2 NTP (1995) Chronic Oral (Drinking Water) Toxicity Study in Rats, respectively, as is the NOAEL (2100 ppm) for reproductive toxicity. Additionally, USEPA (2021a) indicated that although minimal effects were observed at otherwise toxic dose levels, the available evidence is considered insufficient to identify developmental, including neurodevelopmental, effects or reproductive effects as a potential human health hazard of TBA exposure (see Sections 1.2.3, 1.2.4, and 1.2.5 of USEPA 2021a).

4.1.2 Selection of the Key Study and Critical Effect

The 13-week inhalation study in rats (NTP 1997) and 2-year oral (drinking water) study in rats (NTP 1995) were selected as potential key studies.

As noted in Section 4.1 Noncarcinogenic Potential, the TCEQ reviewed the noncarcinogenic dose-response assessment in USEPA (2021a,b) for potential adoption under TCEQ guidelines (TCEQ 2015a). The TD decided to use the USEPA's POD_{HEC} values and adopt the candidate RfC associated with the lowest POD_{HEC} (i.e., BMD_{HEC} or $LOAEL_{HEC}$) as the chronic ReV as per TCEQ guidelines (TCEQ 2015a). As noted in Section 3.10 of the *TCEQ Guidelines to Develop Toxicity Factors* (TCEQ 2015a), dose-response assessments for each potential critical health effect are performed with selection of the critical effect associated with the lowest HEC (i.e., BMD_{HEC} or $LOAEL_{HEC}$). Based on the dose-response data (and relevant toxicokinetic adjustments), this is indicative of the first effect that may be expected to occur in humans as the dose rate increases. Note that the chronic ReV adopted by TCEQ differs from the RfC adopted by USEPA because USEPA did not utilize the lowest POD_{HEC} when deriving their final RfC. Route-to-route extrapolation (oral route to inhalation route) from the RfD to the RfC was performed by USEPA using a physiologically based pharmacokinetic (PBPK) model.

USEPA identified kidney effects as a potential human hazard of TBA-induced toxicity based on findings in female rats. Because kidney findings in male rats are complicated by α2u-globulin

nephropathy, which is not relevant to humans, male kidney findings were not considered in deriving reference values. Although there is disagreement among scientists regarding the relevance of CPN to humans, USEPA did consider this finding potentially relevant to human risk when observed in female rats. Therefore, the findings of absolute kidney weight, findings consistent with CPN (kidney suppurative inflammation, kidney transitional epithelial hyperplasia), and increased severity of nephropathy in female rats in the 2-year oral (drinking water) study (NTP 1995) were chosen for dose-response analysis in derivation of the RfD, with subsequent route-to-route extrapolation using a PBPK model to derive the RfC. The study selected was of high quality. In light of the conflicting evidence regarding the relevance of CPN to humans (see Appendix 2), the use of female rat kidney endpoint data is a conservative choice to ensure the protection of public health in the face of uncertainty.

The models in USEPA's Benchmark Dose Software (BMDS) were applied. Consistent with USEPA's *Benchmark Dose Technical Guidance* (USEPA 2012a), the benchmark dose (BMD) and the BMDL (BMD 95% lower confidence limit at a specified response level) 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 endpoint BMDLs were used as candidate PODs. When modeling was not feasible, the study endpoint NOAEL or LOAEL was used as the candidate POD.

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. Note that absolute and/or relative kidney weights were increased in females at the 15-month sacrifice in the 2-year oral (drinking water) study. USEPA did not use relative kidney weight because the decrease in body weight observed at the 15-month interim sacrifice was believed to result in exaggerated kidney weight differences from control. For the increased severity of CPN, the lowest dose, associated with a statistically significant increase in average severity, was the POD for this endpoint.

The following sections delineate the potential PODs (renal findings in female rats) from the relevant studies (13-week inhalation study in rats, 2-year oral [drinking water] study in rats) that are included in USEPA (2021a).

4.1.3 MOA Analysis and Dose Metric

CPN is a common, spontaneous age-related background finding observed in chronic toxicity studies in rats (Hard and Khan 2004, Melnick et al. 2012). The MOA for CPN is incompletely understood and there is disagreement among scientists regarding the potential human relevance of exacerbation of CPN observed in rats (USEPA 2021a, also refer to Appendix 2 TCEQ Position Paper on Human Relevance of Chronic Progressive Nephropathy Observed in Rats). The renal findings in female rats were considered appropriate for derivation of a chronic ReV because: (1) the MOA for CPN is currently unknown; (2) there is no consensus that the exacerbation of CPN observed in rats is not relevant to humans; and (3) potential human relevance of the renal findings observed in female rats cannot be excluded.

Exposure concentrations are available for the 13-week inhalation study in rats (NTP 1997), and the oral doses (mg/kg-day) are available for the 2-year oral (drinking water) study in rats (NTP 1995). Because the RfC is an air exposure concentration, USEPA employed route-to-route extrapolation using a PBPK model was used to determine the RfC that would be equivalent to the RfD which was derived from the 2-year oral (drinking water) study in rats.

4.1.4 Adjustments to the POD

4.1.4.1 BMC and BMD Modeling

Consistent with USEPA's *Benchmark Dose Technical Guidance* (USEPA 2012), the benchmark concentration (BMC) and the 95% lower confidence limit on the BMC (BMCL) were estimated using a BMR of 10% change from the control mean for absolute kidney weights of females in the 13-week inhalation study (NTP 1997). Modeling was attempted, but as shown in Appendix C of the supplemental information (pp. C-12 to C-13 in USEPA 2021b), no model adequately fit the data. As noted in Section 4.1.1.2.1 NTP (1997) Subchronic Inhalation Toxicity Study in Rats, there were no statistically significant differences in absolute kidney weights of females in comparison to controls and at the highest exposure concentration, where the mean absolute kidney weight of females was increased by 3.9% relative to controls. Therefore, the NOAEL of 2100 ppm (6368 mg/m^3) for absolute kidney weight increase in female rats was selected by USEPA as a potential POD for the 13-week inhalation study (NTP 1997).

In the 2-year oral (drinking water) study (NTP 1995), the findings of absolute kidney weight, kidney suppurative inflammation, kidney transitional epithelial hyperplasia, and increased severity of nephropathy in female rats were chosen for dose-response analysis in derivation of the RfD. The models in USEPA's Benchmark Dose Software were applied. Consistent with USEPA's *Benchmark Dose Technical Guidance* (USEPA 2012) and as described above, the BMD and the BMDL are estimated using a BMR to represent a minimal, biologically significant level of change. When modeling was feasible, the estimated BMDLs were used as candidate PODs. When modeling was not feasible, the study NOAEL or LOAEL was used as the candidate POD.

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. Note that both absolute and relative kidney weights were increased in females at the 15-month sacrifice in the 2-year oral (drinking water) study (NTP 1995). USEPA did not use relative kidney weight because the decrease in body weight observed at the 15-month interim sacrifice was believed to result in exaggerated kidney weight differences from control.

The BMD modeling for the 2-year oral (drinking water) study in rats is shown in Appendix C of the IRIS document (USEPA 2021b). BMD modeling was used to derive PODs for increased absolute kidney weight at the 15-month interim sacrifice, suppurative inflammation and transitional epithelial hyperplasia in female rats. For the increased severity of CPN, the lowest dose, which was associated with a statistically significant increase in average severity, was the POD for this endpoint.

The potential inhalation exposure concentration and oral dose PODs considered for derivation of USEPA's RfC, adopted herein as candidate PODs for derivation of the TCEQ chronic ReV, are shown in Table 6.

Table 6. Candidate Points of Departure for Derivation of USEPA's RfC: Female Rat Kidney Findings

Kidney Endpoint	**Study**	**Model**	**P value**	**AIC**	**POD type**	**BMD_{10}**	**$BMDL_{10}$**	**POD**
↑ absolute weight	13-wk inhalation study (NTP 1997)	N/A	--	--	NOAEL	--	--	2100 ppm
↑ absolute weight at 15-month interim sacrifice	2-yr oral study (NTP 1995)	Exponential (M4) (constant variance)	0.176	-145.81	$BMDL_{10}$	164 mg/kg-d	91 mg/kg-d	91 mg/kg-d
Suppurative inflammation	2-yr oral study (NTP 1995)	Log-Probit	0.243	167.6	$BMDL_{10}$	254 mg/kg-d	200 mg/kg-d	200 mg/kg-d

Kidney Endpoint	Study	Model	P value	AIC	POD type	BMD_{10}	$BMDL_{10}$	POD
Transitional epithelial hyperplasia	2-yr oral study (NTP 1995)	Multistage (3 degree)	0.92	89.73	$BMDL_{10}$	412 mg/kg-d	339 mg/kg-d	339 mg/kg-d
↑ severity chronic progressive nephropathy	2-yr oral study (NTP 1995)	N/A	--	--	LOAEL	--	--	180 mg/kg-d

4.1.4.2 Default Exposure Duration Adjustments

For the 13-week inhalation study (NTP 1997), the NOAEL of 2100 ppm 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

POD_{ADJ} = POD (ppm) × (6 ÷ 24) × (5 ÷ 7)

= 2100 ppm × 0.1786 = 375 ppm (1137 mg/m^3)

For the 2-year oral (drinking water) study in rats (NTP 1995), where animals were offered water continuously throughout the day and night, no duration adjustment was necessary. Therefore, the POD_{ADJ} for the 2-year oral (drinking water) study is equivalent to the BMDL or LOAEL.

