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

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Supplemental Information

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ABBREVIATIONS

ADAF	age-dependent adjustment factors
ATH	acute tubule hyperplasia
AIC	Akaike's information criterion
atm	atmosphere
BEC	blood ethanol concentration
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence level
$BMCL_{ADJ}$	duration-adjusted benchmark concentration lower confidence level
$BMCL_{HEC}$	benchmark concentration lower confidence level, human equivalent concentration
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
$BMDL_{BMR}$	benchmark dose lower confidence limit, benchmark response
BMDS	Benchmark Dose Software
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
BW	body weight
$BW^{3/4}$	body weight to the ¾ power
BW_a	animal body weight
BW_h	human body weight
CAAC	Chemical Assessment Advisory Committee
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service registry number
C_{avg}	average blood concentration
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary (cell line)
CL	confidence limit
CL	liver concentration
CNS	central nervous system
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
CSL	continuous simulation language
CVL	concentration in the venous blood leaving the liver
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
df	degrees of freedom
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EHC	Environmental Health Criteria
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ETBE	ethyl tertiary butyl ether
F	female
FDA	Food and Drug Administration
Fe-EDTA	iron-catalyzed oxidation of ascorbic acid
GD	gestation day
GLP	good laboratory practice
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HBA	2-hydroxyisobutyrate
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
HL-60	human promyelocytic leukemia
i.p.	intraperitoneal
i.v.	intravenous
IARC	International Agency for Research on Cancer
IC_{50}	half-maximal inhibitory concentration
IRIS	Integrated Risk Information System
JPEC	Japan Petroleum Energy Center
Km	Michaelis-Menten constant
LA	animal blood:air partition concentration
LH	human blood:air partition concentration
LOAEL	lowest-observed-adverse-effect level
M	male
M_{avg}	average rate of metabolism
MFO	mixed function oxidase
MPD	2-methyl-1,2-propanediol
mRNA	messenger ribonucleic acid
MTBE	methyl *tert* butyl ether
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate
MTD	maximum tolerated dose
MW	molecular weight
NADPH	reduced form of nicotinamide adenine dinucleotide phosphate
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
·OH	hydroxyl radical
OSF	oral slope factor
PBPK	physiologically based pharmacokinetic
PECO	populations, exposures, comparators, and outcomes
PK	pharmacokinetic

PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
POD_{HEC}	point of departure, human equivalent concentration
POD_{HED}	point of departure, human equivalent dose
QA	quality assurance
QSAR	quantitative structure-activity relationship
QA	quality assurance
RfC	inhalation reference concentration
RfD	oral reference dose
rho	Spearman rank correlation coefficient
RTR	route-to-route
SAB	Science Advisory Board
SD	standard deviation
SULT1A1	sulfotransferase 1A1
T3	triiodothyronine
T4	thyroxine
TBA	*tert*-butyl alcohol; *tert*-butanol
tk	thymidine kinase
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substances Control Act Test Submissions
TSH	thyroid-stimulating hormone
TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S.	United States
w/v	weight by volume
WHO	World Health Organization
XMET	xenobiotic metabolizing enzyme and transporter

APPENDIX A. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Table A-1. Health assessments and regulatory limits by other national and international health agencies

Organization	Toxicity value
National Institute of Occupational Safety and Health (NIOSH, 2007)	Recommended exposure limit—100 ppm (300 mg/m^3) TWA for up to a 10-h workday and a 40-h workweek.
Occupational Safety and Health (OSHA, 2006)	Permissible exposure limit for general industry—100 ppm (300 mg/m^3) TWA for an 8-h workday.
Food and Drug Administration (FDA, 2015a, b)	*tert*-Butyl alcohol—Indirect food additive that may be safely used in surface lubricants employed in the manufacture of metallic articles that contact food, subject to the provisions of this Section (21 Code of Federal Regulations [CFR] 178.3910); substance may be used as a defoaming agent (21 CFR 176.200).

TWA = time-weighted average.

APPENDIX B. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-RESPONSE ANALYSIS

B.1. TOXICOKINETICS

Little information is available on the absorption, distribution, metabolism, or excretion of *tert*-butyl alcohol (*tert*-butanol) in humans. The studies identified for this Toxicological Assessment were conducted in conjunction with methyl *tert*-butyl ether (MTBE) or ethyl *tert*-butyl ether (ETBE) because *tert*-butanol is a metabolite of both compounds. Several studies examining some aspect of the toxicokinetic behavior of *tert*-butanol in animals have been identified. Many of these toxicokinetic studies were carried out in conjunction with other specific endpoints (e.g., developmental). ARCO (1983) did not observe differences in the pharmacokinetics of *tert*-butanol following either oral (i.e., gavage) or inhalation exposure. Although some information is available for both oral and inhalation exposures, many studies administered *tert*-butanol via intraperitoneal (i.p.) or intravenous (i.v.) injection. Although these studies do not inform the absorption of *tert*-butanol, they can provide information on its distribution, metabolism, and excretion.

B.1.1. Absorption

Toxicity data on *tert*-butanol submitted by industry to the U.S. Environmental Protection Agency (EPA) under Section 8(e) of the Toxic Substances Control Act and other reporting requirements indicate that *tert*-butanol is rapidly absorbed after oral administration. Very little of the administered dose was excreted in the feces of rats, indicating that 99% of the compound was absorbed. Comparable blood levels of *tert*-butanol and its metabolites have been observed after acute oral (350 mg/kg) or inhalation (6,060 mg/m^3 for 6 hours) exposure in male Sprague-Dawley rats (ARCO, 1983); the absorption rate after inhalation exposure could not be determined, however, because the blood was saturated with radioactivity after 6 hours of exposure to 6,060 mg/m^3. In another study (Faulkner et al., 1989), blood concentrations indicated that absorption was complete at 1.5 hours following the last of six oral gavage doses of 10.5 mmoles *tert*-butanol/kg (twice daily) in female C57BL/6J mice. There was an apparent zero-order decline in *tert*-butanol concentration for most of the elimination phase, and no differences in absorption or elimination rates were observed between mice on a repeated dosing regimen and mice administered equivalent volumes of tap water every 12 hours before administration of a single dose of 10.5 mmoles *tert*-butanol/kg.

The study therefore concluded that previous exposures did not affect the absorption or elimination of *tert*-butanol (Faulkner et al., 1989).

B.1.2. Distribution

The available animal data suggest that *tert*-butanol is distributed throughout the body following oral, inhalation, and i.v. exposures (Poet et al., 1997; Faulkner et al., 1989; ARCO, 1983). Nihlén et al. (1995) calculated partition coefficients for *tert*-butanol using blood from human volunteers and available information about the relative content of water and fat in each tissue. The calculated tissue:blood partition coefficients for *tert*-butanol were slightly above 1 (from 1.02 to 1.06) for most tissues, except for fat:blood, which had a partition coefficient of 0.646. The same study evaluated the partition coefficients of three oxygenated ethers, including MTBE and ETBE, which are metabolized to *tert*-butanol (see Section B.1.4). The study concluded that, although *tert*-butanol preferentially distributes in body water, the ethers distribute uniformly throughout the body with a preference for fatty tissues (Nihlén et al., 1995).

In a study aimed at determining whether *tert*-butanol (or metabolites) can bind to alpha 2u-globulin, Williams and Borghoff (2001) exposed F344 rats to a single gavage dose of 500 mg/kg ^{14}C-*tert*-butanol and evaluated tissue levels at 12 hours. They found the radiolabel in three tissues (kidney, liver, and blood) in both sexes, but male rats retained more of the *tert*-butanol equivalents than females (Williams and Borghoff, 2001). Radioactivity was found in the low-molecular-weight protein fraction isolated from the kidney cytosol in male rats but not in female rats, indicating that *tert*-butanol, or one of its metabolites, was bound to alpha 2u-globulin. Further analysis determined that *tert*-butanol, and not its metabolite acetone, was bound. Most *tert*-butanol in the kidney cytosol was eluted as the free compound in both males and females, but a small amount was associated with the high-molecular-weight protein fraction in both males and females. In another study on alpha 2u-globulin nephropathy, Borghoff et al. (2001) found similar results after F344 rats were exposed to 0, 250, 450, or 1,750 ppm *tert*-butanol by inhalation for 8 consecutive days (with tissue levels measured at 2, 4, 6, 8, and 16 hours postexposure). Male rat *tert*-butanol kidney-to-blood ratios were significantly elevated over ratios in females at all dose levels and exposure durations. Although the female *tert*-butanol kidney-to-blood ratio remained similar with both duration and concentration, the male *tert*-butanol kidney-to-blood ratio increased with duration. The liver-to-blood ratios were similar, regardless of exposure duration, concentration, or sex. Both of these studies indicate distribution of *tert*-butanol to the liver and kidney, with kidney retention of *tert*-butanol in the male rat.

B.1.3. Metabolism

A general metabolic scheme for *tert*-butanol, illustrating the biotransformation in rats and humans, is shown in Figure B-1. Urinary metabolites of *tert*-butanol in a human male volunteer who ingested a gelatin capsule containing 5 mg/kg [^{13}C]-*tert*-butanol were reported to be 2-methyl-1,2-propanediol (MPD) and 2-hydroxyisobutyrate [HBA; Bernauer et al. (1998)]. Minor

metabolites of unconjugated *tert*-butanol, *tert*-butanol glucuronides, and traces of the sulfate conjugate also were detected. The study was approved by an ethical review board, but no information regarding informed consent was reported. In the same study, HBA, MPD, and *tert*-butanol sulfate were identified as major metabolites in rats, whereas acetone, *tert*-butanol, and *tert*-butanol glucuronides were identified as minor metabolites (Bernauer et al., 1998). Baker et al. (1982) found that *tert*-butanol was a source of acetone, but acetone production might have been stimulated from other sources.

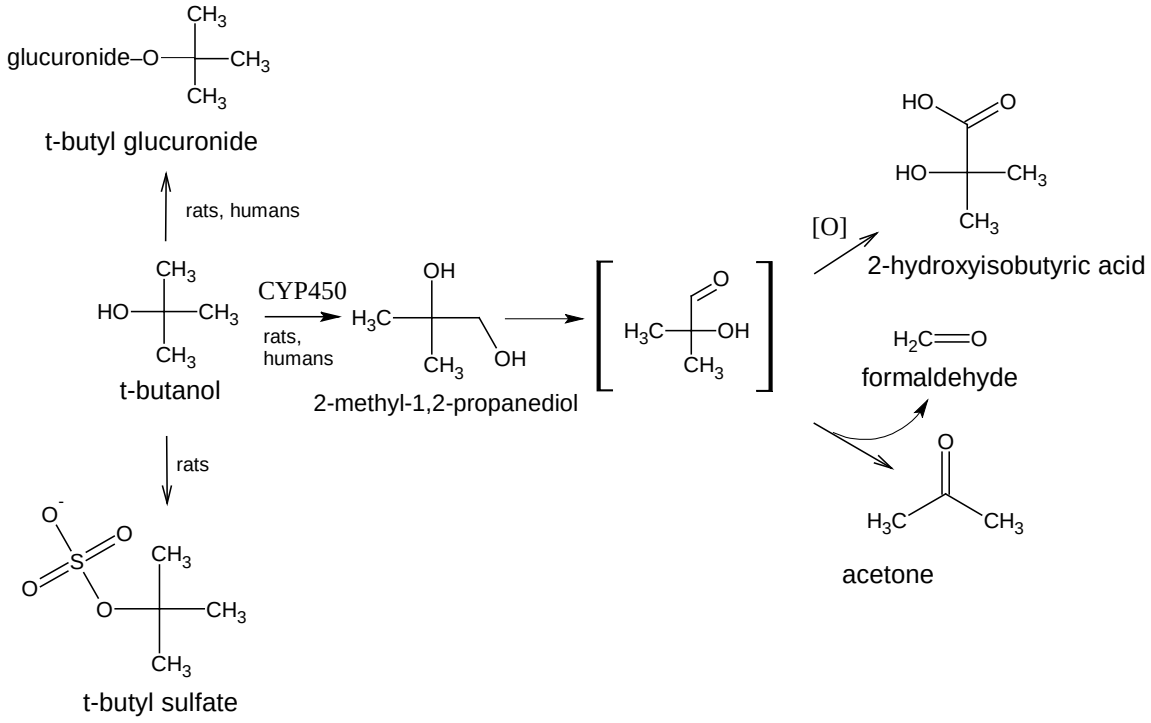


Figure B-1. Biotransformation of *tert*-butanol in rats and humans.

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

No studies identified specific enzymes responsible for the biotransformation of *tert*-butanol. Using a purified enzyme from Sprague-Dawley rats or whole-liver cytosol from Wistar rats, alcohol dehydrogenase had negligible or no activity toward *tert*-butanol (Videla et al., 1982; Arslanian et al., 1971). Other in vitro studies have implicated the liver microsomal mixed function oxidase (MFO) system, namely cytochrome P450 [CYP450; Cederbaum et al. (1983); Cederbaum and Cohen (1980)]. In the 1983 study, incubation of *tert*-butanol at 35 mM with Sprague-Dawley rat liver microsomes and a reduced nicotinamide adenine dinucleotide phosphate (NADPH)-generating system resulted in formaldehyde production at a rate of approximately 25 nmoles/mg protein/30 minutes. According to the study authors, the amount of formaldehyde generated from *tert*-butanol was approximately 30% of the amount of formaldehyde formed during the metabolism

of 10 mM aminopyrene in a similar microsomal system. The rate of formaldehyde generation from *tert*-butanol increased to about 90 nmol/mg protein/30 minutes upon addition of azide, which inhibits catalase and thereby prevents the decomposition of hydrogen peroxide (H_2O_2). In other experiments in the same study, formaldehyde formation was greatly reduced when H_2O_2 was included but NADPH was absent or when the microsomes were boiled prior to incubation. Additionally, the rate of formaldehyde formation in the microsomal oxidizing system depended on the concentration of *tert*-butanol, with apparent Michaelis-Menten constants (Km) and maximum velocity values of 30 mM and 5.5 nmol/minute/mg protein, respectively. The study authors concluded that *tert*-butanol is metabolized to formaldehyde by a mechanism involving oxidation of NADPH, microsomal electron flow, and the generation of hydroxyl radicals (·OH) from H_2O_2, possibly by a Fenton-type or a Haber-Weiss iron-catalyzed reaction involving CYP450, which might serve as the iron chelate (Cederbaum and Cohen, 1980).

In a follow-up study, *tert*-butanol was oxidized to formaldehyde and acetone by various systems known to generate ·OH radical, including rat liver microsomes or other nonmicrosomal ·OH-generating systems (Cederbaum et al., 1983). The nonmicrosomal tests included two chemical systems: (1) the iron-catalyzed oxidation of ascorbic acid (ascorbate-Fe-EDTA [ethylenediaminetetraacetic acid]) and (2) the Fenton system of chelated ferrous iron and H_2O_2. In both Fenton-type systems, H_2O_2 served as a precursor for ·OH. Additionally, a Haber-Weiss enzymatic system involving xanthine oxidation by xanthine oxidase in the presence of Fe-EDTA was used. In this system, ·OH is thought to be produced by the interaction of H_2O_2 and superoxide (O_2^-). Further experiments demonstrated the involvement of ·OH in either the ascorbate-Fe-EDTA or the xanthine oxidation systems based on inhibition of formaldehyde and acetone production from *tert*-butanol when ·OH-scavenging agents (e.g., benzoate, mannitol) were added. Some experiments in this study of the oxidation of *tert*-butanol by the microsomal metabolizing system of the liver were similar to those in the previous study (Cederbaum and Cohen, 1980) except that, in addition to formaldehyde, acetone formation was measured. Again, these experiments showed the dependence of the microsomal metabolizing system on an NADPH-generating system and the ability of H_2O_2 to enhance, but not replace, the NADPH-generating system. Addition of chelated iron (Fe-EDTA) boosted the microsomal production of formaldehyde and acetone, while ·OH-scavenging agents inhibited their production. The study authors noted that neither Fe-EDTA nor ·OH-scavenging agents are known to affect the CYP450-catalyzed oxidation of typical MFO substrates such as aminopyrene or aniline. The study also showed that known CYP450 inhibitors, such as metyrapone or SKF-525A, inhibited the production of formaldehyde from aminopyrene but not from *tert*-butanol. Finally, typical inducers of CYP450 and its MFO metabolizing activities, such as phenobarbital or 3-methylcholanthrene, had no effect on microsomal metabolism of *tert*-butanol to formaldehyde and acetone. According to the study authors, the oxidation of *tert*-butanol appears to be mediated by ·OH (possibly via H_2O_2), which can be produced by any of the tested systems by a Fenton-type reaction as follows:

$$H_2O_2 + Fe^{2+} - \text{chelate} \rightarrow \cdot OH + \cdot OH^- + Fe^{3+} - \text{chelate} \quad \text{(B-1)}$$

According to this reaction, reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) is required for continuous activity. The study authors concluded that the nature of the iron and the pathway of iron reduction within the microsomes remains unclear, even though an NADPH-dependent electron transfer or O_2^- might be involved (Cederbaum et al., 1983).

B.1.4. Excretion

Human data on the excretion of *tert*-butanol derives from studies of MTBE and ETBE (Nihlén et al., 1998a, b). Eight or 10 male human volunteers were exposed to 5, 25, or 50 ppm MTBE (18.0, 90.1, and 757 mg/m^3) or ETBE (20.9, 104, and 210 mg/m^3) by inhalation during 2 hours of light exercise. The half-life of *tert*-butanol in urine following MTBE exposure was 8.1 ± 2.0 hours (average of the 25- and 50-ppm MTBE doses); the half-life of *tert*-butanol in urine following ETBE exposure was 7.9 ± 2.7 hours (average of 25- and 50-ppm ETBE doses). In both studies, the urinary excretion of *tert*-butanol was less than 1% of the uptake or absorption of MTBE or ETBE. The renal clearance rate of *tert*-butanol was 0.67 ± 0.11 mL/hour-kg with MTBE exposure (average of 25- and 50-ppm MTBE doses); the renal clearance rate was 0.80 ± 0.34 mL/hour-kg with ETBE exposure (average of 25- and 50-ppm ETBE doses).

Amberg et al. (2000) exposed six volunteers (three males and three females, 28 ± 2 years old) to 18.8 and 170 mg/m^3 ETBE. Each exposure lasted 4 hours, and the two concentrations were administered to the same volunteers 4 weeks apart. Urine was collected at 6-hour intervals for 72 hours following exposure. *tert*-Butanol and two metabolites of *tert*-butanol, HBA and MPD, also were identified in the urine. At an ETBE level of 170 mg/m^3, *tert*-butanol had a half-life of 9.8 ± 1.4 hours. At the low-exposure ETBE concentration, the *tert*-butanol half-life was 8.2 ± 2.2 hours. The predominant urinary metabolite identified was HBA, excreted in urine at 5–10 times the amount of MPD and 12–18 times the amount of *tert*-butanol (note: urine samples had been treated with acid before analysis to cleave conjugates). HBA in urine showed a broad maximum at 12–30 hours after exposure to both concentrations, with a slow decline thereafter. MPD in urine peaked at 12 and 18 hours after exposure to 170 and 18.8 mg/m^3 ETBE, respectively, while *tert*-butanol peaked at 6 hours after exposure to both concentrations.