4.1.4.3 Default Dosimetry Adjustments from Animal-to-Human Exposure

The POD identified in the 13-week inhalation study was increased absolute kidney weight in females. TBA is rapidly absorbed and distributed throughout the body following inhalation exposure (USEPA 2021a). The increase in kidney weight is a systemic effect, therefore, TBA is considered a Category 3 gas (USEPA 2021a). For Category 3 gases, the default dosimetric adjustment from an animal concentration to a POD_{HEC} is calculated using the following equation:

POD_{HEC} = POD_{ADJ} x regional gas dose ratio (RGDR)

where: RGDR =$[(H_{b/g})_A / (H_{b/g})_H]$

$H_{b/g}$ = ratio of the blood:gas partition coefficient

A = animal

H = human

The measured blood/air partition coefficients in human ($(H_{b/g})_H$) and in the rat ($(H_{b/g})_A$ for TBA are 481 and 462, respectively, resulting in a RGDR of 1.04 (Borghoff et al. 1996, Nihlén et al. 1995). Because the ratio of the animal-to-human blood:gas partition coefficients (481/462 = 1.04) is greater than one, a default value of one typically is used as the regional gas dose ratio as recommended by TCEQ (see section 3.9.1 of the *TCEQ Guidelines to Develop Toxicity Factors* [TCEQ 2015a]). The USEPA also used a RGDR of one (Table 2-4 in USEPA 2021a). Therefore, the resulting POD_{HEC} from the POD_{ADJ} of 375 ppm is 375 ppm.

USEPA used a PBPK model for route-to-route extrapolation (oral route to inhalation route) of the PODs selected from the 2-year oral (drinking water) study (NTP 1995). A critical decision in the route-to-route extrapolation is selection of the internal dose metric that establishes "equivalent" oral and inhalation exposures. The internal dose metric was the blood concentration of TBA. Note that using the kidney concentration of TBA will lead to the same route-to-route extrapolation relationship as TBA in blood because the distribution from blood to kidney is independent of route. Moreover, without evidence suggesting otherwise, TBA is assumed to be the active toxicological agent. The PBPK model was used for the route-to-route extrapolation of the oral BMDLs or LOAEL to derive inhalation PODs. The POD_{HEC} values were then calculated by USEPA based on an RGDR of 1.04 (Table 2-5 in USEPA 2021a), because the effects seen at the PODs were systemic effects.

A PBPK model for TBA in rats has been modified, as described in Appendix B of USEPA (2021b). Using the PBPK model, route-to-route extrapolation of the oral BMDLs or LOAEL to derive inhalation PODs was 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." 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 resultant PODs were then converted to POD_{HEC} as follows.

As noted above, TBA is a Category 3 gas because extrarespiratory effects were observed. USEPA used the RGDR ratio of 1.04 was used to calculate the HEC as follows.

POD_{HEC} = POD (ppm) × (1.04)

Note that in the TCEQ guidelines (TCEQ 2015), the RGDR default value is one when the calculated RGDR value is greater than one. However, the procedure used by USEPA (2021a) is deemed sufficiently similar.

The POD_{HEC} values from the 2-year oral (drinking water) study (NTP 1995) are shown in Table 7 (refer to Table 2-5 of USEPA 2021a). Additionally, POD_{HEC} values using a RGDR value of one, as per TCEQ guidelines, also are shown in Table 7.

Table 7. Inhalation Points of Departure Derived from Route-to-Route Extrapolation of Oral Exposures in the 2-Year Oral (Drinking Water) Study in Rats (NTP 1995)

Kidney endpoint in female rats	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	POD (mg/kg-d) [a]	Internal Dose (mg/L) [b]	Equivalent POD (ppm) [c]	POD_{HEC} (ppm) [d]
↑ absolute weight at 15-month interim sacrifice	164 [e]	91	91	21.5	78.8	82.0
Suppurative inflammation	254	200	200	61.9	173	180
Transitional epithelial hyperplasia	412	339	339	127	292	304
↑ severity chronic progressive nephropathy	---	---	180 [f]	53.6	156	162

[a] POD that USEPA used for derivation of the RfD. For absolute kidney weight, suppurative inflammation and transitional epithelial hyperplasia, this refers to a $BMDL_{10}$. For severity of chronic progressive nephropathy, this is the LOAEL.
[b] Average rat blood concentration of TBA under circadian drinking water ingestion at the $BMDL_{10}$ or LOAEL
[c] Continuous inhalation equivalent concentration that leads to the same average blood concentration of TBA as circadian drinking water ingestion at the POD in the rat
[d] USEPA calculation of POD_{HEC} using a RGDR of 1.04
[e] The exposure concentration associated with the BMD_{10} (164 mg/kg-day) is 148 ppm as the ratio of POD_{HEC} values (ppm) to POD (mg/kg-d) values is 0.9 (i.e., 164 mg/kg-day × 0.9 oral-to-inhalation POD_{HEC} conversion factor = 148 ppm).
[f] LOAEL

As previously mentioned, under the *TCEQ Guidelines to Develop Toxicity Factors* (TCEQ 2015a), dose-response assessments for each potential critical health effect are performed and the effect associated with the lowest POD_{HEC} (i.e., BMD_{HEC} or $LOAEL_{HEC}$) is selected by TCEQ as the critical effect. The LOAEL-based POD_{HEC} in Table 7 for increased severity of CPN in female rats is 162 ppm, whereas the exposure concentration associated with BMD_{10} for increased absolute kidney weight is lower at 148 ppm. Thus, increased absolute kidney weight in female rats is the critical effect as determined under TCEQ guidelines and USEPA's POD_{HEC} of 82.0 ppm (based on the $BMDL_{HEC}$) for increased absolute kidney weight in the 2-year oral (drinking water) study will serve as the basis for TCEQ's chronic ReV. As shown Section 4.1.5 Adjustments to the PODHEC, importantly, this endpoint is also associated with less overall uncertainty.

4.1.5 Adjustments to the POD_{HEC}

USEPA calculated candidate RfC values for all PODs (refer to Table 2-6 in USEPA 2021a), as shown in Table 8.

Table 8. Candidate RfC Values Based on Kidney Findings in Female Rats

Kidney Endpoint	Study	POD_{HEC} (ppm)	POD type	UF_H	UF_A	UF_S	UF_L	UF_D	Composite UF	Candidate value (ppm)
↑ absolute weight at 15-month interim sacrifice	**2-yr oral study (NTP 1995)**	**82.0** [a]	**$BMCL_{10}$**	**10**	**3**	**1**	**1**	**1**	**30**	**2.7**
↑ absolute weight	13-wk inhalation study (NTP 1997)	375	NOAEL	10	3	10	1	1	300	1.2
Suppurative inflammation	2-yr oral study (NTP 1995)	180	$BMCL_{10}$	10	3	1	1	1	30	6.0

Kidney Endpoint	**Study**	**POD_{HEC} (ppm)**	**POD type**	**UF_H**	**UF_A**	**UF_S**	**UF_L**	**UF_D**	**Composite UF**	**Candidate value (ppm)**
Transitional epithelial hyperplasia	2-yr oral study (NTP 1995)	304	$BMCL_{10}$	10	3	1	1	1	30	10
↑ severity chronic progressive nephropathy	2-yr oral study (NTP 1995)	162	LOAEL	10	3	1	3	1	100	1.6

[a] POD_{HEC} for the derivation of TCEQ's chronic ReV, as the $BMD_{10\text{-}HEC}$ of 148 ppm for increased absolute kidney weight in female rats is lower than the $LOAEL_{HEC}$ of 162 ppm for increased severity of CPN. Therefore, increased absolute kidney weight in the 2-year oral (drinking water) study is identified as the critical effect under TCEQ guidelines (TCEQ 2015a).

The following uncertainty factors (UFs) were applied to the candidate $PODs_{HEC}$:

- A UF_H of 10 was used to account for variation in susceptibility among members of the human population;
- A UF_A 3 of for interspecies uncertainty because a default dosimetric adjustment was conducted to account for toxicokinetic differences between animals and humans;
- A UF_L of 3 for LOAEL to NOAEL extrapolation for the severity of nephropathy only. Not applied to the PODs derived from a NOAEL or benchmark dose modeling;
- A UF_{SUB} of 10 for subchronic to chronic uncertainty factor was used for the POD derived from the 13-week inhalation study only (NTP 1997); and
- A UF_D of 1 for database uncertainty.

In light of the conflicting evidence regarding the relevance of CPN to humans (see Appendix 2), the TCEQ's consideration of this and other POD_{HEC} values based on female rat kidney endpoints is a conservative choice to ensure the protection of public health (in the face of uncertainty) against the full spectrum of potential TBA-induced adverse effects. USEPA (2021a) selected the critical effect of the increase in severity of CPN in female rats in the 2-year oral (drinking water) study (NTP 1995) for derivation of their RfD, resulting in a RfC of 1.6 ppm. However, this candidate RfC was not appropriate for adoption by TCEQ for the chronic ReV because USEPA did not select the effect associated with the lowest POD_{HEC} (i.e., BMD_{HEC} or $LOAEL_{HEC}$) as the critical effect for derivation of the RfC, as per TCEQ guidelines (Section 3.10 in TCEQ 2015a). The

TCEQ considers selection of the critical effect as that associated with the lowest POD_{HEC} (i.e., BMD_{HEC} or $LOAEL_{HEC}$) as more consistent with the critical effect as the first adverse effect that occurs as the dose rate increases, as opposed to allowing the greater uncertainty associated with a given candidate critical effect such as the increase in severity of CPN to override the dose-response data (along with relevant toxicokinetic adjustments) that indicate which effect may be expected to occur first in humans as the dose rate increases. This is also consistent with recent USEPA guidance where sensitivity of the POD is a key factor for selecting the final toxicity value (USEPA 2022, p. 8-18).[a]

Therefore, TCEQ adopted the candidate RfC for increased absolute kidney weight (2.7 ppm) in female rats in the 2-year oral (drinking water) study (NTP 1995) as the chronic ReV.

$$\text{chronic ReV} = POD_{HEC} / (UF_H \times UF_A)$$

$$= 82.0 \text{ ppm} / (10 \times 3)$$
$$= 82.0 \text{ ppm} / 30$$
$$= 2.7 \text{ ppm}$$
$$= 2.7 \text{ ppm or } 2{,}700 \text{ ppb (rounded to two significant figures)}$$

Accordingly, the TCEQ chronic ReV is 2.7 ppm. Given the significant margin of exposure of 60 (i.e., POD_{HEC} of 162 ppm for increased severity of CPN in female rats/chronic ReV of 2.7 ppm), the chronic ReV is also considered adequately protective against the adverse endpoint of increased severity of CPN in female rats that might occur as a result of a human-relevant MOA(s). Furthermore, it is noted that USEPA's RfC of 1.6 ppm is in between TCEQ's chronic ReV value (2.7 ppm) and the TCEQ chronic ESL value (0.81 ppm) calculated in Section 4.1.6 Health-Based Chronic ReV and $^{chronic}ESL_{nonlinear(nc)}$.

4.1.6 Health-Based Chronic ReV and $^{chronic}ESL_{nonlinear(nc)}$

In deriving the chronic ReV, no numbers were rounded between equations until the ReV was calculated. The chronic ReV was rounded to two significant figures, and then used to calculate the $^{chronic}ESL_{threshold(nc)}$ using a target hazard quotient of 0.3.

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[a] Sensitivity of POD: Concerning the identification of the most sensitive outcome or toxicity value, USEPA (2022) notes that BMDs (not BMDLs) should be the starting point for evaluating relative sensitivity (p. 8-18), and the BMD_{10} for increased absolute kidney weight (164 mg/kg-d) in Table 7 is lower than the LOAEL for the increase in severity of CPN (180 mg/kg-d). Therefore, increased absolute kidney weight is the basis for the POD selected for derivation of the chronic ReV.

Table 9. Derivation of the Chronic ReV and $^{chronic}ESL_{threshold(nc)}$

Parameter	Summary
Study	2-year oral (drinking water) study in rats (NTP 1995)
Study Population	Female F344/N rats (60/group)
Study Quality	High
Exposure Method	Oral (drinking water) at concentrations of 0, 2.5, 5 and 10 mg/mL resulting in average daily doses of 0, 180, 330 and 650 mg/kg-day
Critical Effects	Increased absolute kidney weight in female rats at the 15-month interim sacrifice
Exposure Duration	103 weeks
$BMDL_{10}$	91 mg/kg-day
Internal dose	21.5 mg/L
Equivalent air concentration POD (oral route to inhalation route extrapolation using a PBPK model)	78.8 ppm
POD_{HEC}	82.0 ppm (calculated using a RGDR of 1.04 as per USEPA 2021a)
Total UFs	30
Interspecies UF	3
Intraspecies UF	10
Incomplete Database UF *Database Quality*	1 High
Chronic ReV (HQ = 1)	**2,700 ppb (8,200 µg/m^3)**
$^{chronic}ESL_{threshold(nc)}$ (HQ = 0.3)	**810 ppb (2,400 µg/m^3)**

4.1.7 Chronic Noncarcinogenic IOAEL

The exposure concentration associated with the BMD_{10} (164 mg/kg-day) for the critical effect of increased absolute kidney weights in female rats at the 15-month interim sacrifice in the 2-year oral (drinking water) study of TBA (NTP 1995) is 148 ppm, as the ratio of POD_{HEC} values (ppm) to POD (mg/kg-d) values is 0.9. This POD will be used for the ^{chronic}IOAEL.

Risk assessors, and the general public, often ask to have information on the levels in air where health effects would be expected to occur. So, when possible, the TCEQ provides chemical-specific observed adverse effects levels in DSDs (TCEQ 2015a). As the basis for development of IOAELs is limited to available data, future studies could possibly identify a lower POD for this purpose. The chronic IOAEL is provided for informational purposes only (TCEQ 2015a).

The margin of exposure between the estimated chronic IOAEL (150 ppm, equivalent to 150,000 ppb or 450,000 µg/m^3) and the chronic ReV (2,700 ppb or 8,200 µg/m^3) for TBA is a factor of 55.

4.2 Carcinogenic Potential

4.2.1 Carcinogenic Weight of Evidence and Hazard Assessment

TCEQ guidelines (TCEQ 2015a) indicate that TD generally performs carcinogenic dose-response assessments for chemicals considered "carcinogenic to humans" or "likely to be carcinogenic to humans" under the USEPA cancer guidelines (USEPA 2005). USEPA (2021a) indicated that relevant considerations under USEPA (2005) support the weight of evidence classification of *suggestive evidence of carcinogenic potential* for TBA (see Section 1.3.2 of USEPA 2021a).

No human data relevant to an evaluation of the carcinogenicity of TBA are available. USEPA (2021a) indicated that available laboratory animal study results for TBA raise a concern for cancer but none of the effects is particularly strong. The thyroid tumors observed in male and female mice were almost entirely benign. The kidney tumors resulted, at least in part, from an MOA that is specific to male rats, while no kidney tumors occurred in female rats. Additionally, exacerbation of CPN, which may not be relevant to humans, has been proposed as an MOA for the development of renal tubule tumors in rats, calling into question the human relevance of these tumors (Hard et al. 2013, p. 270).

TBA is a metabolite of ETBE and MTBE. Some of the toxicological effects observed for these compounds are attributed to TBA. In addition, while MTBE was also associated with male rat kidney tumorigenesis, results between TBA- and ETBE-associated tumorigenesis in rats have little coherence (USEPA 2021c). 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 TBA exposure in B6C3F1 mice. These considerations, particularly that none of the tumor responses were strong or coherent with the results for ETBE, supported USEPA's classification of *suggestive evidence of carcinogenic potential* for TBA.

Because TCEQ concurs with USEPA (2021a) that the carcinogenic weight of evidence best supports the *suggestive evidence of carcinogenic potential* descriptor for TBA, consistent with

TCEQ guidelines (TCEQ 2015a), the TD will not perform or adopt a carcinogenic dose-response assessment for TBA.

4.3 Welfare-Based Chronic Evaluation

No data were found regarding adverse effects observed in plants due to chronic air exposure to TBA. Therefore, no chronic vegetation-based ESL ($^{chronic}ESL_{veg}$) was derived.

4.4 Summary of the Chronic Values

The chronic evaluation resulted in the derivation of the following values:

- Chronic ReV = 8,200 µg/m^3 (2,700 ppb)
- $^{chronic}ESL_{threshold(nc)}$ = 2,400 µg/m^3 (810 ppb)

Although TBA is not currently analyzed for in the TCEQ ambient air monitoring network, the chronic ReV of 8,200 µg/m^3 is the critical long-term health-based AMCV for the evaluation of any future long-term ambient air data (Table 2). The long-term ESL for air permit reviews is the $^{chronic}ESL_{threshold(nc)}$ of 2,400 µg/m^3.