Amberg et al. (2000) exposed F344 NH rats to 18.8 and 170 mg/m^3 ETBE. Urine was collected for 72 hours following exposure. Like humans, rats excreted mostly HBA in urine, followed by MPD and *tert*-butanol. The half-life for *tert*-butanol in rat urine was 4.6 ± 1.4 hours at ETBE levels of 170 mg/m^3, but half-life could not be calculated at the ETBE concentration of 18.8 mg/m^3. Corresponding half-lives were 2.6 ± 0.5 and 4.0 ± 0.9 hours for MPD and 3.0 ± 1.0 and 4.7 ± 2.6 hours for HBA. In Sprague-Dawley rats treated with radiolabeled *tert*-butanol by gavage at 1, 30, or 500 mg/kg, a generally constant fraction of the administered radioactivity (23–33%) was recovered in the urine at 24 hours postdosing. Only 9% of a 1,500-mg/kg administered dose

was recovered in urine, however, suggesting that the urinary route of elimination is saturated following this dose (ARCO, 1983). Among all tested doses, most of the urinary radiolabel was attributed to a polar fraction that was not characterized, while only 0.3–5.5% of the administered dose was considered *tert*-butanol. The saturation in urinary elimination of radioactivity with the increased dose was considered a manifestation of saturated metabolic capacity; however, no further information was provided on the fate or balance of the administered radiolabel at any of the tested *tert*-butanol doses (ARCO, 1983).

Borghoff and Asgharian (1996) evaluated the disposition of ^{14}C radiolabel in F344 rats and CD-1 mice after nose-only inhalation exposure to 500, 1,750, or 5,000 ppm ^{14}C-ETBE for 6 hours. Recovery of total radioactivity in urine, feces, and expired air was measured, and air and urine samples were analyzed for ETBE and *tert*-butanol. Urine samples were also analyzed for *tert*-butanol metabolites HBA and MPD, and $^{14}CO_2$ was measured in exhaled air. Results were also obtained in rats after 13 days of exposure to 500 or 5,000 ppm ETBE. Total ETBE equivalents in exhaled air and excreted urine were found to increase linearly with exposure level, with over 90% eliminated by 48 hours (with the majority of exhalation occurring by 8 hours postexposure). Elimination shifted from being primarily in the urine at 500 ppm to occurring primarily by exhalation at 5,000 ppm in naïve rats, indicating a saturation of metabolism of ETBE to *tert*-butanol; this shift was greater in female rats than in males. In rats preexposed to 5,000 ppm ETBE for 13 days, most of the excretion was in urine, even at 5,000 ppm. The rats preexposed to 500 ppm ETBE also showed a shift from exhalation to urinary excretion compared with naïve rats, but to a lesser degree than that elicited by the 5,000-ppm preexposure group.

The results for the CD-1 mice were similar to those for the rats. The fraction of radiolabel in exhaled volatiles increased with exposure level while the fraction excreted in urine decreased. The exhalation pattern observed in rats showed levels of ETBE falling approximately 90% in the first 8 hours postexposure, while levels of *tert*-butanol exhaled rose between 0 and 3 hours postexposure and then fell more slowly between 3 and 16 hours, particularly at 5,000 ppm ETBE. The increase in *tert*-butanol between 0 and 3 hours postexposure can be explained by the continued metabolism of ETBE during that period. The slower decline after 3 hours likely results from a generally slower clearance of *tert*-butanol, which is saturated by the higher ETBE exposure levels.

B.1.5. Physiologically Based Pharmacokinetic Models (PBPK)

Although no models of *tert*-butanol have been created independently of other chemicals from which it arises as a metabolite (e.g., MTBE, ETBE), submodels have been adapted specifically to estimate internal doses for administration of *tert*-butanol. These models are parameterized using pharmacokinetic studies with *tert*-butanol exposures. Three PBPK models have been developed specifically for administration of *tert*-butanol in rats: Leavens and Borghoff (2009), Salazar et al. (2015), and Borghoff et al. (2016); other models have incorporated *tert*-butanol as a

submodel following MTBE administration. In Leavens and Borghoff (2009), *tert*-butanol is incorporated as a metabolite of MTBE and in Salazar et al. (2015) and Borghoff et al. (2016), it is incorporated as a metabolite of ETBE. In all three models, inhalation and oral exposure to *tert*-butanol can be simulated in rats. A detailed summary of these toxicokinetic models is provided in a separate report evaluating the pharmacokinetic (PK)/PBPK modeling of ETBE and *tert*-butanol (U.S. EPA, 2017).

The PBPK model described in Borghoff et al. (2016), with parameters modified as described by U.S. EPA (2017), was applied to conduct oral-to-inhalation route extrapolation based on an equivalent internal dose (the average concentration of *tert*-butanol in the blood). The time to reach a consistent periodic pattern of *tert*-butanol blood concentrations ("periodicity"), given the drinking water ingestion pattern described below, was much shorter than the duration of the oral bioassay studies. To allow for possible metabolic induction, computational scripts used a simulated time of 7 weeks, although periodicity was achieved in only a few days without metabolic induction. The average blood concentration was calculated over the last week of the simulation and was considered representative of the bioassays. To calculate steady-state values for continuous inhalation exposure, the simulations were run until the blood concentration had a <1% change between consecutive days. The continuous inhalation exposure equivalent to a given oral exposure was then selected by identifying the inhalation concentration for which the final (steady-state) blood concentration of *tert*-butanol matched the average concentration from water ingestion, as described above.

For simulating exposure to drinking water, the consumption was modeled as episodic, based on the drinking pattern observed in rats (Spiteri, 1982). In particular, rats were assumed to ingest water in pulses or "bouts," which were treated as continuous ingestion, interspersed with periods of no ingestion. Eighty percent of total daily ingestion (45-minute bouts with alternating 45-minute periods of other activity) was assumed to occur during the active dark period (12 hours/day). The remaining 20% of daily ingestion was assumed to occur during the relatively inactive light period (12 hours/day), when bouts were assumed to last 30 minutes with 2.5 hours in between. This resulting pattern of drinking water ingestion is thought to be more realistic than assuming continuous 24 hours/day ingestion (see Figure B-2).

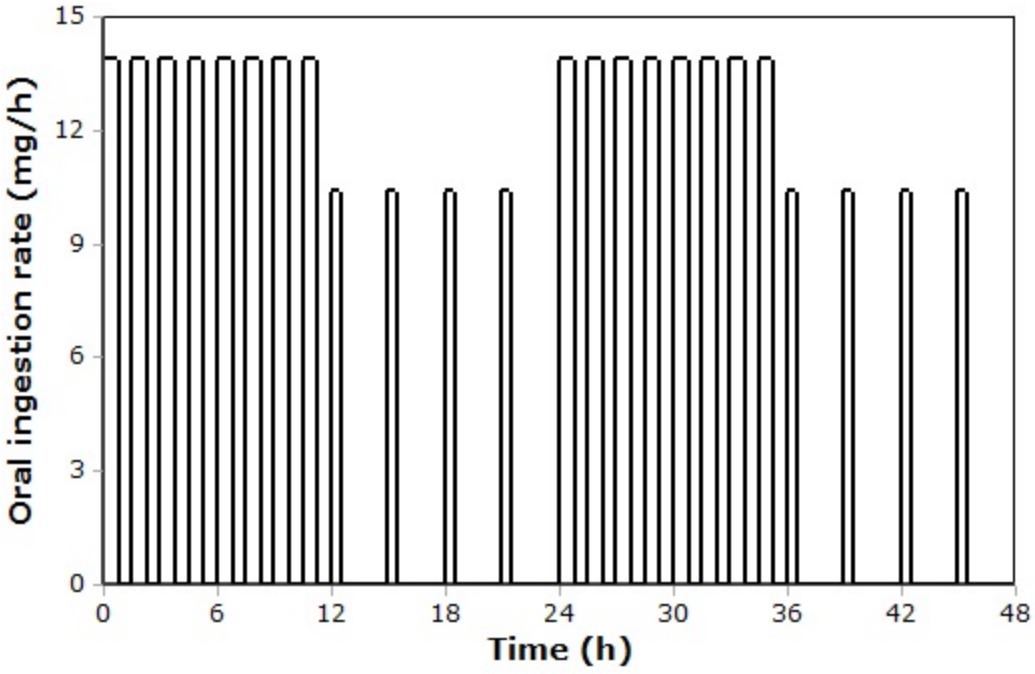
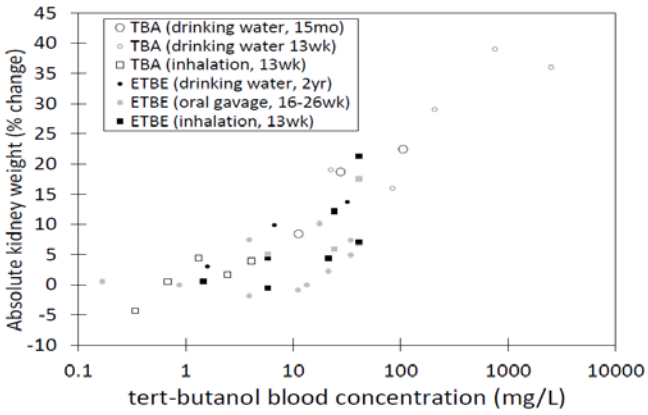
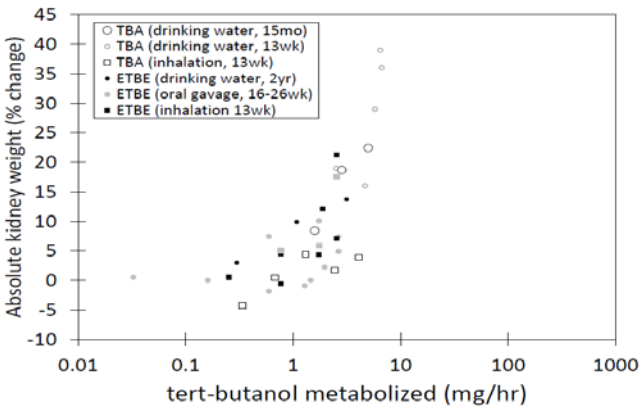


Figure B-2. Example oral ingestion pattern for rats exposed via drinking water.

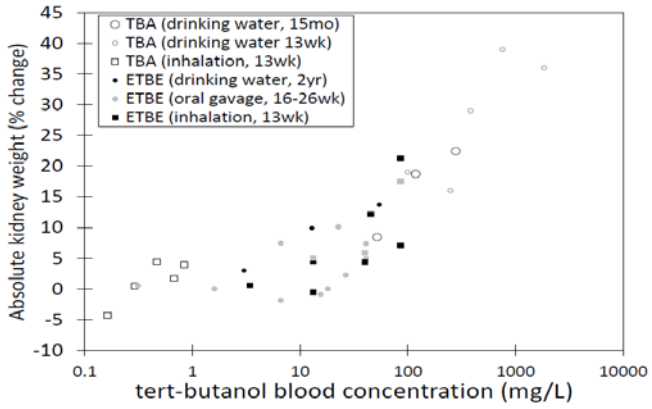
PBPK modeling was also used to evaluate a variety of internal dose metrics (daily average *tert*-butanol blood concentration, daily amount of *tert*-butanol metabolized in liver, daily average of ETBE blood concentration, and daily amount of ETBE metabolized in liver) to assess their correlation with different endpoints following exposure to ETBE or *tert*-butanol (Salazar et al., 2015). Administering ETBE either orally or via inhalation achieved similar or higher levels of *tert*-butanol blood concentrations or *tert*-butanol metabolic rates as those induced by direct *tert*-butanol administration (see Figure B-3). Altogether, the PBPK model-based analysis by Salazar et al. (2015) [which applied a model structurally similar to Borghoff et al. (2016)] indicates that kidney weight, urothelial hyperplasia, and chronic progressive nephropathy (CPN) yield consistent dose-response relationships using *tert*-butanol blood concentration as the dose metric for both ETBE and *tert*-butanol studies. For kidney and liver tumors, however, a consistent dose-response pattern was not obtained using any dose metric. These data are consistent with *tert*-butanol mediating the noncancer kidney effects following ETBE administration, but additional factors besides internal dose are necessary to explain the induction of liver and kidney tumors.



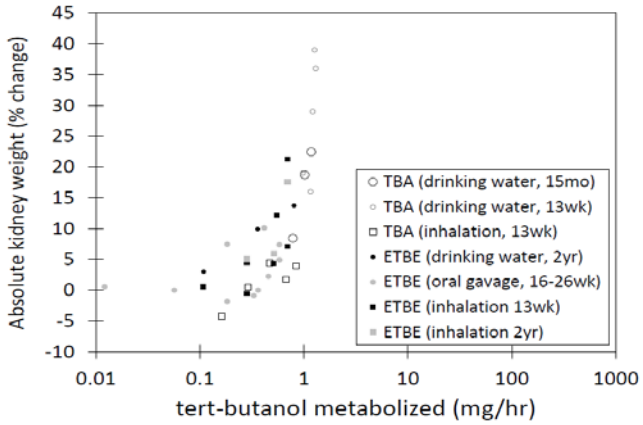
A. *tert*-butanol blood dose-metric [Salazar et al. (2015) model]



B. *tert*-butanol metabolized dose-metric [Salazar et al. (2015) model]



C. *tert*-butanol blood dose metric [Borghoff et al. (2016) model]



D. *tert*-butanol metabolized dose-metric [Borghoff et al. (2016) model]

Figure B-3. Change in absolute kidney weight in female rats as a function of estimated *tert*-butanol blood concentration (average mg/L) and *tert*-butanol metabolism (average mg/h) for two structurally similar PBPK models.

tert-Butanol inhalation data are from NTP (1997) (exposure for 6 hours/day, 5 days a week for 13 weeks). *tert*-Butanol oral data are from subchronic and chronic bioassays in NTP (1995) (ad libitum drinking water exposure for 13 weeks or 15 months). ETBE inhalation data are from two subchronic bioassays that exposed rats for 6 hours/day, 5 days/week for 13 weeks (JPEC, 2008a; Medinsky et al., 1999; Bond et al., 1996) and from one chronic bioassay that exposed rats for 6 hours/day, 5 days/week for 2 years (Saito et al., 2013; JPEC, 2010b). Oral gavage ETBE data are from bioassays that exposed rats via a single daily oral gavage for 16−26 weeks (Miyata et al., 2013; Fujii et al., 2010; JPEC, 2008b, c; Gaoua, 2004). Drinking water ETBE data are from one bioassay that exposed rats via drinking water (ad libitum) for 2 years (Suzuki et al., 2012; JPEC, 2010a).

B.1.6. Physiologically Based Pharmacokinetic (PBPK) Model Code

The PBPK acslX™ model code is available electronically through EPA's Health and Environmental Research Online (HERO) database. All model files may be downloaded in a zipped workspace from HERO (U.S. EPA, 2016).

B.1.7. Pharmacokinetic/Physiologically Based Pharmacokinetic (PK/PBPK) Model Evaluation

PBPK models can be used to perform route-to-route extrapolation of toxicological data. For *tert*-butanol, oral-to-inhalation extrapolation was performed using the concentration of *tert*-butanol in blood as the internal dose metric. An overview of *tert*-butanol toxicokinetics, as well as the scientific rationale for selecting the internal dose metric, is available in the Toxicological Review. Because the existing human PBPK model was not considered adequate (see below), default methodologies were applied to extrapolate toxicologically equivalent exposures from adult laboratory animals to adult humans. For inhalation exposures, the interspecies conversion was the ratio of animal/human blood:air partition coefficients (LA/LH), according to reference concentration (RfC) guidelines for Category 3 gases (U.S. EPA, 1994). For oral exposures, extrapolation is performed by body-weight scaling to the ¾ power ($BW^{3/4}$) (U.S. EPA, 2011).

All available PBPK models of ETBE and its principal metabolite *tert*-butanol were evaluated for potential use in the assessments. A PBPK model of ETBE and its principal metabolite *tert*-butanol has been developed for humans exposed while performing physical work (Nihlén and Johanson, 1999). The Nihlén and Johanson model is based on measurements of blood concentrations of eight individuals exposed to 5, 25, or 50 ppm ETBE for 2 hours while physically active. This model differs from conventional PBPK models in that the tissue volumes and blood flows were calculated from individual data on body weight and height. Additionally, to account for physical activity, blood flows to tissues were expressed as a function of the workload. These differences from typical PBPK models preclude allometric scaling of this model to other species for cross-species extrapolation. Because there are no oral exposure toxicokinetic data in humans, this model does not have a mechanism for simulating oral exposures, which prevents using the model in animal-to-human extrapolation for that route.

A number of PBPK models were developed previously for ETBE's related compound, methyl tertiary butyl ether (MTBE) and the metabolite *tert*-butanol that is common to both parent compounds (Borghoff et al., 2010; Leavens and Borghoff, 2009; Blancato et al., 2007; Kim et al., 2007; Rao and Ginsberg, 1997; Borghoff et al., 1996). EPA (Salazar et al., 2015) developed a PBPK model for ETBE and *tert*-butanol in rats by integrating information from across these earlier models. Another model for ETBE and *tert*-butanol was published by Borghoff et al. (2016), adapted with modest structural differences from the Leavens and Borghoff (2009) MTBE/*tert*-butanol model. Brief descriptions below highlight the similarities and differences between the

MTBE/*tert*-butanol models of Blancato et al. (2007) and Leavens and Borghoff (2009), and the ETBE/*tert*-butanol models of Salazar et al. (2015) and Borghoff et al. (2016).

The models of Blancato et al. (2007) and Leavens and Borghoff (2009)

The Blancato et al. (2007) model is an update of the earlier Rao and Ginsberg (1997) model, and the Leavens and Borghoff (2009) model is an update of the Borghoff et al. (1996) model. Both the Blancato et al. (2007) and Leavens and Borghoff (2009) models are flow-limited models that predict amounts and concentrations of MTBE and its metabolite *tert*-butanol in blood and six tissue compartments: liver, kidney, fat, brain, and rapidly and slowly perfused tissues. These tissue compartments are linked through blood flow, following an anatomically accurate, typical, physiologically based description (Andersen, 1991). The parent (MTBE) and metabolite (*tert*-butanol) models are linked by the metabolism of MTBE to *tert*-butanol in the liver. Oral and inhalation routes of exposure are included in the models for MTBE; Leavens and Borghoff (2009) also included oral and inhalation exposure to *tert*-butanol. Oral doses are assumed 100% bioavailable and 100% absorbed from the gastrointestinal tract, represented with a first-order rate constant. After inhalation, MTBE or *tert*-butanol is assumed to enter the systemic blood supply directly, and the respiratory tract is assumed to be at pseudo-steady state. Metabolism of MTBE by CYP450s to formaldehyde and *tert*-butanol in the liver is described with two Michaelis-Menten equations representing high- and low-affinity enzymes. *tert*-Butanol is conjugated either with glucuronide or sulfate, or further metabolized to acetone through 2-methyl-1,2-propanediol (MPD) and hydroxyisobutyric acid (HBA); the total metabolic clearance of *tert*-butanol by both processes is described by a single Michaelis-Menten equation in the models. All model assumptions are considered valid for MTBE and *tert*-butanol.

In addition to differences in fixed parameter values between the two models and the addition of exposure routes for *tert*-butanol, the Leavens and Borghoff (2009) model has three features not included in the Blancato et al. (2007) model: (1) the alveolar ventilation was reduced during exposure, (2) the rate of *tert*-butanol metabolism increased over time to account for induction of CYP enzymes, and (3) binding of MTBE and *tert*-butanol to alpha 2u-globulin was simulated in the kidney of male rats. The Blancato et al. (2007) model was configured through EPA's PBPK modeling framework, Exposure-Related Dose Estimating Model, which includes explicit pulmonary compartments. The modeling assumptions related to alveolar ventilation, explicit pulmonary compartments, and induction of metabolism of *tert*-butanol are discussed in the model evaluation section below.

MTBE and *tert*-butanol binding to alpha 2u-globulin in the kidneys of male rats were incorporated in the PBPK model of MTBE by Leavens and Borghoff (2009). Binding to alpha 2u-globulin is one hypothesized mode of action (MOA) for the observed kidney effects in MTBE-exposed animals. For a detailed description of the role of alpha 2u-globulin and other modes of action for kidney effects, see the kidney mode-of-action section of the Toxicological Review (Section 1.2.1). In the Leavens and Borghoff (2009) model, binding of MTBE to alpha 2u-globulin

was applied to describe sex differences in kidney concentrations of MTBE and *tert*-butanol, but acceptable estimates of MTBE and *tert*-butanol pharmacokinetics in the blood are predicted in other models that did not consider alpha 2u-globulin binding. Moreover, as discussed below, EPA's implementation of the Leavens and Borghoff (2009) model did not adequately fit the available *tert*-butanol i.v. dosing data, adding uncertainty to the parameters they estimated.