Chapter 5 Uncertainty in Derivation of Acute and Chronic Toxicity Factors

There are several uncertainties associated with the derivation of toxicity factors, particularly those derived from toxicity data in laboratory animals. Consequently, the application of several UFs is considered in the derivation of toxicity factors. The following UFs were utilized in the derivation of the acute ReV based on CNS depression in rats exposed via inhalation for 12 exposure days (NTP 1997):

- A UF_H to account for potential variation in susceptibility among members of the human population;
- A UF_A to account for potential toxicodynamic differences between animals and humans (dosimetric adjustments had already been made to account for interspecies toxicokinetic differences); and
- A UF_D for database uncertainty.

Although mice and rats were exposed via inhalation to the same target concentrations of TBA for 12 exposure days (NTP 1997, summarized in Table 4), mice had a NOAEL for CNS depression of 900 ppm, which is 2-fold higher than the NOAEL of 450 ppm observed in rats. In these studies, the rat was more sensitive to the CNS depressant effects of TBA. In each species, there were no sex-related differences in these effects. The same uncertainty factors would apply to

the NOAEL POD in mice and would result in an acute ReV that is 2-fold higher than that derived from the rat.

In addition to a UF_H and UF_A, two additional UFs were utilized in the derivation of candidate chronic ReVs based on kidney effects in female rats. These UFs were applied as follows for increased severity of CPN observed in NTP (1995) and increased absolute kidney weight observed in NTP (1995):

- A UF_L to account for extrapolation from the LOAEL to NOAEL for the severity of CPN, because the LOAEL was the POD; and
- A UF_{SUB} to account for subchronic to chronic extrapolation for increased absolute kidney weight in the 13-week inhalation study (NTP 1997).

Thus, a total of five areas of uncertainty were duly accounted for through selection and application of appropriate values for these UFs in the acute and chronic ReV derivations.

There are also other areas associated with uncertainty for this assessment, such as residual uncertainty associated with the PBPK modeling used for route-to-route extrapolation (i.e., oral-to-inhalation route) for the derivation of the chronic toxicity factors. Most importantly, which animal species or sex might be more comparable to humans in terms of the dose-response for chronic effects is unknown. Generally, rats were more susceptible than mice, and males more susceptible than females to TBA toxicity (USEPA 2021). Note that only male rats developed α2u-globulin nephropathy, a male rat-specific finding that is not relevant to humans. Therefore, renal toxicity data in male rats was not used in the derivation of toxicity factors. There is also uncertainty associated with the assumption that the MOA causing exacerbation of CPN in rats may be relevant to a disease process in humans (Appendix 2). The Science Advisory Board that reviewed the draft USEPA IRIS assessment for TBA (USEPA 2017) was unable to reach a consensus regarding whether the exacerbation of CPN observed in rats exposed to ETBE or TBA could be attributed to a biological process not relevant to humans. However, because scientific justification frequently cannot be provided as to the best animal model for prediction of adverse effects in humans, as a matter of policy (in the interest of the protection of public health), the most sensitive adverse effect(s) in the most sensitive laboratory animal species (and sex) is typically used for toxicity factor derivation by regulatory agencies in the absence of information that shows that the given species, MOA and/or endpoint is not relevant to humans. At the same time, it should be recognized that use of the most sensitive laboratory animal species can be a large and key area of uncertainty (e.g., where significant interspecies differences in sensitivity exist in the absence of data to inform identification of the most human-relevant laboratory animal species).

In the present case, laboratory animal interspecies differences in kidney toxicity appear to be significant. For example, in contrast to results for rats in the key study for derivation of the

chronic ReV (NTP 1995), for mice, study authors indicated no treatment-related changes in kidney-related histopathology. As a specific example relevant to the chronic ReV critical effect, in the 13-week oral (drinking water) studies, increased absolute kidney weight only occurred at the highest dose (11,620 mg/kg-d) in female mice, whereas all doses (≥ 290 mg/kg-d) resulted in increased absolute kidney weight in female rats (NTP 1995, summarized in A.3 Data Extraction). This suggests an approximately 39-fold interspecies (rat versus mouse) difference in sensitivity to this effect after 13 weeks of oral dosing based on the tested doses. The 13-week female mouse NOAEL for increased kidney weight (6,430 mg/kg-d) is approximately 71-fold higher than the chronic POD ($BMDL_{10}$) in female rats (91 mg/kg-d; Table 7, data from 15-month interim sacrifice in the 2-year oral [drinking water] study) and would still be around an order of magnitude higher after adjustment to a chronic scenario through use of a UF_{SUB} (value of 3-10). Thus, where there are significant interspecies differences in susceptibility to a chemical's toxicity, in the absence of data to inform identification of the most human-relevant laboratory animal species, selection of the laboratory animal species to represent the dose-response in humans can have important implications for derivation of a toxicity factor based on a given critical effect (e.g., the magnitude of the value itself and that of the associated level of uncertainty). It is not known which laboratory animal species (or sex) might be more comparable to humans in terms of the dose-response for TBA-related effects on the kidney.

Chapter 6 References

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Appendix 1 Systematic Evidence Map

A.1 Problem Formulation and Protocol

Problem formulation identifies and defines the causal questions and describes the extent of the evaluation. These questions structured the systematic review for *tert*-butyl alcohol:

- What are the physical and chemical properties of *tert*-butyl alcohol?
- What is/are the critical effect(s) following exposure to *tert*-butyl alcohol?
- Are there potentially sensitive subpopulations?
- What is the mode of action (MOA)?
- Does route of exposure play a role in toxicity?
- Is *tert*-butyl alcohol a reproductive or developmental toxicant?
- Is *tert*-butyl alcohol carcinogenic, and if so, is it only carcinogenic by a specific route of exposure?
- Does *tert*-butyl alcohol exposure result in vegetative toxicity?

Protocol development is another important aspect in the initial process. A protocol is typically developed around a PECO statement: Populations, Exposure, Comparator/Control, and Outcomes. These identifiers are used to lay out the framework for the literature search and inclusion/exclusion criteria. The PECO statement for *tert*-butyl alcohol followed these criteria:

Table 10. PECO Statement Used by the TCEQ to Develop Toxicity Factors for *tert*-Butyl Alcohol

Population	Any human population and any relevant sensitive subpopulations, mammalian animal species, and vegetation
Exposure	Inhalation exposure to *tert*-butyl alcohol, surrogates with demonstrated similar MOAs, and any identified metabolites (oral route studies may be tracked during screening as potentially relevant)
Comparator/Control	Populations not exposed or exposed to concentrations below the concentration that causes the most sensitive critical effect
Outcome(s)	The most sensitive critical effect directly related to *tert*-butyl alcohol exposure

The protocol used for the systematic review and the development of toxicity factors for *tert*-butyl alcohol is as follows:

1. Identify the chemical of interest and define the causal questions
2. Conduct a systematic review for the dose-response assessment
    a. Conduct a systematic literature search
    b. Identify the inclusion/exclusion criteria
    c. Extract the relevant data from each data stream (human, animal, mechanistic)
    d. Assess the study quality and conduct a risk of bias analysis

   e. Weigh the evidence in each data stream and then integrate the evidence across the data streams
3. Derive toxicity factors (TCEQ 2015a)
   a. Review the essential data, including chemical/physical properties and selected key studies from the systematic review
   b. Conduct MOA analysis
   c. Choose the appropriate dose metric considering toxicokinetics and MOA
   d. Select critical effect, based on human equivalent exposure considering each key study
   e. Extrapolate from the adjusted POD to lower exposures based on MOA analysis
4. Rate the confidence in the evaluation

A.2 Systematic Literature Review and Study Selection

As a first step, publically available databases were searched using explicitly stated search criteria. Please see TCEQ (2015a) for a list of available databases that were searched. The search terms used in literature review for *tert*-butyl alcohol, along with the number of results from PubMed, are found in Table 11. Additional references were also identified using the reference sections from some of the selected studies. This literature review was conducted on August 26, 2020, and therefore studies published after this date were not available at the time of the initial review.

Table 11. Search Strings Used in the Literature Review of *tert*-Butyl Alcohol

Search Term/String	PubMed Results
"tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol"	1,027
"tertiary butanol" OR "tert-butanol" OR "t-butanol"	1,021
TBA	4,726
75-65-0	470
2-methyl-2-propanol	1,272
2-methylpropan-2-ol OR 2-methyl-propan-2-ol	33
"trimethyl carbinol"	1
"1,1-dimethylethanol"	0
"trimethyl methanol"	0
NCI-C55367	0
"t-butyl hydroxide"	4
"tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol" OR "tertiary butanol" OR "tert-butanol" OR "t-butanol"	1,858

Search Term/String	PubMed Results
"tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol" OR "tertiary butanol" OR "tert-butanol" OR "t-butanol" OR "75-65-0"	1,858
"tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol" OR "tertiary butanol" OR "tert-butanol" OR "t-butanol" OR "75-65-0" OR "2-methyl-2-propanol"	1,921
"tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol" OR "tertiary butanol" OR "tert-butanol" OR "t-butanol" OR "75-65-0" OR "2-methyl-2-propanol" OR "2-methylpropan-2-ol" OR "2-methyl-propan-2-ol"	1,945
"tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol" OR "tertiary butanol" OR "tert-butanol" OR "t-butanol" OR "75-65-0" OR "2-methyl-2-propanol" OR "2-methylpropan-2-ol" OR "2-methyl-propan-2-ol" OR "trimethyl carbinol"	1,945
"tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol" OR "tertiary butanol" OR "tert-butanol" OR "t-butanol" OR "75-65-0" OR "2-methyl-2-propanol" OR "2-methylpropan-2-ol" OR "2-methyl-propan-2-ol" OR "trimethyl carbinol" OR "t-butyl hydroxide"	1,948

The search term "TBA" resulted in inclusion of studies unrelated to *tert*-butyl alcohol (e.g., thiobarbituric acid, thrombin binding aptamer, tetrabutylammonium, total bile acids, transluminal balloon angioplasty, traditional birth attendants); and, therefore, "TBA" was not used as a search term for this systematic review. The 1,948 studies using the search terms in the last row were imported into the desktop application SWIFT-Review by Sciome and briefly searched to ensure that the key studies used in several other reviews were present in the data set. The data set was further narrowed down using the tag levels created by the SWIFT-Review software. The tags used and the number of studies with certain tagged studies removed are shown in Table 12.

Table 12. SWIFT-Review Tags and Results

Data Set/Tag	Number of Studies
PubMed Search: "tertiary butyl alcohol" OR "tert-butyl alcohol" OR "t-butyl alcohol" OR "tertiary butanol" OR "tert-butanol" OR "t-butanol" OR "75-65-0" OR "2-methyl-2-propanol" OR "2-methylpropan-2-ol" OR "2-methyl-propan-2-ol" OR "trimethyl carbinol" OR "t-butyl hydroxide"	1,948
Tag – Evidence stream, excluded studies with no tag, environmental fate (beta) and physical chemistry (beta)	1,018

Additionally, several governmental and private sector organizations were searched for published literature and toxicity values for *tert*-butyl alcohol (Table 13), and the available documents along with their relevant references were added to the pool of selected material as needed.

Table 13. Available Reviews and Inhalation Toxicity Values for *tert*-Butyl Alcohol

Organization	Year	Toxicity Value
US EPA External Review Draft: Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol) (CAS No. 75-65-0)	2017 (final 2021)	RfC = 5 mg/m^3

The final USEPA assessment of *tert*-butyl alcohol (USEPA 2021a,b) also was used as a reference; the RfC of 5 mg/m^3 is the same as that proposed in the draft assessment (USEPA 2017).

NOTE: NSF International and the American Petroleum Institute each derived RfD (oral toxicity factors) of 1000 µg/kg-day and 220 µg/kg-day, respectively, for *tert*-butyl alcohol. USEPA (IRIS) has a RfD of 400 µg/kg-day. The International Council for Harmonization has a permitted daily exposure of 35 mg/day (i.e., 500 µg/kg-day for 70 kg human).

In SWIFT Review duplicate results were eliminated prior to screening in SWIFT Screener. This resulted in a total of 1,016 studies for screening. Following this initial review, specific inclusion and exclusion criteria were used to narrow down the pool of available data. The criteria along with examples of the kinds of studies that were excluded are shown in Table 14.

Based on review of the USEPA External Review Draft: Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol), the following references were included:

Nelson, BK, WS Brightwell, A Khan, PB Shaw, EF Krieg, Jr, VJ Massari. 1991. Behavioral teratology investigation of *tertiary*-butanol administered by inhalation to rats. Pharmacopsychoecologia. 4:1-7.

Huntingdon Life Sciences (East Millstone, NJ). 2004. Reproductive and developmental toxicity screening test in rats by oral gavage. Lyondell Chemical Company. Report No. 03-4254.

The Nelson et al. 1991 study is included (not found on PubMed) as it was listed as one of the studies in the summary of experimental animal database.

The Huntingdon Life Sciences 2004 study also is listed in the summary of experimental animal database.

The Borghoff et al. 2016 reference was retained as a supplementary study, as it was used by US EPA in the derivation of the RfC. This reference describes a PBPK model used for route-to-route extrapolation from the POD of an oral study.

Borghoff, SJ, C Ring, MI Banton, TL Leavens. 2016. Physiologically based pharmacokinetic model for ethyl *tertiary*-butyl ether and *tertiary*-butyl alcohol in rats: Contribution of binding to α2u-

globulin in male rats and high-exposure nonlinear kinetics to toxicity and cancer outcomes. J Appl Toxicol. 37:621-640.

Table 14. Inclusion/exclusion criteria used in the review of *tert*-butyl alcohol

Study Type	Inclusion Criteria	Exclusion Criteria
General	Complete study available for review	- Only abstract is available - Study in a language other than English - Unpublished report/unable to retrieve
	Study contains original data or utilizes existing data in a novel way	- Study is a review article or meta-analysis - Study comments on a previous method without providing a sufficient alternative - Reference is not the source document, but presents some draft data from the source document prior to its finalization - Reference is a not the source document, but is a commentary on the study
	Exposure concentration (inhalation studies) or dose (oral studies) is known or can be reasonably estimated	- Exposure concentration unknown - Dose unknown/cannot be estimated for oral studies - Exposure environment/conditions unsuitable to concentration estimation
	Study examines effects related to chemical exposure	- Study only measures concentration in air, factories, etc. - Study does not examine health effects
	Study focused on the chemical of concern	- Study examined mixture effects - Study on treatment following *tert*-butyl alcohol exposure
	Route of exposure is relevant to exposure and toxicity factor development [a]	- Exposure through intravenous, intraperitoneal, or subcutaneous injection - Study examining dermal exposure
Animal	Relevant animal model and endpoints examined	- Study used non-mammalian animal models - All endpoints studied not relevant to human health - All endpoints not applicable to toxicity factor development
	Appropriate study populations and methods were used	- Study lacked appropriate numbers or doses - Exposure method unsuitable for dose-response
Human/Epi	Relevant endpoints examined	- Study focused solely on biomarkers or cytogenetic changes
	Study populations allowed for significant findings and follow ups	- Case studies examining single high-dose exposures - Studies without appropriate follow-up studies - Historical studies that have been updated

a: Oral studies were included because the USEPA used route-to-route extrapolation (oral to inhalation) in the derivation of the RfC (US EPA External Review Draft: Toxicological Review of *tert*-Butyl Alcohol [*tert*-Butanol]).
epi – epidemiology

Studies were then divided into groups by route of exposure (i.e., inhalation, oral) and study type/duration (i.e., general toxicology: acute, chronic non-carcinogenic, carcinogenic; developmental toxicity; developmental and reproductive toxicity; physical dependence; mode of action). No relevant human studies were available.

After full text review and screening with the inclusion/exclusion criteria listed above, 16 references describing animal studies were identified for further use in this systematic review. Of these 16 references, 4 used inhalation as the route of exposure, and 12 used oral (either via offering in drinking water or via oral gavage) as the route of exposure. Both NTP references (NTP 1995, NTP 1997) each comprised 4 studies (2 mouse and 2 rat studies in each NTP report).

A total of 14 plant and animal studies was reviewed and excluded for various reasons (see Table 15). Three studies in plants or seeds were screened and excluded as these studies were inadequate for derivation of a vegetation-based reference value.

Table 15. Excluded plant and animal studies

Reason for Exclusion	Study	
Reference is not the source document, but presents some draft data from the source document prior to its finalization	Cirvello et. al. 1995 Lindamood III et al. 1992	
Reference is a not the source document, but is a commentary on the study	McGregor and Hard 2001	
Exposure through intravenous, intraperitoneal, or subcutaneous injection	Belknap and Deutsch 1982 Hong and Krauss 2017 McComb and Goldstein 1979	
Study examined mixture effects	Clark et al. 2014 Gray et al. 2014 O'Callaghan et al. 2014	Roberts et al. 2014 White et al. 2014
Study in plants not adequate to derive a vegetation-based reference value	An and Lee 2007 Soriano et al. 2008 Yu and Gu 2006	

Therefore, when one includes the additional two references from USEPA 2017 (Huntingdon Life Sciences 2004 and Nelson et al. 1991), the total number of references included for study quality evaluation was 18.

Following the literature search conducted by TCEQ in August 2020, the final USEPA IRIS assessment of *tert*-butyl alcohol (USEPA 2021a,b) became available in August 2021. In USEPA (2021a), USEPA noted that the last formal literature search they conducted was in 2019 while the draft IRIS assessment was in external peer review, after which they monitored the literature in PubMed through January 2021. USEPA stated that no animal bioassays or epidemiological studies were identified in the post peer review literature that would change any major conclusions in their assessment. Additionally, TCEQ performed an additional literature search in PubMed on June 1, 2022 and did not identify any animal bioassays or epidemiological studies that would change any conclusions in this assessment.