The Blancato et al. (2007) and Leavens and Borghoff (2009) PBPK models for MTBE were specifically evaluated by comparing predictions from the *tert*-butanol portions of the models with the *tert*-butanol i.v. data of Poet et al. (1997) (see Figure B-4). Neither model adequately represented the *tert*-butanol blood concentrations. Modifications of model assumptions for alveolar ventilation, explicit pulmonary compartments, and induction of metabolism of *tert*-butanol did not significantly improve model fits to the data.

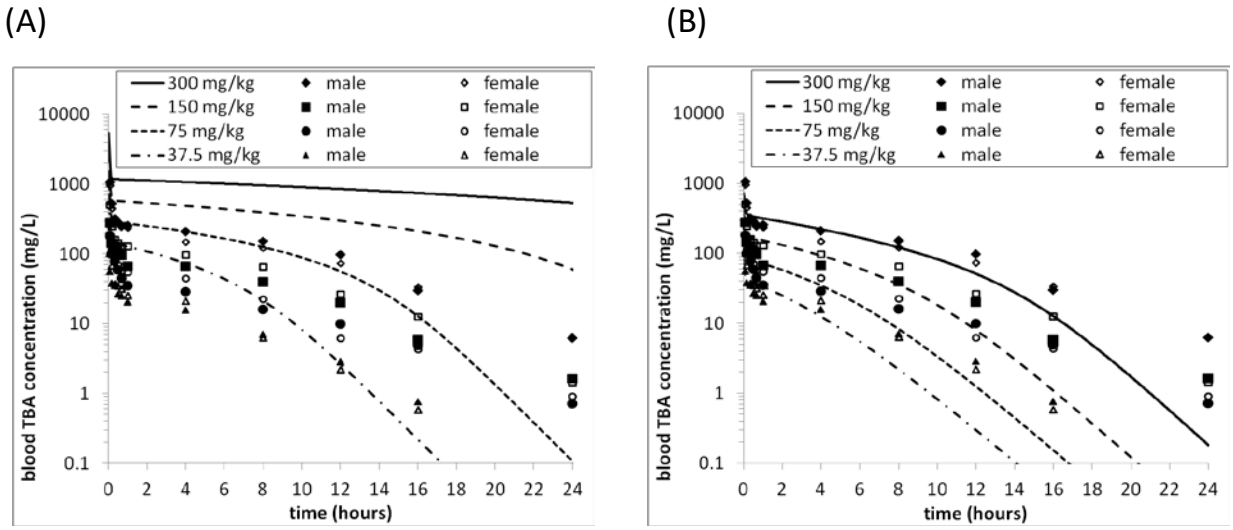


Figure B-4. Comparison of the *tert*-butanol portions of existing MTBE models with *tert*-butanol blood concentrations from i.v. exposure by Poet et al. (1997). Neither the (A) Blancato et al. (2007) nor the (B) Leavens and Borghoff (2009) model adequately represents the measured *tert*-butanol blood concentrations.

The model of Salazar et al. (2015)

To better account for the *tert*-butanol blood concentrations after i.v. *tert*-butanol exposure, the model by Leavens and Borghoff (2009) was modified by adding a pathway for reversible sequestration of *tert*-butanol in the blood (Salazar et al., 2015). Sequestration of *tert*-butanol was modeled using an additional blood compartment, which *tert*-butanol can enter reversibly, represented by a differential mass balance (see Figure B-5). Other differences in model structure are that the brain was included in the other richly perfused tissues compartment and binding to alpha 2u-globulin was not included. Binding to alpha 2u-globulin was neglected because it was assumed to not significantly affect the blood concentration or metabolic rate of ETBE or

tert-butanol, the two dose metrics being used for route-to-route extrapolation. This model improved the fit to *tert*-butanol blood concentrations after *tert*-butanol i.v. exposures [see Salazar et al. (2015)]. Additionally, the model adequately estimated the *tert*-butanol blood concentrations after inhalation and oral gavage exposures. The ETBE submodel was based on the MTBE component of the Leavens and Borghoff (2009) model. The model assumed two pathways for metabolism of ETBE to *tert*-butanol, and the metabolic parameters were optimized to fit toxicokinetic data. Partition coefficients of ETBE were based on data of Nihlén and Johanson (1999).

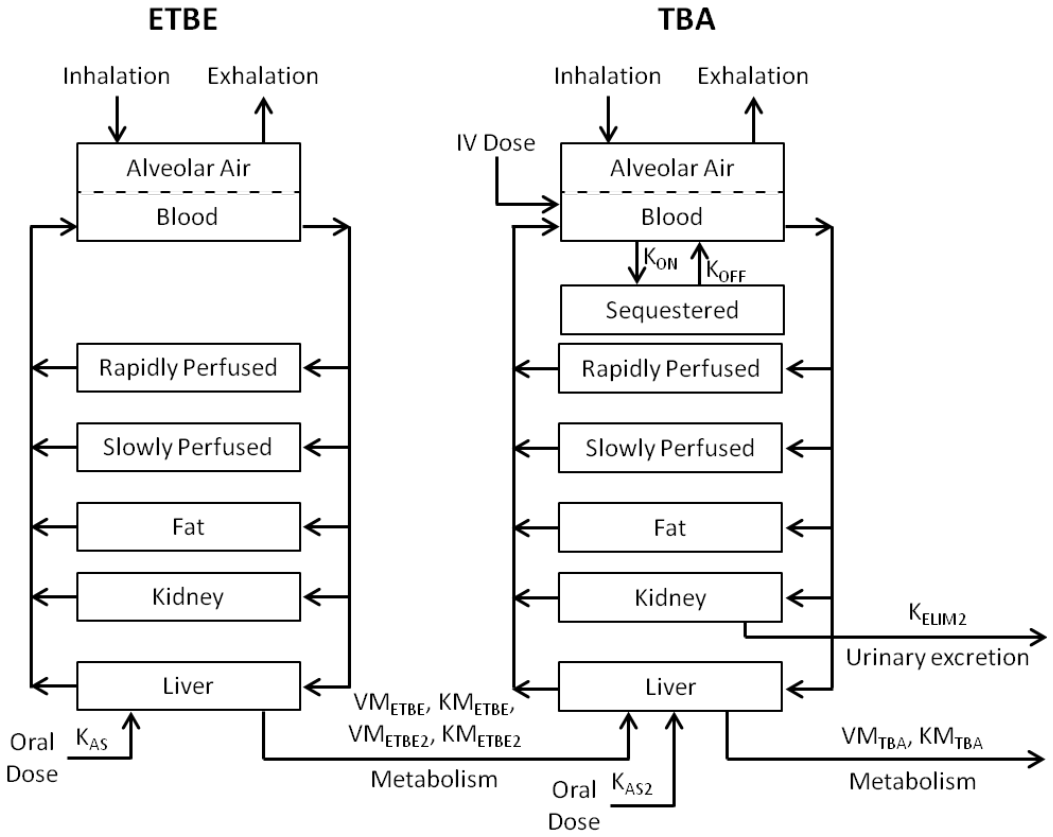


Figure B-5. Schematic of the Salazar et al. (2015) PBPK model for ETBE and its major metabolite *tert*-butanol in rats. Exposure can be via multiple routes including inhalation, oral, or i.v. dosing. Metabolism of ETBE and *tert*-butanol occurs in the liver and is described by Michaelis-Menten equations with two pathways for ETBE and one for *tert*-butanol. ETBE and *tert*-butanol are cleared via exhalation, and *tert*-butanol is additionally cleared via urinary excretion.

The model of Borghoff et al. (2016)

The Borghoff et al. (2016) models for ETBE and *tert*-butanol were based on Leavens and Borghoff (2009), including binding of ETBE and *tert*-butanol to alpha 2u-globulin and induction of *tert*-butanol metabolism, but with some structural changes. The revised model lumped gastrointestinal tract tissue and brain tissue into the richly perfused compartment [Leavens and

Borghoff (2009) modeled these compartments separately]. Borghoff et al. (2016) assumed that urinary clearance was a function of central venous blood concentration and effectively occurs from that compartment, as opposed to clearance from the kidney venous blood assumed by Leavens and Borghoff (2009). Using the new structure, urinary clearance was reparameterized to fit the intravenous data by Poet et al. (1997). The model assumed a single oxidative metabolic pathway for metabolism of ETBE to *tert*-butanol using parameters from Rao and Ginsberg (1997), instead of the two-pathway models assumed by Leavens and Borghoff (2009) (for MTBE) and Salazar et al. (2015). The model did not incorporate the *tert*-butanol blood sequestration kinetics included in the *tert*-butanol model. It did, however, incorporate the oral absorption rate of *tert*-butanol estimated by Salazar et al. (2015). Partition coefficients for ETBE were obtained from Kaneko et al. (2000). Rate constants for binding of ETBE to alpha 2u-globulin and its dissociation were assumed to be the same as estimated for MTBE by Leavens and Borghoff (2009). Finally, unlike the Leavens and Borghoff (2009) model, the Borghoff et al. (2016) model assumed a lower-bound alveolar ventilation for all times and exposures, not just during periods of inhalation exposure.

To simulate induction of *tert*-butanol metabolism, the default metabolic rate of *tert*-butanol clearance is multiplied by an exponential function of the form $[1 + A(1 - e^{-kt})]$, where A is the maximum fold increase above baseline metabolism, k is the rate constant for the ascent to maximum induction, and t is time. Because metabolic induction does not occur instantaneously, but involves a delay for induction of ribonucleic acid transcription and translation, Borghoff et al. (2016) assumed that induction did not begin until 24 hours after the beginning of exposure. But the computational implementation then treated the effect as if the enzyme activity suddenly jumped each 24 hours to the level indicated by the time-dependent equation shown in the paper. This stepwise increase in activity was not considered realistic. Therefore, in evaluating the effect of induction, EPA treated the induction as occurring continuously with time but beginning at 12 hours after the start of exposure. This change would not affect long-term steady-state or periodic simulations, in particular those used to characterize bioassay conditions. But it does have a modest effect on simulations between 12 hours and 24 hours, which are compared with experimental data below for the purpose of model validation. However, with further review of the existing data on liver histology (which would also reflect metabolic induction if it occurs, as detailed below), EPA determined that histological changes are likely to occur only at the very highest exposure levels and hence not at levels where the model is applied for route-to-route extrapolation. Therefore, the maximal induction was set to zero unless otherwise noted.

The form of the equations for hepatic metabolism in the Borghoff et al. (2016) model was revised to be a function of the free liver concentration (CL), specifically the concentration in the venous blood leaving the liver (CVL), rather than the concentration in the liver tissue (see Figure B-6). In order to maintain the integrity of all prior model simulations and parameter estimations, EPA updated the Km's for ETBE and *tert*-butanol by scaling them by the liver:blood

partition coefficients. As a result, the model produces identical results as before without re-estimating a fitted parameter.

Finally, a discrepancy between the pulmonary ventilation value as described by Borghoff et al. (2016), in particular as the lower limit of values reported by Brown et al. (1997), should be noted. Borghoff et al. (2016) claim that an allometric coefficient of 18.9 L/hour/kg$^{3/4}$ (allometric coefficient provided here reflects actual use in model code) is the lower limit. For a 0.25 kg rat, this value yields an absolute ventilation rate of 6.6822 L/hour or 111.3 mL/minute. In Table 31 of Brown et al. (1997), the mean and range of values given for the rat are 52.9 and 31.5–137.6 mL/minute/100 g BW). From the text immediately following this table, it is clear that the mean and range are not scaled to BW$^{3/4}$, but exactly as indicated. Hence, for a 250 g rat, they correspond to 132.25 and 78.75–344 mL/minute. Use of 18.9 L/hour/kg$^{3/4}$corresponds to a ventilation rate 61% of the way between the lower limit and the mean for a 0.25 kg rat. It can be noted that 31.5 mL/minute/100 g BW, the actual lower limit, equals 18.9 L/hour/kg$^{1.0}$ (i.e., the respiration per kg BW, not per kg BW$^{3/4}$). Thus, the discrepancy appears due to a mistaken translation in allometric scaling.

The fact that Borghoff et al. (2016) and Leavens and Borghoff (2009) used a ventilation rate closer to the mean than the lower limit may explain why it was also necessary to incorporate a fraction of *tert*-butanol available for alveolar absorption of 0.6. When considering the plots of model simulations versus data below, it seems that model fits to the data would be improved by further decreasing ventilation, which could now be justified. But EPA has chosen to keep the value of alveolar ventilation (Q_{pc}) and absorption fraction as published by Borghoff et al. (2016).

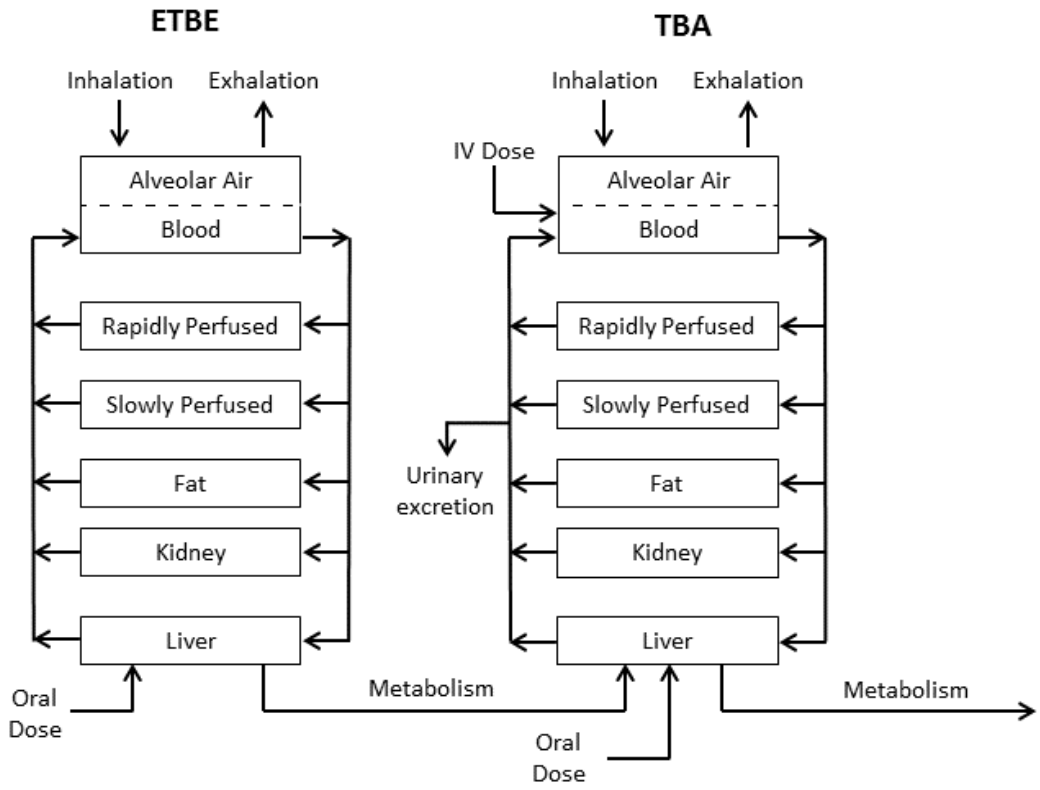


Figure B-6. Schematic of the Borghoff et al. (2016) PBPK model for ETBE and its major metabolite *tert*-butanol in rats.

Table B-1. PBPK model physiologic parameters and partition coefficients*

Body weight and organ volumes as fraction of body weight		
Body weight (kg)	0.25	Brown et al. (1997)
Liver	0.037	Brown et al. (1997)
Kidney	0.0073	Brown et al. (1997)
Fat	0.35 × BW + 0.00205	Brown et al. (1997)
Richly perfused (total)	0.136	Brown et al. (1997)
Richly perfused	0.0177	[a]
Poorly perfused (total)	0.757	Brown et al. (1997)
Poorly perfused	0.75495 − 0.35 × BW	
Blood	0.074	Brown et al. (1997)
Rest of body (not perfused)	0.107	Brown et al. (1997)
Cardiac output and organ blood flows as fraction of cardiac output		
Cardiac output (L/h-kg)	18.9	Brown et al. (1997)[b]
Alveolar ventilation (L/h-kg)	18.9	Brown et al. (1997)[b]
Liver	0.174	Brown et al. (1997)[c]
Kidney	0.141	Brown et al. (1997)
Fat	0.07	Brown et al. (1997)
Richly perfused (total)	0.47	[d]
Richly perfused	0.155	[e]
Poorly perfused (total)	0.53	Brown et al. (1997)
Poorly perfused	0.46	[f]
Partition coefficients for ETBE		
Blood:air	11.6	Kaneko et al. (2000)
Liver:blood	2.9	Kaneko et al. (2000)
Fat:blood	11.7	Kaneko et al. (2000)
Richly perfused:blood	2.9	Kaneko et al. (2000)
Poorly perfused:blood	1.9	[g]
Kidney:blood	2.9	[h]

Table B-1. PBPK model physiologic parameters and partition coefficients* (continued)

Partition coefficients for *tert*-butanol		
Blood:air	481	Borghoff et al. (1996)
Liver:blood	0.83	Borghoff et al. (1996)
Fat:blood	0.4	Borghoff et al. (1996)
Richly perfused:blood	0.83	Borghoff et al. (1996)
Poorly perfused:blood	1.0	Borghoff et al. (1996)
Kidney:blood	0.83	Borghoff et al. (2001)

*Values have been updated to incorporate corrections from a quality assurance (QA) review and to include values to the number of digits used in the model code.
[a]0.165—Σ(kidney,liver.blood).
[b]Lower limit of alveolar ventilation for rat reported in Brown et al. (1997); alveolar ventilation is set equal to cardiac output.
[c]Sum of liver and gastrointestinal blood flows.
[d]Brown et al. (1997) only accounts for 94% of the blood flow. This assumes unaccounted 6% is richly perfused.
[e]0.47—Σ(kidney, liver).
[f]0.53—fat.
[g]Set equal to muscle tissue (Borghoff et al., 2016).
[h]Set equal to richly perfused tissue (Borghoff et al., 2016).

Table B-2. PBPK model rate constants

Parameter	Value	Source or reference
***tert*-butanol rate constants**		
TBA first-order absorption constant (1/h)	5.0	Salazar et al. (2015)
Fraction of TBA absorbed in alveolar region	0.6	Medinsky et al. (1993)
Urinary clearance of TBA (L/h/kg$^{0.75}$)	0.015	Borghoff et al. (2016)
Scaled maximum metabolic rate of TBA (µmol/h/kg)	54	Borghoff et al. (1996), Rao and Ginsberg (1997)
Michaelis-Menten constant (µmol/L)	457[a]	Borghoff et al. (1996), Rao and Ginsberg (1997)
Maximum percentage increase in metabolic rate	0.0	124.9 used by Leavens and Borghoff (2009)
Rate constant for ascent to maximum (1/d)[b]	0.3977	Leavens and Borghoff (2009)
ETBE rate constants		
ETBE first-order absorption constant (1/h)	1.6	Leavens and Borghoff (2009)
Scaled maximum metabolic rate of ETBE (µmol/h/kg$^{0.75}$)	499	Rao and Ginsberg (1997)
Michaelis-Menten constant for ETBE (µmol/L)	430[a]	Rao and Ginsberg (1997)
alpha 2u-globulin binding parameters		
Steady-state free kidney alpha 2u-globulin (µmol/L)	550[c]	Leavens and Borghoff (2009)
First-order constant for hydrolysis of free alpha 2u-globulin (1/h)	0.31	Leavens and Borghoff (2009)
First-order constant for hydrolysis of bound alpha 2u-globulin (1/h)	0.11	Leavens and Borghoff (2009)
Second-order binding constant for TBA to alpha 2u-globulin (L/µmol/h)	1.3	Leavens and Borghoff (2009)
Alpha 2u-globulin dissociation constant for TBA (µmol/L)	120	Leavens and Borghoff (2009)
First-order constant for unbinding of TBA from alpha 2u-globulin (1/h)	Calculated[d]	
Second-order binding constant for ETBE to alpha 2u-globulin (L/µmol/h)	0.15	Leavens and Borghoff (2009)
Alpha 2u-globulin dissociation constant for ETBE (µmol/L)	1	Leavens and Borghoff (2009)

Table B-2. PBPK model rate constants (continued)

Parameter	Value	Source or reference
First order constant for unbinding of ETBE from alpha 2u-globulin (1/h)	Calculated[e]	

[a]Based on dividing the original values in Borghoff et al. (1996) and Rao and Ginsberg (1997) [used by Borghoff et al. (2016)] by the corresponding liver partition coefficients: 379/0.83 = 457 for *tert*-butanol kinetics, and 1,248/2.9 = 430 for ETBE kinetic pathway 1.
[b]Note: Model revised from a daily stepwise induction change to a continuous change (with a 12-h time lag), while still maintaining the default parameters.
[c]Based on values ranging from ~160 to 1,000 µmol/L (Carruthers et al., 1987; Charbonneau et al., 1987; Olson et al., 1987; Stonard et al., 1986).
[d]Product of alpha 2u-globulin dissociation constant for *tert*-butanol and second-order binding constant for *tert*-butanol to alpha 2u-globulin.
[e]Product of alpha 2u-globulin dissociation constant for ETBE and second-order binding constant for ETBE to alpha 2u-globulin.

B.1.8. Toxicokinetic Data Extraction and Selected Model Outputs

Data extraction and adjustments

The ARCO (1983) study reported *tert*-butanol blood levels after oral gavage exposure primarily as *tert*-butanol equivalents based on total ^{14}C activity, which does not distinguish between *tert*-butanol and its metabolites. However, for oral doses of 1 and 500 mg/kg, the fractions of activity identifiable as *tert*-butanol were also reported, although not at identical time points. Therefore, empirical bi-exponential curves (see Figure B-7) were used to interpolate between the time points when total *tert*-butanol equivalents were measured to estimate total equivalents at other times. The total equivalents calculated this way were then multiplied by the fraction of *tert*-butanol reported at 0.5, 3, 6, and 12 hours for 1 mg/kg (ARCO (1983), see Table 24) and 500 mg/kg (ARCO (1983), see Table 25) to obtain the data used for PBPK modeling (see Table B-4).

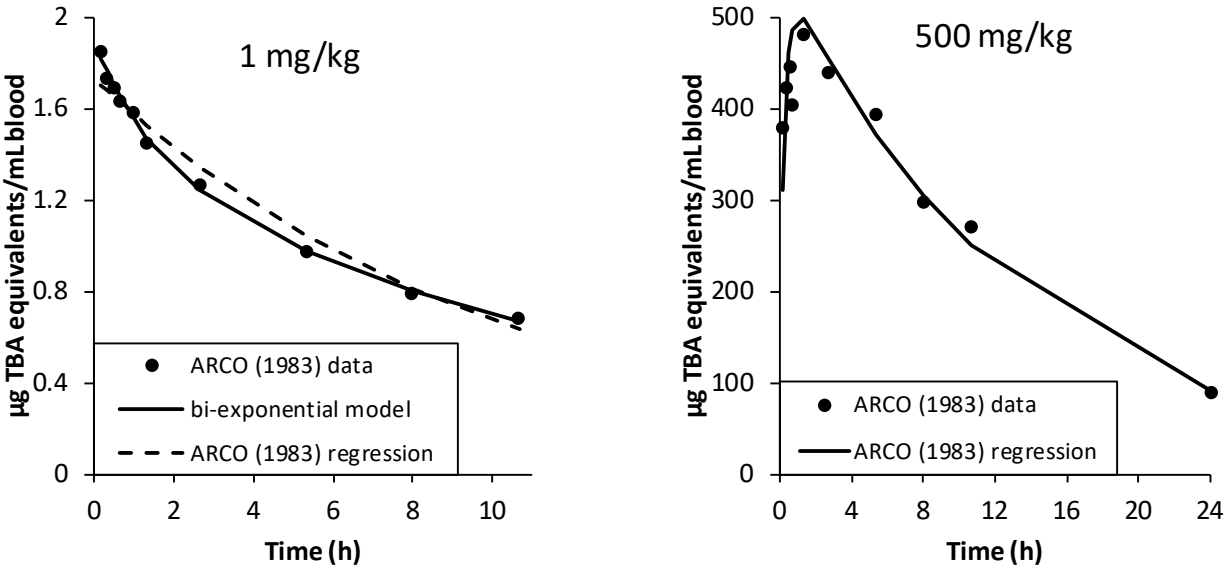


Figure B-7. *tert*-Butanol PK data for 1 and 500 mg/kg oral exposures from ARCO (1983).

Time-course data and empirical regressions for *tert*-butanol equivalents in rats following oral exposure to 1 or 500 mg/kg ^{14}C-TBA (ARCO, 1983). For 1 mg/kg, the single exponential regression reported by ARCO (1983) was 1.73*exp(−0.0946**t*; dashed line), but it did not appear to adequately fit the data. A bi-exponential regression (solid line) was found by minimizing the sum of square errors between the regression and data in Excel: 0.4874*exp(−0.7055**t*) + 1.404*exp(−0.06983**t*). For 500 mg/kg the bi-exponential regression reported by ARCO (1983) appeared sufficient: 554*exp(−0.0748**t*) − 426*exp(−3.51**t*).

The single-dose data from JPEC (2008a) were taken from Appendix Table 12 of that report. The values for the P-5 component were converted from ETBE equivalents to mg/L *tert*-butanol. For example, at 5 mg/kg-day, 416 ng ETBE-eq/mL is reported for P-5 in animal #17. The corresponding concentration in mg/L for *tert*-butanol is then calculated as (416 ng ETBE-eq/mL) × (1,000 mL/L) × (10^{-6} mg/ng) × (74.12 [molecular weight (MW) *tert*-butanol])/(102.17 [MW ETBE]) = 0.302 mg *tert*-butanol-eq/L. Likewise, the data for the repeated-dose study (JPEC, 2008d), Days 7 and 14, were converted from the P-5 values in Appendix Table 7, p. 53 of that report. (The data from the single-dose study were combined with the Days 7 and 14 data from the multiple-dose study for comparison with model simulations of 14-day dosing.)

The JPEC (2008a) studies measured *tert*-butanol in plasma only, unlike the Poet et al. (1997) and Leavens and Borghoff (2009) studies, which measured *tert*-butanol in whole blood. Based on the measurements of plasma and whole blood by JPEC (2008a), the concentration of *tert*-butanol in plasma is approximately 130% of the concentration in whole blood (see Table B-5). The *tert*-butanol plasma concentrations measured by Japan Petroleum Energy Center (JPEC) were therefore divided by 1.3 to obtain the expected concentration in whole blood for comparison with the PBPK model.

Table B-3. Summary of pharmacokinetic data used for model calibration and evaluation

Exposure		Measured		Data source	Figure Number in Salazar et al. (2015)	Conversion	Notes
Chemical	Route	Chemical	Medium				
TBA	i.v.	TBA	Blood	Poet et al. (1997) Figures 1 and 2	3A	µM to mg/L	Digitized from the figure
	Inhalation	TBA	Blood	Leavens and Borghoff (2009) Figure 8A-B	3B	µM to mg/L	Digitized from the figure showing only 1 d of exposure
	Gavage	TBA	Blood	ARCO (1983), percentage total TBA, Tables 24–25; TBA equivalents, Figure 6	3C	TBA equivalents to TBA concentration	
ETBE	Gavage	TBA	Blood	JPEC (2008a) Appendix 12	4A	ETBE equivalents to mg/L TBA	"P5" is TBA
		TBA	Urine	JPEC (2008a) Appendix 13	4B	ETBE equivalents to mg/L TBA	"P5" is TBA
ETBE	Inhalation	ETBE	Blood	Amberg et al. (2000) Table 5	4C	µM to mg/L	
		TBA	Blood	Amberg et al. (2000)Table 5	4D	µM to mg/L	
		TBA	Urine	Amberg et al. (2000) Table 6 and Figure 4	4E	µM to mg/L	
		ETBE	Exhaled air	Borghoff et al. (1996)	4F	µmoles to mg	Cumulative mass
		TBA	Exhaled air	Borghoff et al. (1996)	4G	µmoles to mg	Cumulative mass
TBA	Inhalation	TBA	Blood	Leavens and Borghoff (2009) Figure 8B	5A–B	µM to mg/L	Digitized from the figure
		TBA	Blood	Leavens and Borghoff (2009) Figure 8A	5C–D	µM to mg/L	Digitized from the figure
ETBE	Gavage	TBA	Blood	JPEC (2008a) Appendix 12	5E	ETBE equivalents to mg/L TBA	"P5" is TBA

Table B-4. Conversion of ARCO (1983) total *tert*-butanol equivalents and serum fraction data to *tert*-butanol concentrations

Time (h)	% TBA[a]	Total TBA equivalents interpolated (µg/mL)[b]	TBA concentration using interpolated equivalents (µg/mL = mg/L)[c]	Total TBA equivalents measured at nearest time-point (time measured; h)[d]	TBA concentration using nearest time-point (mg/L)[e]
1 mg/kg data					
0.5	57.3	1.6982	0.9731	1.69 (0.5)	0.9684
3	25	1.1972	0.2993	1.26 (2.67)	0.3150
6	18.1	0.9304	0.1684	0.97 (5.33)	0.1756
12	1	0.6074	0.006074	0.68 (10.67)	0.006800
500 mg/kg data					
0.5	22.9	460.0	105.34	445 (0.5)	101.91
3	20.4	442.6	90.30	438 (2.67)	89.35
6	18.7	353.7	66.14	393 (5.33)	73.49
12	18.5	225.8	41.77	269 (10.67)	49.77

[a]From Table 24, p. 48 of ARCO (1983) (1 mg/kg) and Table 25, p. 49 of ARCO (1983) (500 mg/kg).
[b]Using bi-exponential functions given in the legend of Figure B-7.
[c]Values used in PBPK modeling; %TBA × total TBA equivalents interpolated.
[d]From Table 14, p. 32 of ARCO (1983) (1 mg/kg) and Table 11, p. 27 of ARCO (1983) (500 mg/kg).
[e]%TBA × total TBA equivalents at nearest time-point.

Table B-5. Ratio of ^{14}C activity in blood versus plasma after ^{14}C-ETBE exposures in rats (JPEC, 2008a)

Time (h)	Animal number	Plasma (ng ^{14}C-eq/mL)	Blood (ng ^{14}C-eq/mL)	Plasma/blood (%)
Single dose, JPEC (2008a) Appendix Table 5, p. 94				
8	97	78,133	40,667	192.1
	98	95,533	80,000	119.4
	99	89,367	64,667	138.2
	100	72,400	62,333	116.2
24	37	10,900	8,800	123.9
	38	19,133	14,433	132.6
	39	19,433	15,400	126.2
	40	30,767	22,967	134.0
72	41	2,133	1,600	133.3
	42	2,833	3,033	93.4
	43	4,033	3,200	126.0
	44	3,167	2,333	135.7
			Mean ± SD	**130.9 ± 22.8**
Single dose, JPEC (2008a) Appendix Table 3, p. 91				
8	17	2,853	1,784	159.9
	18	2,850	1,802	158.2
	19	2,629	1,568	167.7
	20	3,918	2,718	144.2
24	21	1,692	1,255	134.8
	22	846.7	642.9	131.7
	23	1,048	785	133.5
	24	761.7	591.3	128.8
72	25	49.6	40	124.0
	26	34.2	29.2	117.1
	27	79.2	60.8	130.3
	28	107.9	84.6	127.5

Table B-5. Ratio of ^{14}C activity in blood versus plasma after ^{14}C-ETBE exposures in rats (JPEC, 2008a) (continued)

Time (h)	Animal number	Plasma (ng ^{14}C-eq/mL)	Blood (ng ^{14}C-eq/mL)	Plasma/blood (%)
168	29	12.9	13.3	97.0
	30	17.5	13.8	126.8
	31	26.7	24.2	110.3
	32	40	35.8	111.7
			Mean ± SD	**131.5 ± 18.9**
Repeated dose, JPEC (2008a), Appendix Table 3, p. 49				
8 (7 d dosing)		3,789	3,029	125.1
		5,041	3,988	126.4
		4,914	3,938	124.8
		5,608	4,638	120.9
24 (7 d dosing)		2,740	1,908	143.6
		3,433	2,575	133.3
		2,488	1,888	131.8
		963.3	812.5	118.6
8 (14 d dosing)		5,665	4,546	124.6
		5,175	4,075	127.0
		3,889	3,058	127.2
		5,090	3,858	131.9
24 (14 d dosing)		2,003	1,508	132.8
		2,121	1,692	125.4
		1,948	1,354	143.9
		1,037	804.2	128.9
72 (14-d dosing)		1,378	1,138	121.1
		301.3	245.8	122.6
		110	N.D.	
		421.3	337.5	124.8
			Mean ± SD	**128.1 ± 6.85**

N.D. = not detected; SD = standard deviation.

Selected model comparisons applying the Borghoff et al. (2016) model

The modeling code was obtained by the authors of Borghoff et al. (2016). The modeling language and platforms is acslX (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, Alabama).

The following modifications were made:

1) The periodic drinking water pathway was incorporated into the continuous simulation language (CSL) file, and the continuous oral dose rate function was modified slightly to improve flexibility of the model.

2) For simulations showing the effect of including enzyme induction, the code was modified slightly in the CSL file to improve continuity. Daily step functions in metabolic chances were replaced with a continuous function but delayed by 12 hours.

3) Otherwise enzyme induction was not used (set to zero).

4) In the PBPK model code, the changes to the Michaelis-Menten constants described as footnotes in Table B-2 above were not made in the PBPK parameter script (MTBEparam.m). Instead, parameters were redefined in the core model *CSL file as scaling calculations in the parameters section of the INITIAL bloc:

    a. Km1vetbe = Km1etbe/Pletbe

    b. Km2vetbe = Km2etbe/Pletbe

    c. Kmvtba = Kmtba/Pltba

5) Tissue volumes and the rate of hydrolysis of free alpha 2u-globulin were corrected (slightly) to values shown in Table B-1.

6) All model scripts previously used to evaluate model fits of the Salazar et al. (2015) model were adapted to run the Borghoff et al. (2016) model. Model parameters were set to uniform values for all simulations highlighted in this section, unless otherwise noted.

7) Digitized data from Amberg et al. (2000) were updated subsequent to a quality assurance (QA) review.

8) Tabulated data from Borghoff and Asgharian (1996) were updated subsequent to a QA review.

The PBPK acslX model code is available electronically through EPA's HERO database. All model files may be downloaded in a zipped workspace from HERO (U.S. EPA, 2016). The model contains workspaces for the EPA implementation of the Salazar et al. (2015) model, the unchanged version of the Borghoff et al. (2016) model, and the EPA implementation of the Borghoff et al. (2016) model.

Selected model outputs compared with the experimental data sets are provided in Figure B-8.

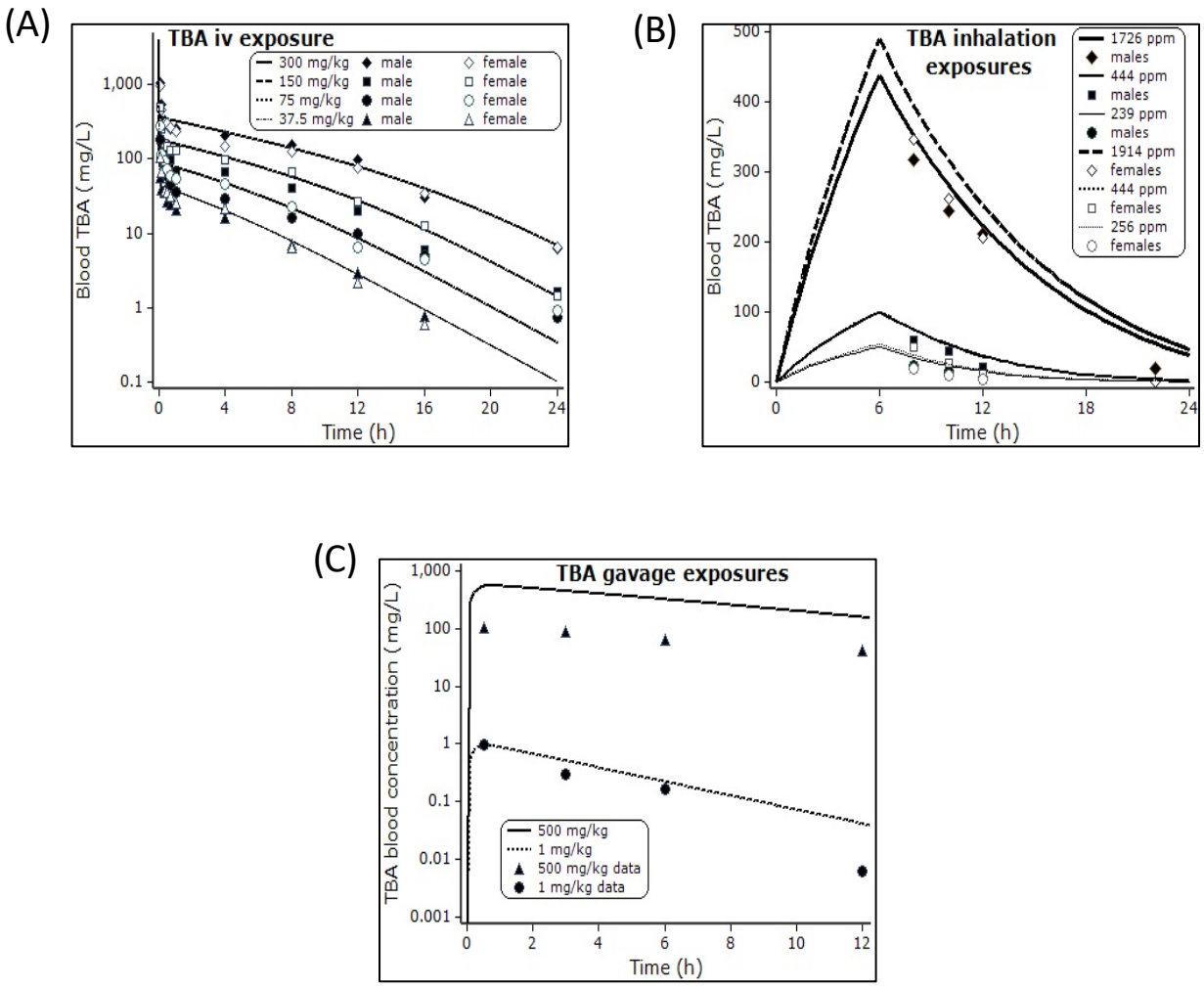


Figure B-8. Comparison of the Borghoff et al. (2016) model predictions with measured *tert*-butanol blood concentrations for i.v., inhalation, and gavage exposure to *tert*-butanol.

Source: (A) i.v. data from Poet et al. (1997); (B) inhalation data from Leavens and Borghoff (2009); and (C) gavage data from ARCO (1983).

The model results for the i.v. data are significantly improved from the Blancato et al. (2007) and Leavens and Borghoff (2009) model results presented previously. As evident here and in the Borghoff et al. (2016) study, the Borghoff et al. (2016) model generally over-predicts *tert*-butanol blood and urine concentrations (see Figure B-9). Some attempts were made to improve model fit in the EPA model implementation (such as adjusting inhalation, urinary, and induction parameter values); however, the default values were maintained in the final model.