A.3 Data Extraction

Each of the identified studies was reviewed in detail and, when relevant, the primary data were extracted for potential use in this DSD. Data from the most of these studies are shown in Table 16. Data that were applicable to the development of the acute and chronic ReVs and ESLs are also in Section 3.1.3 Health-Based Acute 1-h ReV and ESL and Section 4.1.2 Selection of the Key Study and Critical Effect, respectively.

Table 16. Data extraction from animal studies

General Toxicology: Inhalation

Reference	Species (age); n	Exposure Route and Concentration	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
NTP 1997 18-d inhalation study	B6C3F1 mice (6 wk old at dosing initiation); 5/sex/ dose	Inhalation 0, 450, 900, 1750, 3500 or 7000 ppm (nominal)	6 h/d, 5 d/wk (12 exposures total)	900 ppm	1750 ppm	≥ 1750 ppm: clinical signs of alcohol toxicity (e.g., ataxia, hypoactivity, hyperactivity), and urogenital wetness in males and females
NTP 1997 18-d inhalation study	F344/N rats (6 wk old at dosing initiation); 5/sex/ dose	Inhalation 0, 450, 900, 1750, 3500 or 7000 ppm (nominal)	6 h/d, 5 d/week (12 exposures total)	450 ppm	900 ppm	≥ 900 ppm: clinical signs of alcohol toxicity (e.g., ataxia, hypoactivity, hyperactivity) in males and females
NTP 1997 13-wk inhalation study	B6C3F1 mice (7 wk old at dosing initiation); 10/sex/ dose	Inhalation 0, 135, 270, 540, 1080 or 2100 ppm (nominal)	6 h/d, 5 d/wk for 13 wk	540 ppm (females) 1080 ppm (males) Reproductive toxicity: 2100 ppm	1080 ppm (females) 2100 ppm (males)	≥ 1080 ppm: ↓ body weight gain in females (19% and 24% versus controls, at 1080 and 2100 ppm, respectively) ≥ 1080 ppm: ↑ relative liver weights in females (9.3% and 21% higher than controls at 1080 and 2100 ppm, respectively) 2100 ppm: death in one male
NTP 1997 13-wk inhalation study	F344/N rats (7 wk old at dosing initiation); 10/sex/ dose	Inhalation 0, 135, 270, 540, 1080 or 2100 ppm (nominal)	6 h/d, 5 d/wk for 13 wk	Not identified (males) 540 ppm (females) Reproductive toxicity: 2100 ppm	135 ppm (males) 1080 ppm (females)	≥ 135 ppm: ↑ severity (overall minimal to mild) chronic progressive nephropathy in males versus controls ≥ 1080 ppm: ↑ relative liver weight in females (up to 8.8% versus controls)

Reference	Species (age); n	Exposure Route and Concentration	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
Borghoff et al. 2001[a]	F-344 rats (10 wk old at dosing initiation); 10/sex/ dose[b]	Inhalation 0, 250, 450, 1750 ppm (nominal)	6 h/d for 10 d	450 ppm (males) 1750 ppm (females)	1750 ppm (males) Not identified (females)	≥ 250 ppm: α2u-globulin nephropathy in males[c] 1750 ppm: ↓ absolute (9.5%) and relative (14%) liver weights in males

[a] Also mentioned as a "toxicological highlight" in same issue of journal (McGregor D and GC Hard. 2001. Renal tubule tumor induction by *tertiary*-butyl alcohol. Toxicol Sci. 61:1-3.).
[b] Five/sex/dose designated for kidney histopathology and 5/sex/dose designated for biochemical analysis of α2u-globulin in kidney tissue homogenates, and measurement of kidney and liver weights
[c] The TCEQ considers the increase in cell proliferation (Figures 4 and 6 of the publication) at all concentrations consistent with α2u-globulin nephropathy. This finding is not relevant to humans and is not appropriate for derivation of a ReV.

Developmental Toxicity: Inhalation

Reference	Species n	Exposure Route and Concentration	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
Nelson et al. 1989	Pregnant Sprague Dawley rats; 13-18/ dose	Inhalation 0, 2000, 3500, 5000 ppm	7 h/d from Gestation Days 1 to 19	Not identified	Maternal toxicity (clinical signs): 2000 ppm Fetal toxicity (↓ body weights): 2000 ppm	Maternal animals: ≥ 2000 ppm: unsteady gait, Fetuses: ≥ 2000 ppm: ↓ fetal weight

Postnatal Development: Inhalation

Reference	Species	Exposure Concentration	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
Nelson et al. 1991	Sprague-Dawley rat 4-5 pups/sex/ litter from 15 pregnant rats/dose (maternally exposed) 4-5 pups/sex/ litter from 18 exposed males mated to non-exposed females (paternally exposed)	Inhalation 0, 2000, 4000 ppm	Gestation Days 1-20 for pregnant rats 6 weeks in males prior to mating to non-exposed females	Maternal toxicity: 2000 ppm Fetal toxicity: not identified	Maternal toxicity: 4000 ppm Fetal toxicity: tentative LOAEL ≥ 2000 ppm	All litters fostered by non-exposed dams Maternal toxicity: 4000 ppm: ↓ body weights throughout gestation and ↓ food consumption during 1st week of gestation Fetal toxicity: 2000 ppm: effects on ascent on wire test (maternally exposed, ↓ time on wire, distance climbed) Note: Paternal toxicity not described in publication

General Toxicology: Oral

Reference	Species (age); n	Exposure Route and Concentration /Dose	Exposure Duration	NOAEL	LOAEL	Effects at LOAEL
Blanck et al. 2009	B6C3F1 mice (F only, 7-10 wk old at dosing initiation); 15/dose	Oral (drinking water) 0, 2, 20 mg/mL Doses F: 344 and 818 mg/kg-day for 3 days F: 418 and 1616 mg/kg-day for 14 days	3 or 14 days	818 mg/kg-day (3 days) 418 mg/kg-day (14 days)	Not identified (3 days) 1616 mg/kg-day (14 days)	No clinical signs or body weight effects 3 days of dosing: No TBA-related findings 14 days of dosing: minimal (1/5) or slight (1/5) diffuse centrilobular hepatocellular hypertrophy at 1616 mg/kg-day
Lin et al. 2020	Wild type (WT) C57BL/6 male mice (8 wk old at dosing initiation); Aldehyde dehydrogenase 2 knockout (KO) male mice also used; 6/strain/ dose	Oral (drinking water) 0, 5, 20 mg/mL Doses in WT mice: 0, 978, 3431 mg/mg-day Doses in KO mice: 0, 855, 2699 mg/kg-day	6 wk	978 mg/kg-day for WT mice Not identified for KO mice	3431 mg/kg-day for WT mice 855 mg/kg-day for KO mice	Wild type mice 3431 mg/kg-day: ↓ relative epididymides weight (12%); liver histopathology: extremely slight centrilobular hypertrophy and granuloma at 3431 mg/kg-day Knockout mice ≥ 855 mg/kg-day: ↑ relative liver weight (17% and 18% at 855 and 2699 mg/kg-day, respectively)

Reference	Species (age); n	Exposure Route and Concentration/ Dose	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
NTP 1995[a]	B6C3F1 mice (6 wk old at dosing initiation); 10/sex/ dose	Oral (drinking water) 0, 2.5, 5, 10, 20, and 40 mg/mL Average Doses: M: 350, 640, 1590, 3940 and 8210 mg/kg-day F: 500, 820, 1660, 6430 and 11,620 mg/kg-day	13 wk	1590 mg/kg-day (males) 6430 mg/kg-day (females) Reproductive toxicity: 6430 mg/kg-day (females) 8210 mg/kg-day (males)	3940 mg/kg-day (males) 11,620 mg/kg-day (females)	≥ 3940 mg/kg-day (males): ↓ body weight (14% and 25% versus controls) and body weight gain (30% and 54% versus controls) at 3940 and 8210 mg/kg-day, respectively; inflammation and transitional epithelial hyperplasia in urinary bladder 11,620 mg/kg-day (females): ↓ body weight (15% versus controls) and body weight gain (26% versus controls); ↑ absolute (12% versus control) and relative (35% versus control) kidney weight; inflammation and transitional epithelial hyperplasia in urinary bladder; ↑ length of estrous cycle

a: Also presented in Lindamood et al. 1992, which was excluded because it is not the source document and has discrepancies with the source document (NTP 1995).

Reference	Species (age); n	Exposure Route and Concentration	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
Acharya et al. 1995	Male Wistar rats (7 wk old at dosing initiation); 5-6/group	Oral (drinking water) 0 0.5% (v/v, equivalent to 5 mg/mL) TBA	10 wk	Not identified	575 mg/kg-day TBA[a]	↓ terminal body weight (8.1% lower than control group) ↑ serum triglycerides: ~1.6-fold higher than control group. ↑ serum glucose: 1.8-fold higher than control group. ↑ glycogen content (mg/g tissue) in liver: 7.5-fold higher than control group. ↓ glutathione content (mg/g tissue) in kidney: 29% lower than control group
Acharya et al. 1997	Male Wistar rats (7 wk old at dosing initiation); 5-6/group	Oral (drinking water) 0 0.5% (v/v, equivalent to 5 mg/mL) TBA	10 wk	Not identified	575 mg/kg-day TBA[a]	Kidney: degeneration of renal tubules with syncytial arrangement of the nucleus in renal tubular epithelial cells, degeneration of the basement membrane of Bowman's capsule, diffused glomeruli and vacuolation of glomeruli Liver: centrilobular necrosis, periportal proliferation, lymphocytic infiltration, vacuolation in hepatocytes and loss of hepatic architecture

a: The publication did not include the doses administered and did not include water consumption and body weight data for calculation of doses. The dose shown is from USEPA's IRIS assessment in which it is noted that the study authors calculated a dose of 575 mg/kg-day (Table 1-2 of IRIS assessment, USEPA 2021).

Reference	Species (age); n	Exposure Route and Concentration/ Dose	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
NTP 1995[a]	F344/N rats (6 wk old at dosing initiation); 10/sex/ dose	Oral (drinking water) 0, 2.5, 5, 10, 20 and 40 mg/mL Average Doses: M: 230, 490, 840, 1520 and 3610 mg/kg-day F: 290, 590, 850, 1560 and 3620 mg/kg-day in females	13 wk	Not identified in males and females Reproductive toxicity: 3610 mg/kg-day (males); 3620 mg/kg-day (females)	230 mg/kg-day (males) 290 mg/kg-day (females)	≥ 230 mg/kg-day (males): ↑ absolute and relative kidney weights; increased severity of nephropathy with findings consistent with α2u-globulin nephropathy (hyaline droplets) ≥ 290 mg/kg-day (females): ↑ absolute and relative kidney weights; ↑ absolute and relative liver weights; minimal (25%) ↑ serum alanine aminotransferase Dose-related clinical findings: ataxia, emaciation, hypoactivity (males), hyperactivity (females)

a: Also presented in Lindamood et al. 1992, which was excluded because it is not the source document and has discrepancies with the source document (NTP 1995). Additional review, including pathology working group reviews of this study included in Hard et al. 2011, Hard et al. 2019 and Takahashi et al. 1993.

Reference	Species (age); n	Exposure Route and Concentration/ Dose	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
NTP 1995[b]	B6C3F1 mice (7 wk old at dosing initiation); 60/sex/ dose	Oral (drinking water) 0, 5, 10 and 20 mg/mL Average doses M: 540, 1040 and 2070 mg/kg-day F: 510, 1020 and 2110 mg/kg-day	103 wk	Not identified for males 510 mg/kg-day (females)	540 mg/kg-day 1020 mg/kg-day (females)	≥ 540 mg/kg-day (males): ↑ incidence follicular cell hyperplasia in the thyroid gland ≥ 1020 mg/kg-day (females): ↓ body weight (up to 15% versus controls); ↑ incidence follicular cell hyperplasia in the thyroid gland

b: Also presented in Cirvello et al. 1995, which was excluded because it is not the source document and has discrepancies with source document (NTP 1995).

Reference	Species (age); n	Exposure Route and Concentration/ Dose	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
NTP 1995[a]	F344/N rats (7 wk old at dosing initiation); 60/sex/ dose	Oral (drinking water) M: 0, 0, 1.25, 2.5 and 5 mg/mL F: 0, 2.5, 5 and 10 mg/mL Average Doses: M: 90, 200, and 420 mg/kg-day F: 180, 330 and 650 mg/kg-day in females	103 wk	Not identified	90 mg/kg-day (males) 180 mg/kg-day (females)	≥ 90 mg/kg-day (males): ↓ terminal mean body weight (15%, 18%, 24% versus controls) at 90, 200 and 420 mg/kg-day, respectively; ↑ incidence mineralization in kidneys (component of chronic progressive nephropathy) at termination ≥ 180 mg/kg-day (females): ↑ absolute and relative kidney weights at 15-month interim sacrifice; ↑ severity chronic progressive nephropathy at termination

a: Also presented in Cirvello et al. 1995, which was excluded because it is not the source document and has discrepancies with source document (NTP 1995). Additional review, including pathology working group reviews of this study included in Hard et al. 2011 and Hard et al. 2019.

Developmental Toxicity: Oral

Reference	Species n	Exposure Route and Concentration/ Dose	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
Faulkner 1989	Pregnant CBA/J and C57BL/6J mice; 5-12/strain/ dose	Oral (gavage) 0 or 1557 mg/kg-day (0 or 778 mg/kg given twice daily, 12 h apart)	Gestation Days 6 to 19	Not identified	Fetal toxicity (resorptions): 1557 mg/kg-day Maternal toxicity not included in publication	Maternal toxicity: not included in publication Fetal toxicity: ↑ resorptions and litters with all resorbed, ↓ live fetuses Toxicokinetics in satellite non-gravid females confirmed exposure to TBA
Daniel and Evans 1982	Pregnant Swiss Webster mice; 15 per dose	Oral 0, 0.5, 0.75, 1% of liquid diet (calculated doses of 0, 3324, 4879, 6677 mg/kg-day)	Gestation Days 6 to 20	Maternal toxicity: 3324 mg/kg-day Fetal toxicity: not identified	Maternal toxicity: 4879 mg/kg-day Fetal toxicity: 3324 mg/kg-day	Maternal toxicity: 4879 mg/kg-day: ↓ body weight, body weight gain, food consumption Fetal toxicity: 3324 mg/kg-day: ↓ body weight (6.7% lower than control)

Developmental and Reproductive Toxicity: Oral

Reference	Species (age); n	Exposure Route and Dose	Exposure Duration	NOAEL	LOAEL	Effects at LOAEL
Huntingdon Life Sciences 2004	Sprague Dawley rat (8 wk old at dosing initiation) for mating; F_0 animals 12/sex/ dose; F_1 animals 1 weanling/ sex/litter/ dose	Oral gavage 0, 64, 160, 400 and 1000 mg/kg-day formulated in distilled/ deionized water	F_0 animals M: 9 wk (4 wk prior to mating until termination) F: 4 wk prior to mating to PND 21 F_1 animals 7 days (PND 21-27)	64 mg/kg-day (F_0 males) 160 mg/kg-day (F_0 females) Reproductive/ developmental toxicity: 400 mg/kg/day	160 mg/kg-day (F_0 males) 400 mg/kg-day (F_0 females) 1000 mg/kg-day (F_1 animals)	<u>F_0 animals</u> 160 mg/kg-day (males): ↑ relative kidney weights (11-14%) 400 mg/kg-day (females): CNS toxicity (irregular gait, ataxia, ↑ vocalization, rapid breathing) <u>F_1 animals</u> 1000 mg/kg-day: ↓ live born, ↑ stillborn, ↓ survival up to postnatal day (PND) 4, ↓ litter size; ↓ body weight (up to 15% relative to controls) starting on PND 1 (10%) through PND 14 In pups dosed PND 21-27, on PND 21 ↓ body weight (13% and 6% in males and females, respectively, relative to control); ↓ body weight gain and food consumption in 1 male

Abbreviation: CNS = central nervous system

Postnatal Development: Oral

Reference	Species n	Exposure Concentration	Exposure Duration	NOAEL	LOAEL	Findings at LOAEL
Grant and Samson 1982	Long Evans rat 4 pups/litter Number of litters not specified; appears to be 2-3 litters/dose	Not given Oral (via surgically implanted cannula inserted into esophagus) Doses: PND 4: 1.44 g/kg PND 5: 2.16 g/kg PND 6: 0.60 g/kg PND 7: 2.69 g/kg	Postnatal days 4-7	Not identified	The doses administered	TBA-dosed animals: impaired cliff avoidance, ↑ time to righting reflex, abstinence syndrome following cessation of dosing (full body tremors with head bobbing, rigid extension of body extremities, distress vocalization), ↓ total brain weight and total brain/body weight ratio, ↓ total DNA content in hindbrain After dose on PND 7, blood concentrations of TBA ranged from 33 to 66 mg/dL (n=6)

Abbreviation: PND = postnatal day

Physical Dependence: Oral

Reference	Species n	Exposure Route and Concentration	Exposure Duration	NOAEL	LOAEL	Effects at LOAEL
Grant and Samson 1981	Long Evans rats 2-5/group	Oral (drinking water) Dose escalation scheme until maintained at 0 (water control), 1% or 3% TBA Doses of 0, 0.7-0.8, 1.7-2.8 g/kg	60 – 90 days at maintenance concentration	1% TBA (0.7-0.8 g/kg)	3% (1.7-2.8 g/kg)	3% TBA (1.7 – 2.8 g/kg): self-withdrawal (refusing to drink to point of death), self-mutilation, tremors, spontaneous seizures, straub tail, ↑ withdrawal scores
Witkin and Leander 1982	Long Evans rats (males) 4-5/dose	Oral gavage doses of 0, 0.25, 0.5, 1, 1.5, 2, and 3 g/kg	Single dose	0.75 g/kg	1 g/kg	≥ 1 g/kg: ↓ overall and running response rates under a fixed ratio 30 schedule

Appendix 2 TCEQ Position Paper on Human Relevance of Chronic Progressive Nephropathy Observed in Rats

If the mode of action (MOA[s]) for a chemically-induced adverse effect is known, an evaluation of whether the adverse effect is relevant to humans can be conducted (TCEQ 2015a). Specifically, for a chemically-induced adverse effect in a laboratory animal species organ that is also part of human anatomy (e.g., the kidney), only if the underlying MOA(s) is known can an evaluation of whether the adverse effect is relevant to humans be conducted. The α2u-globulin nephropathy observed in male rats exposed to TBA is not considered relevant to humans, and therefore, this finding was not used as a critical effect in the derivation of toxicity factors by the TCEQ.