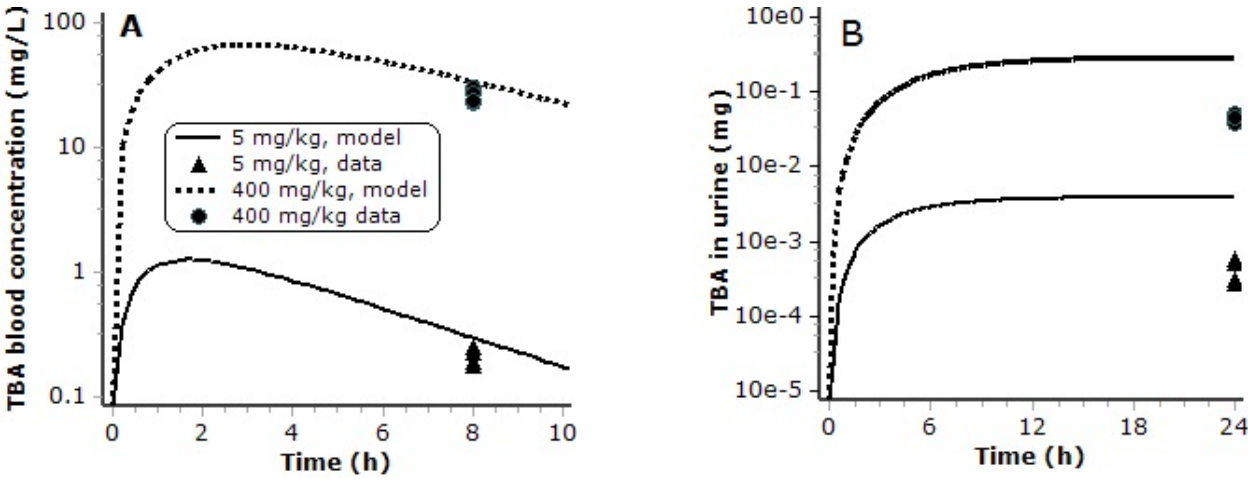


Figure B-9. Comparison of Borghoff et al. (2016) model predictions with measured amounts of *tert*-butanol after gavage of ETBE.

The data points show the measurements from the four individual rats in the JPEC (2008a) study. The concentrations of *tert*-butanol in blood are shown in (A). The amount of *tert*-butanol in urine is shown in (B). Note that the over-prediction of *tert*-butanol in urine (B) is by a factor 3- to 10-fold.

The predictions of the model are compared with amounts measured by Amberg et al. (2000) after ETBE inhalation in Figure B-10. The prediction of the *tert*-butanol blood concentrations are slightly higher than was measured. The *tert*-butanol blood concentration would be reduced if exposed animals were reducing their breathing rate or other breathing parameters, but the exposure concentration of ETBE exposure is unlikely to be high enough to cause a change in breathing parameters because, at the much higher ETBE concentration in the ARCO (1983) study (5,000 ppm), changes in breathing were not noted; the model already uses a lower-bound estimate of respiration rate and cardiac output for all simulations, and the model predictions fit most measured concentrations well. However, the urinary elimination of *tert*-butanol is significantly overestimated (~ 3- to 10-fold) by the *tert*-butanol submodel (see Figure B-11).

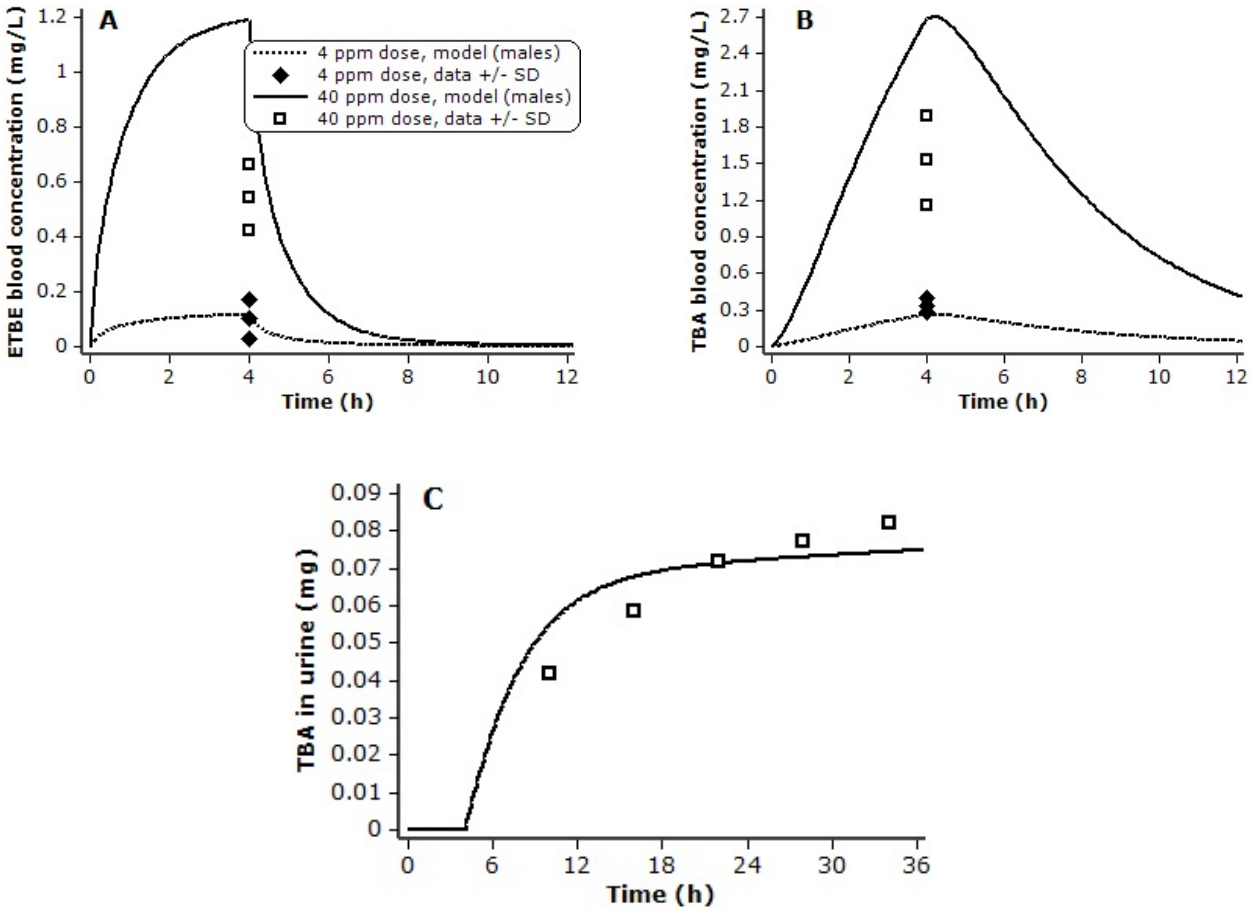


Figure B-10. Comparison of Borghoff et al. (2016) model predictions with measured amounts after a 4-hour inhalation exposure to 4 and 40 ppm ETBE.

Concentrations in blood are shown in (A) for ETBE, (B) for *tert*-butanol. The amount of *tert*-butanol in urine is shown in (C) for the 40 ppm exposure. The data are from Amberg et al. (2000).

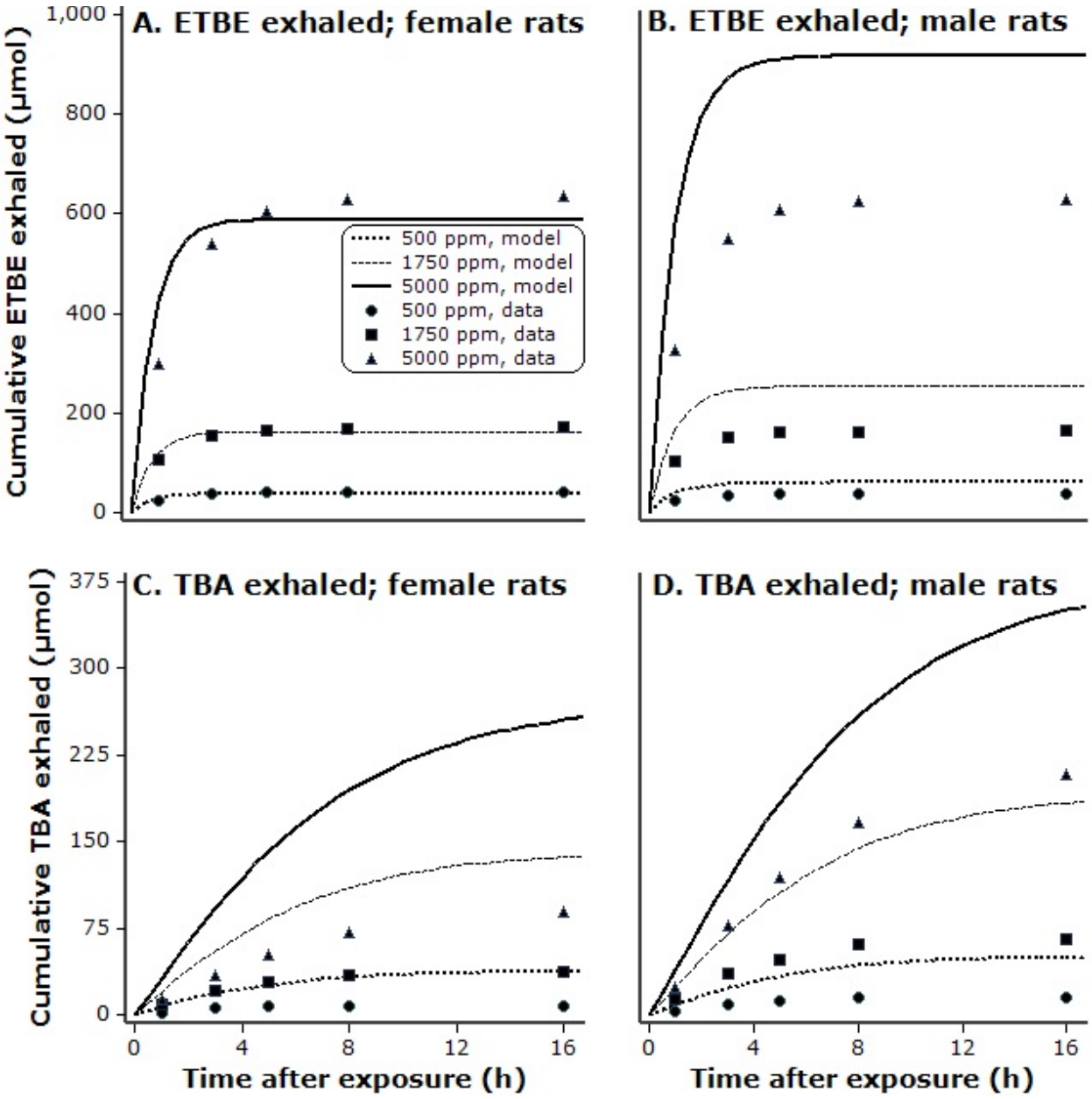


Figure B-11. Comparison of Borghoff et al. (2016) model predictions with measured amounts of ETBE and *tert*-butanol in exhaled breath after a 6-hour inhalation exposure to 500, 1,750, and 5,000 ppm ETBE.

The data points are from the Borghoff and Asgharian (1996) study. The model significantly over-predicted exhaled breath of both ETBE and *tert*-butanol following ETBE inhalation exposure for male rats and the exhaled *tert*-butanol for female rats. The model currently assumes that 100% of inhaled ETBE, but only 60% of inhaled *tert*-butanol, is available for alveolar absorption. The inhalation availability may have a significant impact on estimated exhaled breath amounts but was not adjusted to fit this data set.

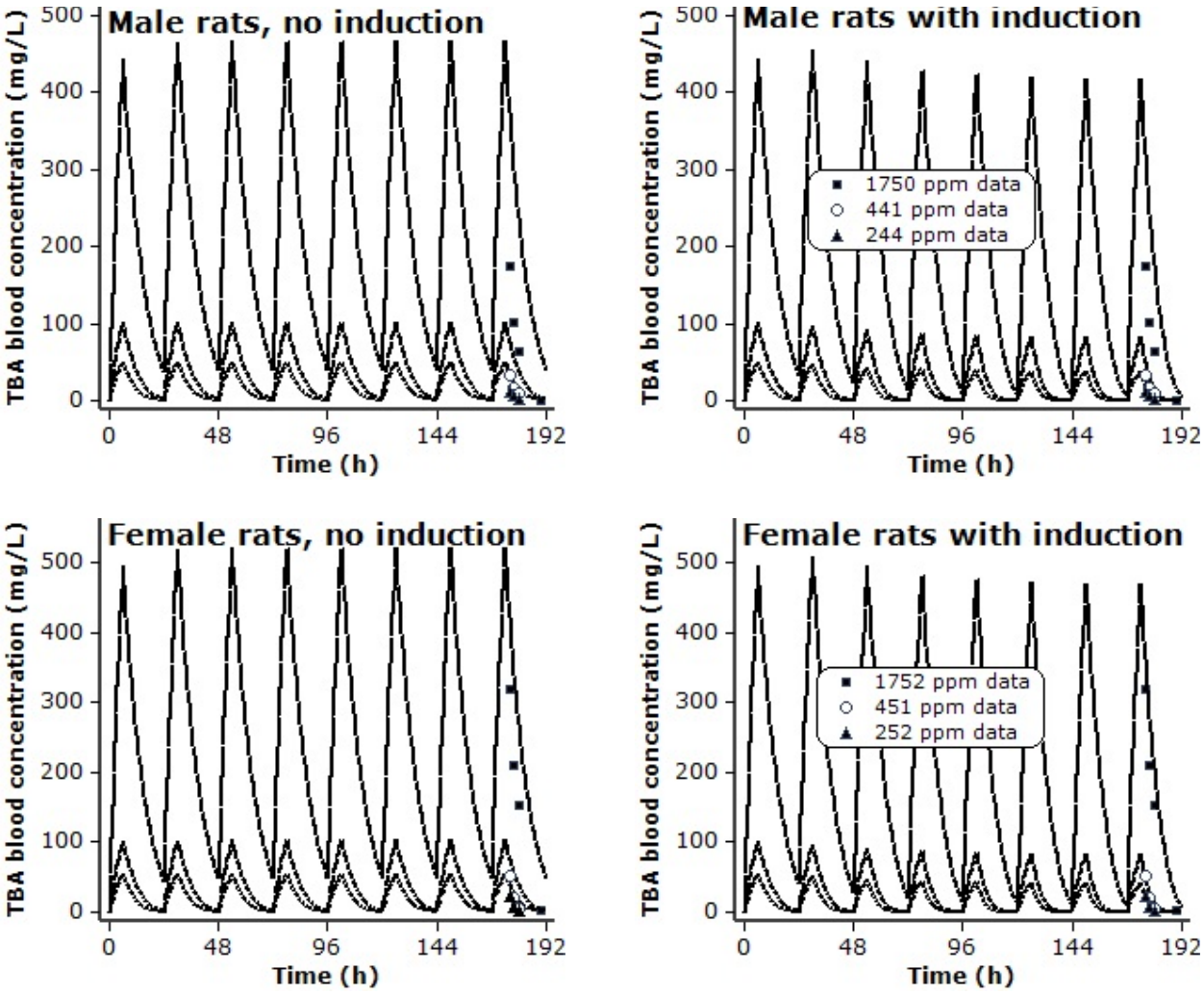


Figure B-12. Comparison of the Borghoff et al. (2016) model predictions with measured amounts of *tert*-butanol in blood after repeated inhalation exposure to *tert*-butanol.

Male rats were exposed to 239, 444, or 1,726 ppm and female rats were exposed to 256, 444, or 1,914 ppm *tert*-butanol for up to 8 consecutive days (Borghoff et al., 2001). *tert*-Butanol blood concentrations are better predicted by the model after 8 days of exposure with enzyme induction (right panels) compared to those without enzyme induction (left panels).

The increased *tert*-butanol metabolism better estimates the measured *tert*-butanol blood concentrations as shown in a comparison of the model predictions and experimental measurements in Figure B-12. The male rats have lower *tert*-butanol blood concentrations after repeated exposures than female rats, and this difference could indicate greater induction of *tert*-butanol metabolism in males or other physiologic changes, such as in ventilation or urinary excretion (see Figure B-13).

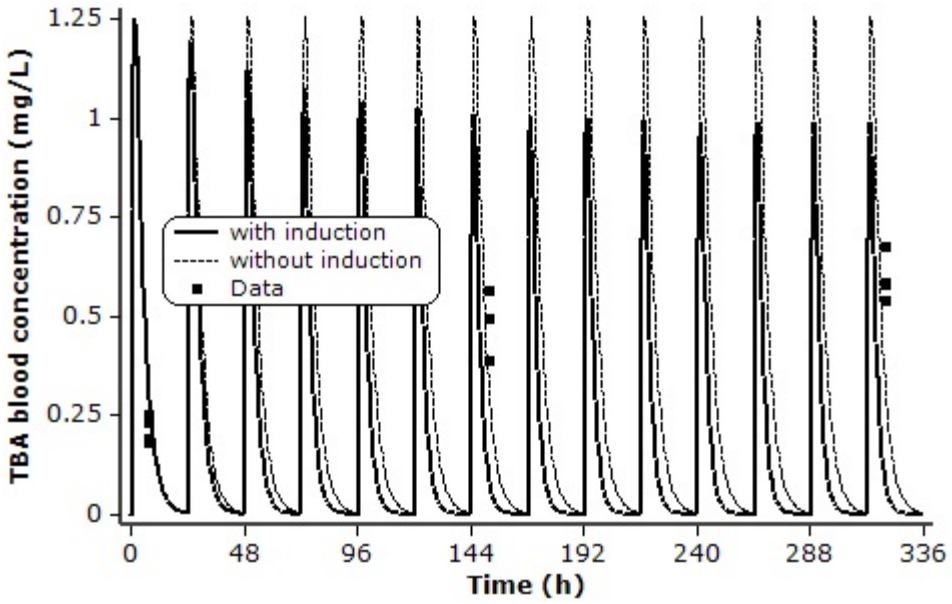


Figure B-13. Comparison of EPA model predictions with measured amounts of *tert*-butanol in blood after 5 mg/kg-day ETBE gavage for up to 14 days in male rats.

The data show the individual measurements of the four rats in the JPEC (2008a) study. Adding enzyme induction to the model has a small effect on the predicted *tert*-butanol blood concentrations, and the model predictions are closer to measured data when induction is not included.

B.2. OTHER PERTINENT TOXICITY INFORMATION

B.2.1. Other Toxicological Effects

Synthesis of Other Effects

Effects other than those related to kidney, thyroid, reproduction, development, and neurodevelopment were observed in some of the available rodent studies. These include liver and urinary bladder effects. As previously mentioned in the *Study Selection* section of the Toxicological Review, all studies discussed employed inhalation, gavage, or drinking water exposures for ≥30 days. Studies are arranged in evidence tables by effect, species, duration, and design. The design, conduct, and reporting of each study were reviewed, and each study was considered adequate to provide information pertinent to this assessment.

Central nervous system effects similar to those of ethanol (i.e., animals appearing intoxicated and having withdrawal symptoms after cessation of oral or inhalation exposure) were observed with *tert*-butanol. Severity of central nervous system symptoms increased with dose and duration of exposure. Study quality and utility concerns associated with these studies [e.g., inappropriate exposure durations, lack of data reporting, small number of animals per treatment group; Grant and Samson (1981); Snell and Harris (1980); Thurman et al. (1980);

McComb and Goldstein (1979a, 1979b); Wood and Laverty (1979)] preclude an understanding of potential neurotoxicity following *tert*-butanol exposure; therefore, central nervous system studies are not discussed further.

Exposure-response arrays of liver and urinary bladder effects are provided in Figure B-14 and Figure B-15 for oral and inhalation studies, respectively.

Kidney effects

Absolute and relative kidney-weight numerical data are presented in Table B-6.

Liver effects

Liver weight and body weight were demonstrated to be proportional, and liver weight normalized to body weight was determined to be optimal for data analysis (Bailey et al., 2004); thus, only relative liver weight is presented and considered in the determination of hazard. Although some rodent studies observed liver effects (organ weight changes and histopathologic lesions), the effects were not consistent across the evidence base. Increases in relative liver weight with *tert*-butanol exposure were observed, but the results pertaining to histopathologic changes were inconsistent (see Table B-7 and Table B-8). The NTP (1995) oral subchronic and chronic studies did not observe treatment-related effects on liver histopathology in either sex of F344 rats. In a 10-week study in Wistar rats, several liver lesions (including necrosis) and increased liver glycogen were observed in male rats (no females were included in the study) at 575 mg/kg-day, the only dose used (Acharya et al., 1997; Acharya et al., 1995). The study provided no incidence or severity data. The dose used in this rat study was in the range of the lower doses used in the NTP (1995) subchronic rat study. An increased incidence of fatty liver was observed in the male mice of the highest dose group in the 2-year mouse bioassay, but no histopathologic changes were seen in the subchronic mouse study (NTP, 1995). No treatment-related effects in liver histopathology were observed in rats or mice in the NTP (1997) subchronic inhalation study.

Urinary bladder effects

Subchronic studies reported effects in the urinary bladder (see Table B-9), although the chronic studies indicated little progression in incidence with increased exposure. Transitional epithelial hyperplasia of the urinary bladder was observed in male rats and male mice after 13 weeks of exposure at doses of 3,610 mg/kg-day (male rats) and ≥3,940 mg/kg-day (male mice). In rats, the increase in transitional epithelial hyperplasia of the urinary bladder was not observed in the 2-year study. Male mice exposed at the high dose (2,070 mg/kg-day) for 2 years exhibited minimal transitional epithelial hyperplasia of the urinary bladder. Neither female rats nor female mice showed increased incidences of this lesion. Both sexes of mice demonstrated incidences of minimal to mild inflammation in the urinary bladder after both subchronic and chronic exposures, with a greater incidence in males than in females.

Mechanistic Evidence

No mechanistic evidence is available for liver and urinary bladder effects.

Summary of Other Toxicity Data

Based on lack of consistency and lack of progression, the available evidence does not support liver and urinary bladder effects, respectively, as potential human hazards of *tert*-butanol exposure.

Table B-6. Changes in kidney weight in animals following exposure to *tert*-butanol

Reference and study design	Results				
Kidney weight (percentage change as compared to control)					
Huntingdon Life Sciences (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d M: 9 wk beginning 4 wk prior to mating F: ≈ 10 wk (4 wk prior to mating through PND 21)	**Males**				
	Dose mg/kg-d	**Left absolute weight**	**Left relative weight**	**Right absolute weight**	**Right relative weight**
	0	0	0	0	0
	64	6	8	6	8
	160	9	14*	6	11*
	400	12*	14*	14*	17*
	1,000	18*	28*	20*	31*
	Females				
	Dose mg/kg-d	**Left absolute weight**	**Left relative weight**	**Right absolute weight**	**Right relative weight**
	0	0	0	0	0
	64	−1	−2	2	0
	160	0	0	1	0
	400	3	2	4	2
	1,000	4	0	7	2

NTP (1995) F344/N rat; 10/sex/treatment Drinking water 0, 2.5, 5, 10, 20, or 40 mg/mL M: 0, 230, 490, 840, 1,520, or 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, or 3,620[a] mg/kg-d 13 wk	**Males**			**Females**		
	Dose mg/kg-d	**Absolute weight**	**Relative weight**	**Dose mg/kg-d**	**Absolute weight**	**Relative weight**
	0	0	0	0	0	0
	230	12*	19*	290	19*	17*
	490	17*	26*	590	16*	15*
	840	16*	32*	850	29*	28*
	1,520	26*	54*	1,560	39*	40*
	3,610	All dead	All dead	3,620	36*	81*

Table B-6. Changes in kidney weight in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results					
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, or 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, or 11,620[a] mg/kg-d 13 wk	Males			Females		
	Dose mg/kg-d	Absolute weight	Relative weight	Dose mg/kg-d	Absolute weight	Relative weight
	0	0	0	0	0	0
	350	1	1	500	0	−3
	640	3	2	820	−3	−1
	1,590	2	8	1,660	1	0
	3,940	6	22*	6,430	6	15*
	8,210	0	48*	11,620	12*	35*
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 mo) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 yr	Males			Females		
	Dose mg/kg-d	Absolute weight	Relative weight	Dose mg/kg-d	Absolute weight	Relative weight
	0	0	0	0	0	0
	90	4	8	180	8*	14*
	200	11	15*	330	18*	21*
	420	7	20*	650	22*	42*
	Only rats sacrificed at 15 mo were evaluated for organ weights.					
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273, or 6,368 mg/m^3) (dynamic whole-body chamber) 6 h/d, 5 d/wk 13 wk Generation method (Sonimist ultrasonic spray nozzle nebulizer), analytical concentration and method were reported *Right kidney weights measured*		Males		Females		
	Concentration mg/m^3	Absolute weight	Relative weight	Absolute weight	Relative weight	
	0	0	0	0	0	
	406	1	1	−4	−1	
	824	−2	−1	0	1	
	1,643	3	2	4	4	
	3,273	11*	8*	2	2	
	6,368	9.8*	9*	4	9*	

Table B-6. Changes in kidney weight in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results				
NTP (1997) $B6C3F_1$ mouse; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole-body chamber) 6 h/d, 5 d/wk 13 wk Generation method (Sonimist ultrasonic spray nozzle nebulizer), analytical concentration and method were reported *Right kidney weights measured*		Males		Females	
	Concentration mg/m^3	**Absolute weight**	**Relative weight**	**Absolute weight**	**Relative weight**
	0	0	0	0	0
	406	−6	−4	1	−3
	824	−1	3	5	9
	1,643	4	3	1	−2
	3,273	−10	−3	0	7
	6,368	3	6	3	15*

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

Table B-7. Changes in liver weight in animals following exposure to *tert*-butanol

Reference and study design	Results					
Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 wk	No significant treatment-related effects (results were only provided in a figure).					
Huntingdon Life Sciences (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d M: 9 wk beginning 4 wk prior to mating F: 4 wk prior to mating through PND21	**Percentage change compared to control**					
	Males			**Females**		
	Dose mg/kg-d	**Absolute weight**	**Relative weight**	**Dose mg/kg-d**	**Absolute weight**	**Relative weight**
	0	-	-	0	-	-
	64	−1	0	64	−4	−4
	160	−3	1	160	−7	−5
	400	−2	−1	400	2	1
	1,000	8	16*	1,000	8	3
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 wk	**Percentage change compared to control**					
	Males			**Females**		
	Dose mg/kg-d	**Absolute weight**	**Relative weight**	**Dose mg/kg-d**	**Absolute weight**	**Relative weight**
	0	-	-	0	-	-
	230	−2	4	290	11*	9*
	490	1	8*	590	10*	9*
	840	5	20*	850	12*	11*
	1,520	8	31*	1,560	15*	16*
	3,610	All dead	All dead	3,620	9*	41*

Table B-7. Changes in liver weight in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results					
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, or 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, or 11,620[a] mg/kg-d 13 wk	**Percentage change compared to control**					
	Males			**Females**		
	Dose mg/kg-d	**Absolute weight**	**Relative weight**	**Dose mg/kg-d**	**Absolute weight**	**Relative weight**
	0	-	-	0	-	-
	350	2	3	500	−1	−4
	640	−1	−2	820	−5	−3
	1,590	−1	5	1,660	−8	−9*
	3,940	0	14*	6,430	−2	6
	8,210	−16	22*	11,620	−6	13*
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 mo) Drinking water (0, 1.25, 2.5, 5 or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 yr	**Percentage change compared to control**					
	Males			**Females**		
	Dose mg/kg-d	**Absolute weight**	**Relative weight**	**Dose mg/kg-d**	**Absolute weight**	**Relative weight**
	0	-	-	0	-	-
	90	2	7	180	−14*	−8
	200	8	11	330	−3	−1
	420	1	14*	650	−6	9*
	Only animals sacrificed at 15 mo were evaluated for organ weights. Organ weights were not measured in the 2-yr mouse study.					
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole-body chamber) 6 h/d, 5 d/wk 13 wk Generation method (Sonimist ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	**Percentage change compared to control**					
		Males		**Females**		
	Concentration mg/m^3	**Absolute weight**	**Relative weight**	**Absolute weight**	**Relative weight**	
	0	-	-	-	-	
	406	−8	−8	0	3	
	824	−2	−1	0	0	
	1,643	1	−1	3	2	
	3,273	10	7	9	9*	
	6,368	5	5	4	8*	

Table B-7. Changes in liver weight in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results				
NTP (1997) $B6C3F_1$ mouse; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole-body chamber) 6 h/d, 5 d/wk 13 wk Generation method (Sonimist ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	Percentage change compared to control				
		Males		Females	
	Concentration mg/m^3	Absolute weight	Relative weight	Absolute weight	Relative weight
	0	-	-	-	-
	406	−1	0	1	−4
	824	4	9	1	5
	1,643	7	5	5	1
	3,273	−8	−2	2	9*
	6,368	5	7	8	21*

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

Table B-8. Changes in liver histopathology in animals following exposure to *tert*-butanol

Reference and study design	Results			
Acharya et al. (1997) Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0, 0.5%), 0 or 575 mg/kg-d 10 wk	↑ liver glycogen (~sevenfold)[a] ↑ incidence of centrilobular necrosis, vacuolation of hepatocytes, loss of hepatocyte architecture, peripheral proliferation, and lymphocyte infiltration (incidences and results of statistical tests not reported).			
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, or 3,610[b] mg/kg-d F: 0, 290, 590, 850, 1,560, or 3,620[b] mg/kg-d 13 wk	No treatment-related effects observed.			
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, or 8,210[b] mg/kg-d F: 0, 500, 820, 1,660, 6,430, or 11,620[b] mg/kg-d 13 wk	No treatment-related effects observed.			
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 mo) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[b] mg/kg-d F: 0, 180, 330, or 650[b] mg/kg-d 2 yr	No treatment-related effects observed.			
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[b] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 yr	**Males**		**Females**	
	Dose (mg/kg-d)	**Incidence of fatty change**	**Dose (mg/kg-d)**	**Incidence of fatty change**
	0	12/59	0	11/60
	540	5/60	510	8/60
	1,040	8/59	1,020	8/60
	2,070	29/59[a]	2,110	6/60

Table B-8. Changes in liver histopathology in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole-body chamber) 6 h/d, 5 d/wk 13 wk Generation method (Sonimist ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No treatment-related effects observed in the high-dose group (only treatment group with liver endpoints evaluated).
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole-body chamber) 6 h/d, 5 d/wk 13 wk Generation method (Sonimist ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	Authors stated that there was no treatment-related microscopic changes, but data were not provided.

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

Table B-9. Changes in urinary bladder histopathology in animals following oral exposure to *tert*-butanol

<table>
<tr><th>Reference and study design</th><th colspan="6">Results</th></tr>
<tr><td rowspan="9">NTP (1995)
F344/N rat; 10/sex/treatment
Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL)
M: 0, 230, 490, 840, 1,520, or 3,610[a] mg/kg-d
F: 0, 290, 590, 850, 1,560, or 3,620[a] mg/kg-d
13 wk</td><td colspan="6">Incidence (severity)</td></tr>
<tr><td colspan="2">Males</td><td colspan="4">Females</td></tr>
<tr><td>Dose (mg/kg-d)</td><td colspan="2">Transitional epithelial hyperplasia</td><td>Dose mg/kg-d</td><td colspan="2">Transitional epithelial hyperplasia</td></tr>
<tr><td>0</td><td colspan="2">0/10</td><td>0</td><td colspan="2">0/10</td></tr>
<tr><td>230</td><td colspan="2">Not evaluated</td><td>290</td><td colspan="2">Not evaluated</td></tr>
<tr><td>490</td><td colspan="2">Not evaluated</td><td>590</td><td colspan="2">Not evaluated</td></tr>
<tr><td>840</td><td colspan="2">0/10</td><td>850</td><td colspan="2">Not evaluated</td></tr>
<tr><td>1,520</td><td colspan="2">1/10 (3.0)</td><td>1,560</td><td colspan="2">0/10</td></tr>
<tr><td>3,610</td><td colspan="2">7/10* (2.9)</td><td>3,620</td><td colspan="2">3/10 (2.0)</td></tr>
<tr><td colspan="6">Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked</td></tr>
<tr><td rowspan="10">NTP (1995)
B6C3F$_1$ mouse; 10/sex/treatment
Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL)
M: 0, 350, 640, 1,590, 3,940, or 8,210[a] mg/kg-d
F: 0, 500, 820, 1,660, 6,430, or 11,620[a] mg/kg-d
13 wk</td><td colspan="6">Incidence (severity)</td></tr>
<tr><td colspan="3">Males</td><td colspan="3">Females</td></tr>
<tr><td>Dose mg/kg-d</td><td>Transitional epithelial hyperplasia</td><td>Inflammation</td><td>Dose mg/kg-d</td><td>Transitional epithelial hyperplasia</td><td>Inflammation</td></tr>
<tr><td>0</td><td>0/10</td><td>0/10</td><td>0</td><td>0/10</td><td>0/10</td></tr>
<tr><td>350</td><td colspan="2">Not evaluated</td><td>500</td><td>0/10</td><td>0/10</td></tr>
<tr><td>640</td><td colspan="2">Not evaluated</td><td>820</td><td colspan="2">Not evaluated</td></tr>
<tr><td>1,590</td><td>0/10</td><td>0/10</td><td>1,660</td><td colspan="2">Not evaluated</td></tr>
<tr><td>3,940</td><td>6/10* (1.3)</td><td>6/10* (1.3)</td><td>6,430</td><td>0/10</td><td>0/10</td></tr>
<tr><td>8,210</td><td>10/10* (2.0)</td><td>10/10* (2.3)</td><td>11,620</td><td>3/9 (2.0)</td><td>6/9* (1.2)</td></tr>
<tr><td colspan="6">Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked</td></tr>
<tr><td>NTP (1995)
F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 mo)
Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL)
M: 0, 90, 200, or 420[a] mg/kg-d
F: 0, 180, 330, or 650[a] mg/kg-d
2 yr</td><td colspan="6">No treatment-related effects observed.</td></tr>
</table>

Table B-9. Changes in urinary bladder histopathology in animals following oral exposure to *tert*-butanol (continued)

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

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

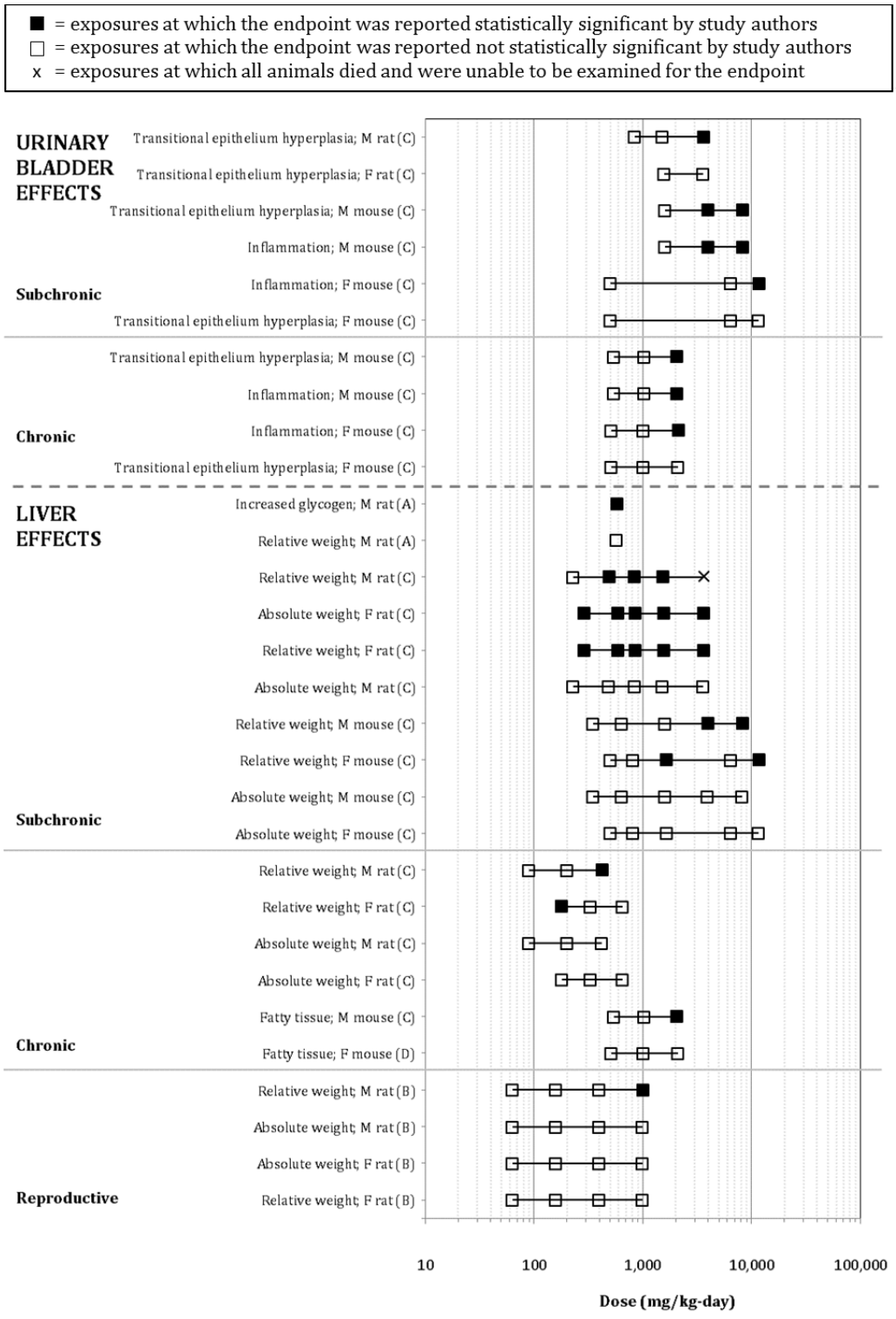


Figure B-14. Exposure-response array of other effects following oral exposure to *tert*-butanol.

Sources: (A) Acharya et al. (1997); Acharya et al. (1995); (B) Huntingdon Life Sciences (2004); (C) NTP (1995).