The Science Advisory Board (SAB) that reviewed the draft USEPA IRIS assessment for TBA (USEPA 2017) was unable to reach a consensus regarding whether the exacerbation of chronic progressive nephropathy (CPN) observed in rats exposed to ethyl *tert*-butyl ether (ETBE) or TBA could be attributed to a biological process not relevant to humans. TBA is a metabolite of ETBE. In the final USEPA IRIS assessment, the renal effects considered as possible critical effects for derivation of the reference dose (RfD) and reference concentration (RfC) were limited to female rats and comprised increased absolute kidney weights, increased severity of CPN, suppurative inflammation and transitional epithelial hyperplasia. The critical effect selected for derivation of the RfD was the increase in severity of CPN observed in female rats in the 2-year oral (drinking water) study. While some SAB members concluded that the possible critical effects selected by USEPA for derivation of the RfD and RfC could occur independently of CPN and were therefore relevant to humans, others concluded that the renal effects in rats exposed to ETBE or TBA were not relevant to humans as they were manifestations of a rat-specific CPN (with possible contribution from α2u-globulin-mediated effects in male rats) and no human relevant MOA has been identified that would clearly lead to renal effects independent of CPN (SAB 2019).[b]

[b] Additionally, exacerbation of CPN, which may not be relevant to humans, has been proposed as an MOA for the development of renal tubule tumors in rats, calling into question the human relevance of these tumors (Hard et al. 2013). The authors of Hard et al. (2013) disagree with and critique various aspects of the Melnick et al. (2012) review that raised questions regarding the validity of exacerbation of CPN as an MOA for rat renal tumorigenesis.

However, CPN involves a spectrum of age-related kidney effects,[c] and simply because an effect is part of that spectrum does not necessarily mean that it, and more specifically the MOA(s) that gave rise to it, can confidently be excluded as relevant to kidney effects or disease in humans generally or in a susceptible human subpopulation (e.g., approximately 13% of the adult population in the US has some degree of chronic kidney disease; Liu 2011). Scientifically it cannot be confidently stated that any TBA-induced kidney effects in female rats including exacerbation of CPN, whether or not they also occur as part of CPN, are not relevant to humans unless the underlying MOA(s) in rats is known and also known not to occur in humans.

Furthermore, the historic scientific burden of proof regarding adverse effects observed in laboratory animals has been on the regulatory agency to demonstrate a MOA that is not relevant to humans (e.g., α2u-globulin nephropathy in male rats). If such a demonstration were made, the regulatory agency would conclude that the effect is not relevant to humans[d], and the animal-specific effect would not be considered a hazard to humans. The converse would not be a conservative approach for the protection of public health; namely to put the burden of proof on the regulatory agency to scientifically demonstrate that the MOA producing a potential critical adverse effect in exposed laboratory animals also occurs in humans in order to utilize the endpoint in dose-response assessment. This would not be prudent from a public health perspective because the MOAs responsible for producing adverse effects resulting from exposure to tested chemicals are often not fully elucidated. It is a common and accepted practice to assume that an adverse effect in animals may occur in humans unless the finding is a known animal-specific finding that is not relevant to humans. Therefore, the TCEQ does not find it deterministic in regard to the potential human relevance of rat kidney effects (apart from α2u-globulin-mediated effects in male rats) or their exacerbation that no human relevant MOA

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[c] Chronic progressive nephropathy (CPN) is a common age-related degenerative-regenerative disease of the kidney that occurs in both sexes of most strains of rats and is characterized by a spectrum of histopathological lesions including multifocal regeneration of renal tubular epithelium, thickening of tubule and glomerular basement membranes, tubule dilatation, tubular proteinaceous casts, chronic inflammatory cell infiltration, and interstitial fibrosis. Progression to an advanced stage of renal disease involves increasing severity of these lesions, with more extensive involvement of the renal parenchyma (Melnick et al. 2012). The disease is one of increased glomerular permeability resulting in protein hyperfiltration, however, the true basic cause of CPN has never been defined and remains incompletely understood (Hard and Khan 2004).

[d] CPN is not an established MOA but rather a spectrum of effects with an unknown etiology (Melnick et al. 2012). By contrast, an MOA is the series of events leading to induction of the critical toxic endpoint; that is, the key and obligatory steps that describe the alterations in cellular or organ function that lead to toxicity (i.e., an adverse health effect; TCEQ 2015a). The MOA for chemically exacerbated CPN in rats (as with TBA or ETBE) is unknown, and without an understanding of the key events it is not possible to determine whether or not the underlying processes that occur in rats may also occur in humans (Melnick et al. 2012), much less confidently state that they do not.

has been specifically identified that would clearly lead to renal effects independent of CPN. In the absence of a sufficiently robust determination that kidney effects in female rats occur via a MOA(s) not relevant to humans, in the interest of public health the TCEQ will conservatively assume that such effects are potentially relevant to humans, including those with chronic kidney disease as a potentially sensitive subpopulation.[e] Just as a chronic chemical exposure may exacerbate CPN through perturbation of one or several physiological parameters to adversely affect the incidence, severity, and/or rate of progression of this disease in rats (Hard et al. 2009), erring on the side of public health protection in the face of uncertainty (e.g., if the unknown MOA(s) underlying CPN might also operate in humans), it is assumed by the TCEQ that chronic TBA exposure might perturb human physiology such that an adverse kidney effect occurs or existing kidney disease is exacerbated.

The TCEQ assumption of potential relevance of female rat kidney effects to humans may be consistent with chronic renal fibrosis, a lesion observed in rat CPN, also being observed in human kidneys and as the common final outcome of almost all progressive chronic kidney diseases (CKDs) in humans (Melnick et al. 2012, Liu 2011).[f] Renal fibrosis is a reliable predictor of prognosis and a major determinant of renal insufficiency. It is considered by and large to be a failed wound healing process that occurs in response to kidney damage, with the cellular and molecular responses in the damaged kidneys largely being dictated by evolutionarily conserved defense programs of wound healing (Liu 2011).[g] It is further noted that end-stage renal disease in humans can show prominent hyaline cast formation similar to that seen in rat CPN (Hard and Khan 2004). Lastly, mice can also develop a form of CPN that bears some resemblance to rat CPN (Hard et al. 2009, Frazier et al. 2012), demonstrating that the MOA(s) involved in CPN may not be exclusively rat specific and may also be chemically induced, albeit perhaps less frequently, to operate in other species.

In summary, in the absence of robust MOA data to the contrary and in consideration of public health, the TCEQ assumes that the MOA(s) that contribute to adverse kidney effects in rats (apart from α2u-globulin-mediated effects in male rats), such as the exacerbation of CPN in

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[e] Consistent with the SAB panel members who noted that in the absence of compelling evidence to the contrary, in order to protect human health, considerations should be conservative and the potential for human relevance should not be discounted.

[f] Consistent with the SAB panel members who pointed out that certain components of the so-called rat CPN response occur in humans, as noted by Melnick and colleagues (Melnick et al. 2012) for chronic renal fibrosis.

[g] Almost all the cell types in the kidneys are involved and participate in some way in the pathogenesis of renal fibrosis. In many aspects, major fibrogenic mechanisms are common and shared by different tissue compartments in the kidneys (Liu 2011).

female rats, may be relevant to humans with the potential to contribute to kidney effects or their exacerbation in humans.

The TCEQ discussion above is relatively consistent with USEPA (2021a), which indicated that although CPN played a role in the renal tubule nephropathy observed following TBA exposure in female rats, exacerbation of one or more of the lesions might reflect a type of injury relevant to the human kidney because the individual lesions associated with the spectrum of toxicities collectively described as CPN can occur in the human kidney (NIEHS 2019) and female rats were not affected by α2u-globulin nephropathy. Thus, the TCEQ and USEPA appear to concur that overall, the female rat kidney effects (suppurative inflammation, transitional epithelial hyperplasia, increased severity of CPN, and increased kidney weights) should be considered TBA-related and considered relevant (based on the current state of scientific knowledge) to human hazard characterization, dose-response analyses, and derivation of chronic reference values (e.g., RfD and RfC).

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