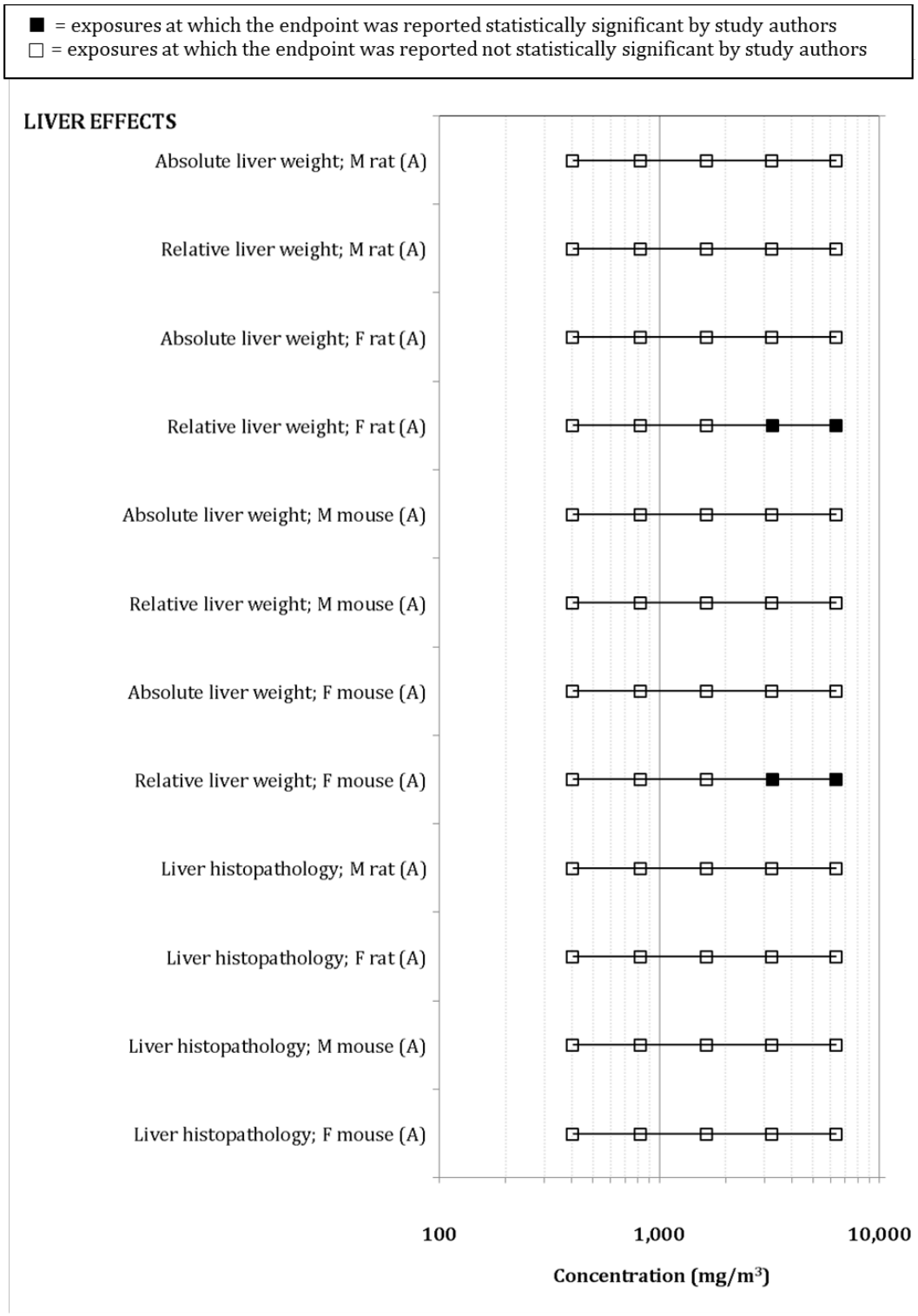


Figure B-15. Exposure-response array of other effects following inhalation exposure to *tert*-butanol.

Source: (A) NTP (1997).

B.2.2. Genotoxicity

The genotoxic potential of *tert*-butanol has been studied using a variety of assays, including bacterial reverse mutation assays, gene mutation assays, chromosomal aberrations, sister chromatid exchanges, micronucleus induction, and deoxyribonucleic acid (DNA) strand breaks and adducts. The available genotoxicity data for *tert*-butanol are discussed below, and the data summary is provided in Table B-10.

Bacterial Systems

The mutagenic potential of *tert*-butanol has been tested by Zeiger et al. (1987) using different *Salmonella typhimurium* strains both in the presence and absence of S9 metabolic activation. The preincubation assay protocol was followed. *Salmonella* strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to five concentrations (100, 333, 1,000, 3,333, or 10,000 µg/plate) and tested in triplicate. No mutations were observed in any of the strains tested, in either the presence or absence of S9 metabolic activation.

Conflicting results have been obtained with *tert*-butanol-induced mutagenicity in *Salmonella* strain TA102, a strain that is sensitive to damage at A-T sites inducible by oxidants and other mutagens and is excision-repair proficient. In a study by Williams-Hill et al. (1999), *tert*-butanol induced an increase in the number of revertants in the first three concentrations with S9 activation in a dose-response manner. The number of revertants decreased in the last two concentrations. No discussion was provided on why the revertants decreased at higher concentrations. The results of this study indicated that test strain TA102 might be a more sensitive strain for monitoring *tert*-butanol levels (Williams-Hill et al., 1999). In another study by McGregor et al. (2005), however, experiments were conducted on TA102 at two different laboratories using similar protocols. *tert*-Butanol was dissolved in dimethyl sulfoxide (DMSO) or distilled water and tested in both the presence and absence of S9 metabolic activation. No statistically significant increase in mutants was observed in either solvent medium.

Mutagenicity of *tert*-butanol has been studied in other systems, including *Neurospora crassa* and *Saccharomyces cerevisiae*. Yeast strain *Neurospora crassa* at the ad-3A locus (allele 38701) was used to test the mutagenic activity of *tert*-butanol at a concentration of 1.75 mol/L for 30 minutes. *tert*-Butanol did not induce reverse mutations in the tested strain at the exposed concentration (Dickey et al., 1949). *tert*-Butanol without exogenous metabolic activation, however, significantly increased the frequency of petite mutations (the mitochondrial DNA deletion rho−) in *Saccharomyces cerevisiae* laboratory strains K5-A5, MMY1, D517-4B, and DS8 (Jiménez et al., 1988). This effect on mitochondrial DNA, also observed with ethanol and other solvents, was attributed by the study authors to the alteration in the lipid composition of mitochondrial membranes, and mitochondrial DNA's close association could be affected by membrane composition (Jiménez et al., 1988).

In Vitro Mammalian Studies

To understand the role of *tert*-butanol-induced genotoxicity in mammalian systems, in vitro studies have been conducted in different test systems and assays. *tert*-Butanol was tested to evaluate its ability to induce forward mutations at the thymidine kinase (tk) locus in the L5178Y $tk^{\pm}$ mouse lymphoma cells using forward mutation assay. Experiments were conducted in both the presence and absence of S9 metabolic activation. The mutant frequency was calculated using the ratio of mutant clones per plate/total clones per plate × 200. *tert*-Butanol did not reliably increase the frequency of forward mutations in L5178Y $tk^{\pm}$ mouse lymphoma cells with or without metabolic activation, although one experiment without addition of S9 yielded a small (1.7-fold) increase in mutant fraction at the highest tested concentration [5,000 µg/mL; Mcgregor et al. (1988)].

To further determine potential DNA or chromosomal damage induced by *tert*-butanol in in vitro systems, NTP (1995) studied sister chromatid exchanges and chromosomal aberrations. Chinese hamster ovary (CHO) cells were exposed to *tert*-butanol in both the presence and absence of S9 activation at concentrations of 160–5,000 µg/mL for 26 hours. *tert*-Butanol did not induce sister chromatid exchanges at any concentration tested, although in one experiment, the percentage of relative change of sister chromatid exchanges per chromosome scored slightly increased. The same authors also studied the effect of *tert*-butanol on chromosomal aberration formation. CHO cells were exposed to four concentrations (160, 500, 1,600, or 5,000 µg/mL) of *tert*-butanol in both the presence and absence of S9. No significant increase in chromosomal aberration was observed at any concentration tested. Of note is that, due to severe toxicity at the highest concentration (5,000 µg/mL), only 13 metaphase cells were scored instead of 100 in the chromosomal aberration assay.

Sgambato et al. (2009) examined the effects of *tert*-butanol on DNA damage using a normal diploid rat fibroblast cell line. Cells were treated with 0- to 100-mM *tert*-butanol for 48 hours to determine the half-maximal inhibitory concentration (IC_{50}; 0.44 ± 0.2 mM). The 48-hour IC_{50} concentration then was used to determine DNA content, cell number, and phases of the cell cycle after 24 and 48 hours of exposure. Total protein and DNA oxidative damage also were measured. A comet assay was used to evaluate DNA fragmentation at time 0 and after 30 minutes, 4 hours, or 12 hours of exposure to the IC_{50} concentration. *tert*-Butanol inhibited cell division as measured by the number of cells after 24 and 48 hours of exposure at IC_{50} concentrations and with concentrations at 1/10th the IC_{50}. Cell death did not increase, suggesting a reduction in cell number due to reduced replication rather than to cytotoxicity. *tert*-Butanol caused an accumulation in the G_0/G_1 phase of replication, related to different effects on the expression of the *cyclin D1*, *p27Kip1*, and *p53* genes. An initial increase in DNA damage as measured by nuclear fragmentation was observed at 30 minutes. The DNA damage declined drastically after 4 hours and disappeared almost entirely after 12 hours of exposure to *tert*-butanol. This reduction in the extent of DNA

fragmentation after the initial increase is likely the result of an efficient DNA repair mechanism activated by cells following DNA damage induced by *tert*-butanol.

DNA damage caused by *tert*-butanol was determined by single-cell gel electrophoresis (comet assay) in human promyelocytic leukemia (HL-60) cells. The cells were exposed to concentrations ranging from 1 to 30 mmol/L for 1 hour, and 100 cells were evaluated for DNA fragmentation. A dose-dependent increase in DNA damage was observed between 1 and 30 mmol/L. No cytotoxicity was observed at the concentrations tested (Tang et al., 1997).

In Vivo Mammalian Studies

Few in vivo studies are available to understand the role of *tert*-butanol on genotoxicity. The National Toxicology Program (NTP) studied the effect of *tert*-butanol in a 13-week toxicity study (NTP, 1995). Peripheral blood samples were obtained from male and female $B6C3F_1$ mice exposed to *tert*-butanol in drinking water at doses of 3,000–40,000 ppm. Slides were prepared to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes. In addition, the percentage of polychromatic erythrocytes among the total erythrocyte population was determined. No increase in micronucleus induction in peripheral blood lymphocytes was observed either in male or female $B6C3F_1$ mice exposed for 13 weeks to *tert*-butanol in drinking water at concentrations as high as 40,000 ppm [2,110 mg/kg-day; NTP (1997, 1995)]. Furthermore, no induction of micronuclei in polychromatic erythrocytes was observed in bone marrow cells of male rats receiving intraperitoneal injections (NTP, 1997).

Male Kunming mice (eight per treatment) were administered 0, 0.099, 0.99, 10, 101, or 997 µg/kg BW ^{14}C-*tert*-butanol in saline via gavage with specific activity ranging from 1.60 to 0.00978 mCi/mol (Yuan et al., 2007). Animals were sacrificed 6 hours after exposure, and liver, kidney, and lung were collected. Tissues were prepared for DNA isolation with samples from the same organs from every two mice combined. DNA adducts were measured using accelerated mass spectrometry. The results of this study showed a dose-response increase in DNA adducts in all three organs measured, although the methodology used to detect DNA adducts is considered sensitive but could be nonspecific. The authors stated that their study was the first to find that *tert*-butanol formed DNA adducts in mouse liver, lung, and kidney. Because this is a single and first-time study, further validation of it will provide certainty in understanding the mechanism of *tert*-butanol-induced DNA adducts.

Table B-10. Summary of genotoxicity (both in vitro and in vivo) studies of *tert*-butanol

Test system	Dose/ concentration	Results[a]		Comments	Reference
		–S9	+S9		
Bacterial systems					
Reverse mutation assay *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	100, 333, 1,000, 3,333, 10,000 µg/plate	–	–	Preincubation procedure was followed; this study was part of the NTP 1995 testing results	Zeiger et al. (1987);NTP (1995)
Reverse mutation assay *Salmonella typhimurium* (TA102)	1,000–4,000 µg/plate	ND	+	Only tested with S9 activation	Williams-Hill et al. (1999)
Reverse mutation assay *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537)	5, 15, 50, 100, 150, 200, 500, 1,000, 1,500, 2,500, 5,000 µg/plate	–	–	Experiments conducted in two different laboratories, two vehicles—distilled water and DMSO were used, different concentrations were used in experiments from different laboratories	McGregor et al. (2005)
Reverse mutation *Neurospora crassa*, ad-3A locus (allele 38701)	1.75 mol/L	–	–	84% cell death was observed; note it is a 1949 study	Dickey et al. (1949)
Mitochondrial mutation *Saccharomyces cerevisiae* (K5-5A, MMY1, D517-4B, and DS8)	4.0% (vol/vol)	+[b]	ND	Mitochondrial mutations, membrane solvent	Jiménez et al. (1988)
In vitro systems					
Gene mutation assay, mouse lymphoma cells L5178Y tk$^{\pm}$	625, 1,000, 1,250, 2,000, 3,000, 4,000, 5,000 µg/mL	–	–	Cultures were exposed for 4 h, then cultured for 2 d before plating in soft agar with or without trifluorothymidine, 3 µg/mL; this study was part of the NTP 1995 testing results	Mcgregor et al. (1988);NTP (1995)
Sister-chromatid exchange, Chinese hamster ovary cells	160, 500, 1,600, 2,000, 3,000, 4,000, 5,000 µg/mL	–	–	This study was part of the NTP 1995 testing results	Galloway et al. (1987); NTP (1995)
Chromosomal aberrations, Chinese hamster ovary cells	160, 500, 1,600, 2,000, 3,000, 4,000, 5,000 µg/mL	–	–	This study was part of the NTP 1995 testing results	Galloway et al. (1987); NTP (1995)

Table B-10. Summary of genotoxicity (both in vitro and in vivo) studies of *tert*-butanol (continued)

Test system	Dose/ Concentration	Results[a]		Comments	Reference
DNA damage (comet assay), rat fibroblasts	0.44 mmol/L (IC_{50})	+[c]	ND	Exposure duration—30 min, 4 h, 12 h; this study provides other information on the effect of cell cycle control genes and mechanism of action for TBA	Sgambato et al. (2009)
DNA damage, (comet assay), HL-60 leukemia cells	1, 5, 10, 30 mmol/L	+	ND	Exposure duration—1 h	Tang et al. (1997)
In vivo animal studies					
Micronucleus induction, $B6C3F_1$ mouse peripheral blood cells	3,000, 5,000, 10,000, 20,000, 40,000 ppm	−		13-wk, subchronic, drinking water study	NTP (1995)
Micronucleus induction, male rats, bone marrow cells	39, 78, 156, 312, 625, 1,250	−		i.p. injections—3 times at 24-h intervals	NTP (1997)
DNA adducts, male Kunming mouse liver, kidney, and lung cells	0.1−1,000 µg/kg body weight	+		Gavage, 6-h exposure, DNA adduct determined by accelerator mass spectrometry	Yuan et al. (2007)

NTP = National Toxicology Program.
[a]+ = positive; − = negative; ND = not determined.
[b]Effect is predicted to be due to mitochondrial membrane composition.
[c]DNA damage was completely reversed with increased exposure time.

B.2.3. Summary

tert-Butanol has been tested for its genotoxic potential using a variety of genotoxicity assays. In general, a positive result in the Ames assay is 73−77% predictive of a positive result in the rodent carcinogenicity assay (Kirkland et al., 2005). *tert*-Butanol did not induce mutations in most bacterial strains; however, when tested in TA102, a strain that is sensitive to damage at A-T sites inducible by oxidants, an increase in mutants was observed at low concentrations, although conflicting results were reported in another study. Furthermore, the solvent (e.g., distilled water or DMSO) used in the genotoxicity assay could influence results. In one experiment where *tert*-butanol was dissolved in distilled water, a significant, dose-related increase in the number of mutants was observed, with the maximum value reaching almost twice the control value. DMSO is known to be a radical scavenger, and its presence in high concentrations might mask a mutagenic response modulated by oxidative damage. Exposure to *tert*-butanol did not produce reverse mutations in other species such as *Neurospora crassa*.

tert-Butanol was tested in several human and animal in vitro mammalian systems for genotoxicity (gene mutation, sister chromatid exchanges, chromosomal aberrations, and DNA damage). No increase in gene mutations was observed in mouse lymphoma cells (L5178Y $tk^{\pm}$). These specific locus mutations in mammalian cells are used to demonstrate and quantify genetic damage, thereby confirming or extending the data obtained in the more widely used bacterial cell tests. Sister chromatid exchanges or chromosomal aberrations were not observed in CHO cells in response to *tert*-butanol treatment. DNA damage was detected using a comet assay in both rat fibroblasts and HL-60 leukemia cells with either an increase in DNA fragmentation at the beginning of the exposure or dose-dependent increase in DNA damage. An initial increase in DNA damage was observed at 30 minutes that declined drastically following 4 hours of exposure and disappeared almost entirely after 12 hours of exposure to *tert*-butanol. This reduction in the extent of DNA fragmentation after an initial increase is likely the result of an efficient DNA repair mechanism activated by cells following DNA damage induced by *tert*-butanol. A dose-dependent increase in DNA damage was observed in human cells tested; however, because the exposure occurred for only 1 hour in this study, whether DNA-repair mechanisms would occur after a longer period of observation cannot be discerned.

Limited in vivo animal studies have been conducted on DNA adduct formation and micronucleus induction. A dose-response increase in DNA adducts was observed in mouse liver, kidney, and lung cells. The authors used accelerated mass spectrometry to detect DNA adducts, but the identity of these adducts was not determined. The method uses ^{14}C-labeled chemical for dosing. Isolated DNA is oxidized to carbon dioxide and reduced to filamentous graphite, and the ratios of $^{14}C/^{12}C$ are measured. The ratio is then converted to DNA adducts based on nucleotide content of the DNA. Confirmation of these data will further the understanding of the mechanism of *tert*-butanol-induced DNA adducts. No increase in micronucleus induction was observed in mouse peripheral blood cells in a 13-week drinking water study conducted by NTP.

Overall, a limited evidence base is available for understanding the role of *tert*-butanol-induced genotoxicity for mode of action and carcinogenicity. The evidence base is limited in terms of either the array of genotoxicity tests conducted or the number of studies within the same type of test. In addition, the results are either conflicting or inconsistent. The test strains, solvents, or control for volatility used in certain studies are variable and could influence results. Furthermore, in some studies, the specificity of the methodology used has been challenged. Given the inconsistencies and limitations of the evidence base in terms of the methodology used, the number of studies in the overall evidence base, the coverage of studies across the genotoxicity battery, and the quality of the studies, the weight-of-evidence analysis is inconclusive. The available data do not inform a definitive conclusion on the genotoxicity of *tert*-butanol and therefore the potential genotoxic effects of *tert*-butanol cannot be discounted.

APPENDIX C. DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant endpoints. The endpoints were modeled using EPA's Benchmark Dose Software (BMDS), version 2.1.2. The preambles for the noncancer and cancer sections below describe the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD as outlined in the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000). In some cases, using alternative methods based on statistical judgment might be appropriate; exceptions are noted as necessary in the summary of the modeling results.

C.1.1. Noncancer Endpoints

Data Sets

Data sets selected for dose-response modeling are provided in Table C-1. In all cases, administered exposure was used in modeling the response data.

Model Fit

All models were fit to the data using the maximum likelihood method. The following procedures were used, depending on whether data were dichotomous or continuous.

- For dichotomous models, the following parameter restrictions were applied: for the Log-Logistic model, restrict slope ≥1; for the Gamma and Weibull models, restrict power ≥1; and for the Multistage models, restrict beta values ≥0. Each model was tested for goodness of fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.10 indicates lack of fit). Other factors also were used to assess model fit, including scaled residuals, visual fit, and adequacy of fit in the low dose region and near the benchmark response (BMR).

- For continuous models, the following parameter restrictions were applied: for Polynomial models, restrict beta values ≥0; and for the Hill, Power, and Exponential models, restrict power ≥1. Model fit was assessed by a series of tests. For each model, the homogeneity of the variances was tested first using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p-value ≥ 0.10), the model was fit to the data assuming constant variance. If Test 2 was rejected (χ^2 p-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance,

models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p-value < 0.10 indicating inadequate fit). Other factors also were used to assess the model fit, including scaled residuals, visual fit, and adequacy of fit in the low-dose region and near the BMR.

Table C-1. Noncancer endpoints selected for dose-response modeling for *tert*-butanol

Endpoint/study	Species/ sex	Doses and effect data					
Kidney transitional epithelial hyperplasia NTP (1995)	Rat (F344)/female	Dose (mg/kg-d)	0	180	330	650	
		Incidence/total	0/50	0/50	3/50	17/50	
Increased absolute kidney weight NTP (1995)	Rat (F344)/female	Dose (mg/kg-d)	0	180	330	650	
		Mean ± SD (*n*)	1.07 ± 0.09 (10)	1.16 ± 0.10 (10)	1.27 ± 0.08 (10)	1.31 ± 0.09 (10)	
Kidney inflammation NTP (1995)	Rat (F344)/female	Dose (mg/kg-d)	0	180	330	650	
		Incidence/total	2/50	3/50	13/50	17/50	
Increased absolute kidney weight NTP (1997)	Rat (F344)/female	Concentration (mg/m^3)	0	406	825	1,643	3,274	6,369
		Mean ± SD (*n*)	0.817 ± 0.136 (10)	0.782 ± 0.063 (10)	0.821 ± 0.061 (10)	0.853 ± 0.045 (10)	0.831 ± 0.054 (10)	0.849 ± 0.038 (10)

Model Selection

For each endpoint, the benchmark dose lower confidence limit (BMDL) estimate (95% lower confidence limit on the benchmark dose [BMD], as estimated by the profile likelihood method) and the Akaike's information criterion (AIC) value were used to select a best-fit model among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by no more than threefold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

Modeling Results

Below are tables summarizing the modeling results for the noncancer endpoints modeled.

Table C-2. Summary of BMD modeling results for kidney transitional epithelial hyperplasia in female F344 rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d	Basis for model selection
	p-value	AIC			

Gamma	0.83	91.41	409	334	Multistage 3rd-order model selected as best-fitting model based on lowest AIC with all BMDL values sufficiently close (BMDLs differed by less than threefold).
Logistic	0.50	92.81	461	393	
Log-Logistic	0.79	91.57	414	333	
Log-Probit	0.89	91.19	400	327	
Multistage 3°	**0.92**	**89.73**	**412**	**339**	
Probit	0.62	92.20	439	372	
Weibull	0.76	91.67	421	337	
Dichotomous-Hill	N/A[b]	117.89	Error[c]	Error[c]	

[a]Scaled residuals for selected model for doses 0, 180, 330, and 650 mg/m^3 were 0.0, −0.664, 0.230, and 0.016, respectively.
[b]No available degrees of freedom to estimate a *p*-value.
[c]BMD and BMDL computation failed for the Dichotomous-Hill model.

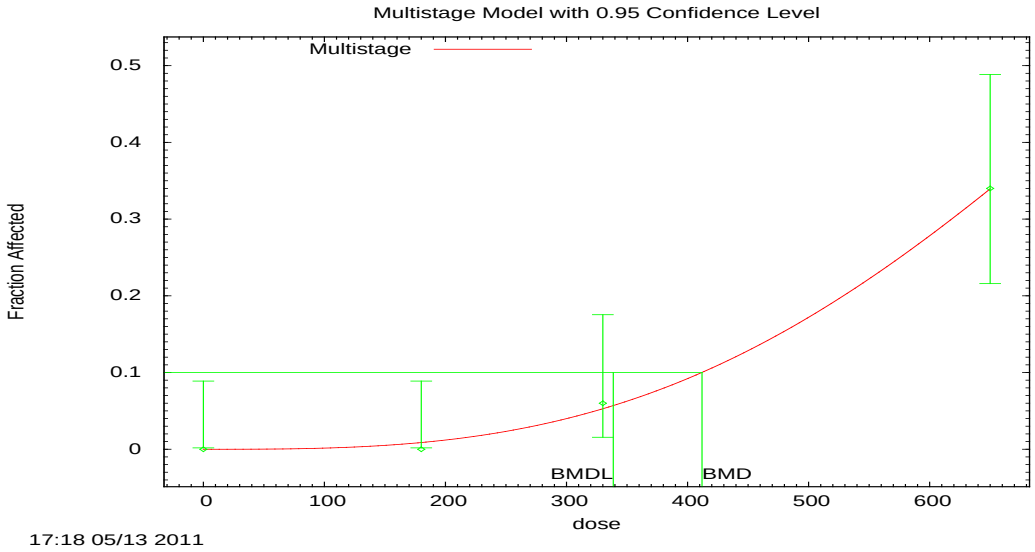


Figure C-1. Plot of incidence by dose, with fitted curve for Multistage 3° model for kidney transitional epithelial hyperplasia in female F344 rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-day.

```
=================================================================
        Multistage Model. (Version: 3.2; Date: 05/26/2010)
        Input Data File: M:\NCEA tert-butanol\BMD modeling\BMDS Output\20 NTP 1995b_Kidney
transitional epithelial hyperplasia, female rats_Multi3_10.(d)
        Gnuplot Plotting File: M:\NCEA tert-butanol\BMD modeling\BMDS Output\20 NTP 1995b_Kidney
transitional epithelial hyperplasia, female rats_Multi3_10.plt
                                                Mon May 09 18:31:33 2011
 =================================================================
```

```
 [notes]
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  The form of the probability function is:

  P[response] = background + (1-background)*[1-EXP(
        -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

  The parameter betas are restricted to be positive


  Dependent variable = Incidence
  Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



         Default Initial Parameter Values
           Background =      0
            Beta(1) =      0
            Beta(2) = 1.51408e-007
            Beta(3) = 1.29813e-009


      Asymptotic Correlation Matrix of Parameter Estimates

      ( *** The model parameter(s) -Background  -Beta(1)  -Beta(2)
         have been estimated at a boundary point, or have been specified by the user,
         and do not appear in the correlation matrix )

        Beta(3)

  Beta(3)      1



                 Parameter Estimates

                             95.0% Wald Confidence Interval
    Variable     Estimate    Std. Err.   Lower Conf. Limit  Upper Conf. Limit
   Background        0      *        *         *
    Beta(1)        0      *        *         *
    Beta(2)        0      *        *         *
    Beta(3)   1.50711e-009      *        *         *

* - Indicates that this value is not calculated.


            Analysis of Deviance Table

    Model   Log(likelihood) # Param's Deviance Test d.f.  P-value
   Full model    -43.4002     4
  Fitted model    -43.8652     1    0.9301   3     0.8182
 Reduced model    -65.0166     1    43.2329   3     <.0001

      AIC:     89.7304


                 Goodness of Fit
```

```
                                  Scaled
   Dose   Est._Prob.  Expected  Observed   Size    Residual
 ----------------------------------------------------------------------
  0.0000   0.0000     0.000   0.000     50    0.000
 180.0000   0.0088     0.438   0.000     50    -0.664
 330.0000   0.0527     2.636   3.000     50    0.230
 650.0000   0.3389    16.946  17.000     50    0.016

 Chi^2 = 0.49   d.f. = 3    P-value = 0.9200


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

Confidence level =      0.95

       BMD =     411.95

      BMDL =    338.618

      BMDU =     469.73

Taken together, (338.618, 469.73 ) is a 90   % two-sided confidence
interval for the BMD
```

Table C-3. Summary of BMD modeling results for absolute kidney weight in female F344 rats exposed to *tert*-butanol in drinking water for 15 months (NTP, 1995); BMR = 10% relative deviation from control mean

Model[a]	Goodness of fit		BMD_{10RD} mg/kg-d	$BMDL_{10RD}$ mg/kg-d	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0594	−144.00	318	249	The Exponential (M4) model was selected as the only model with adequate fit.
Exponential (M4)	**0.176**	**−145.81**	**164**	**91.4**	
Exponential (M5)	N/A[c]	−145.65	207	117	
Hill	N/A[c]	−145.65	202	119	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0842	−144.70	294	224	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.852), selected model in bold; scaled residuals for selected model for doses 0, 180, 330, and 650 mg/kg-d were 0.21, −0.9, 0.94, −0.25, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]No available degrees of freedom to calculate a goodness-of-fit value.

[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

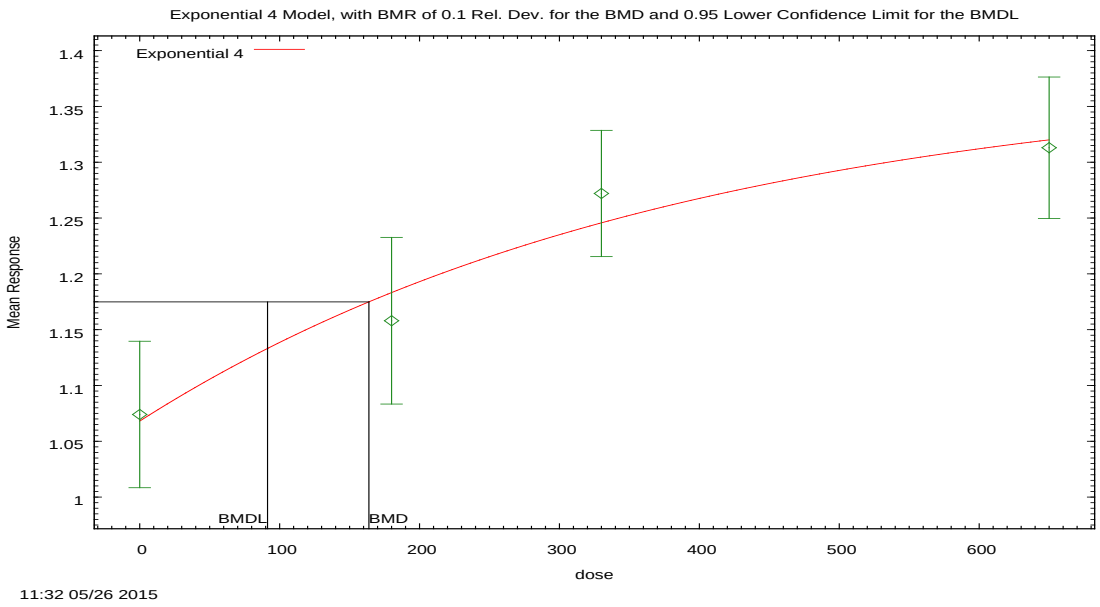

Figure C-2. Plot of mean response by dose, with fitted curve for Exponential (M4) model with constant variance for absolute kidney weight in female F344 rats exposed to *tert*-butanol in drinking water for 15 months (NTP, 1995); BMR = 10% relative deviation from control mean; dose shown in mg/kg-day.

Exponential Model. (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a *[c − (c − 1) *exp(−b * dose)].

A constant variance model is fit.

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 163.803

BMDL at the 95% confidence level = 91.3614

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−4.84526	−4.89115
rho	N/A	0
a	1.06808	1.0203
b	0.00258011	0.00282085
c	1.29013	1.35122
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	*N*	Observed mean	Estimated mean	Observed standard deviation	Estimated standard deviation	Scaled residuals
0	10	1.07	1.07	0.09	0.09	0.2112
180	10	1.16	1.18	0.1	0.09	−0.8984
330	10	1.27	1.25	0.08	0.09	0.9379
650	10	1.31	1.32	0.09	0.09	−0.2507

Likelihoods of Interest

Model	Log(likelihood)	# Parameters	AIC
A1	77.82307	5	−145.6461
A2	78.21688	8	−140.4338
A3	77.82307	5	−145.6461
R	62.21809	2	−120.4362
4	76.90527	4	−145.8105

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	32	6	<0.0001
Test 2	0.7876	3	0.8524
Test 3	0.7876	3	0.8524
Test 6a	1.836	1	0.1755

Table C-4. Summary of BMD modeling results for kidney inflammation in female rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10}% mg/kg-d	$BMDL_{10}$% mg/kg-d	Basis for model selection
	p-value	AIC			
Gamma	0.084	169.9	231	135	LogProbit was selected on the basis of the lowest AIC with all BMDL values for fitting models being sufficiently close (BMDLs differed by less than threefold).
Logistic	0.082	169.7	305	252	
LogLogistic	0.092	169.8	228	124	
LogProbit	**0.243**	**167.6**	**254**	**200**	
Multistage 3°	0.072	170.3	216	132	
Probit	0.108	169.2	285	235	
Weibull	0.081	170.0	226	134	
Dichotomous-Hill	N/A[b]	169.5	229	186	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 180, 330, and 650 mg/kg-d were −0.067, −0.700, 1.347, and −0.724, respectively.
[b]No available degrees of freedom to estimate a *p*-value.

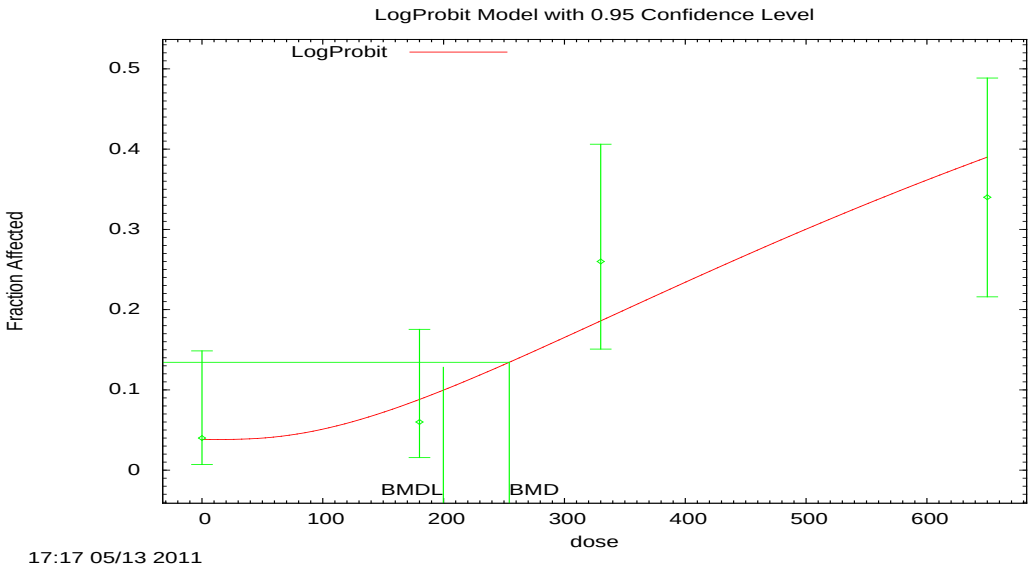

Figure C-3. Plot of incidence by dose, with fitted curve for LogProbit model for kidney inflammation in female rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-day.

```
================================================================
        Probit Model. (Version: 3.2; Date: 10/28/2009)
        Input  Data  File:  M:/NCEA tert-butanol/BMD  modeling/BMDS  Output/19  NTP  1995b_Kidney
inflammation, female rats_LogProbit_10.(d)
        Gnuplot Plotting File: M:/NCEA tert-butanol/BMD modeling/BMDS Output/19 NTP 1995b_Kidney
inflammation, female rats_LogProbit_10.plt
                                                    Fri May 13 17:17:59 2011
 ================================================================

 [notes]
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  The form of the probability function is:

  P[response] = Background
        + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

  where CumNorm(.) is the cumulative normal distribution function


  Dependent variable = Incidence
  Independent variable = Dose
  Slope parameter is restricted as slope >= 1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008



  User has chosen the log transformed model


          Default Initial (and Specified) Parameter Values
            background =     0.04
            intercept =   -8.01425
              slope =   1.18928


      Asymptotic Correlation Matrix of Parameter Estimates

      ( *** The model parameter(s) -slope
         have been estimated at a boundary point, or have been specified by the user,
         and do not appear in the correlation matrix )

       background  intercept

background      1    -0.51

 intercept    -0.51      1



                Parameter Estimates

                            95.0% Wald Confidence Interval
    Variable     Estimate    Std. Err.   Lower Conf. Limit  Upper Conf. Limit
   background    0.0381743    0.0246892     -0.0102155      0.0865642
   intercept     -6.82025     0.161407       -7.1366       -6.5039
     slope        1        NA

NA - Indicates that this parameter has hit a bound
   implied by some inequality constraint and thus
   has no standard error.


            Analysis of Deviance Table
```

```
   Model   Log(likelihood) # Param's Deviance Test d.f.  P-value
  Full model    -80.4502     4
 Fitted model    -81.8218     2    2.7432   2     0.2537
Reduced model    -92.7453     1    24.5902   3     <.0001

     AIC:     167.644


               Goodness of Fit
                                  Scaled
  Dose   Est._Prob.  Expected  Observed   Size    Residual
-----------------------------------------------------------------------
 0.0000   0.0382     1.909   2.000     50    0.067
180.0000   0.0880     4.402   3.000     50    -0.700
330.0000   0.1859     9.295  13.000     50    1.347
650.0000   0.3899    19.495  17.000     50    -0.724

Chi^2 = 2.83   d.f. = 2    P-value = 0.2427


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

Confidence level =      0.95

       BMD =    254.347

      BMDL =    199.789
```

Table C-5. Summary of BMD modeling results for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hours/day, 5 days/week for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean

Model[a]	Goodness of fit		BMC_{10RD} mg/m³	$BMCL_{10RD}$ mg/m³	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0378	−261.52	14,500	7,713	No model adequately fit the data.
Exponential (M4)	0.533	−267.48	Error[c]	0	
Exponential (M5)	0.374	−265.71	Error[c]	0	
Hill	0.227	−265.57	Error[c]	Error[c]	
Power	0.0392	−261.61	14,673	7,678	
Polynomial 3°[d] Polynomial 2°[e] Linear	0.0274	−261.61	14,673	7,678	
Polynomial 5°	0.0274	−261.61	14,673	7,569	
Polynomial 4°	0.0274	−261.61	14,673	7,674	

[a]Modeled variance case presented (BMDS Test 2 *p*-value = 1.90E−04, BMDS Test 3 *p*-value = 0.374), no model was selected as a best-fitting model.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]BMC or BMCL computation failed for this model.

[d]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.

[e]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Note: Graphs of the better fitting models are provided for illustration.

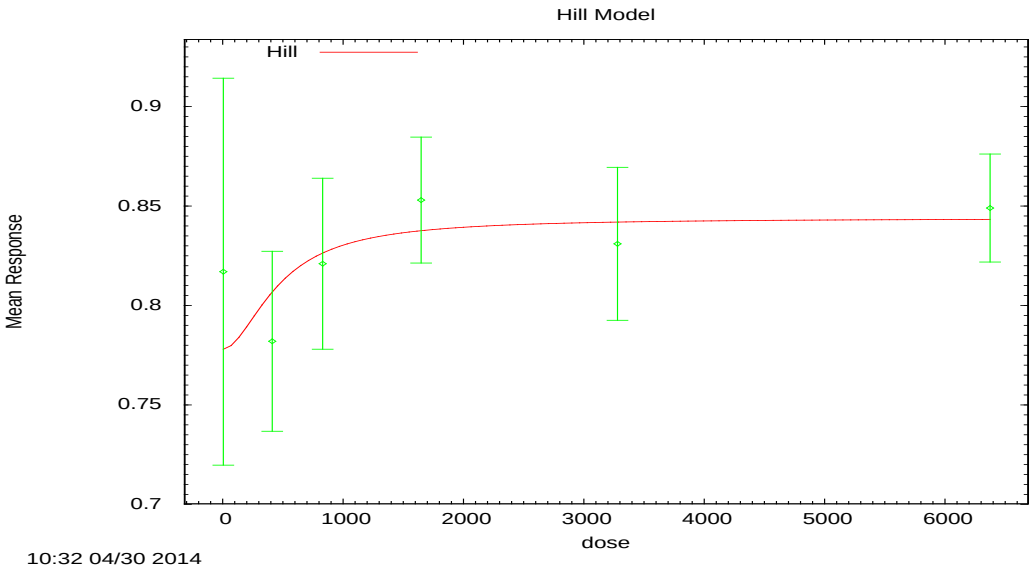

Figure C-4. Plot of mean response by concentration, with fitted curve for Hill model for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hours/day, 5 days/week for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m³.

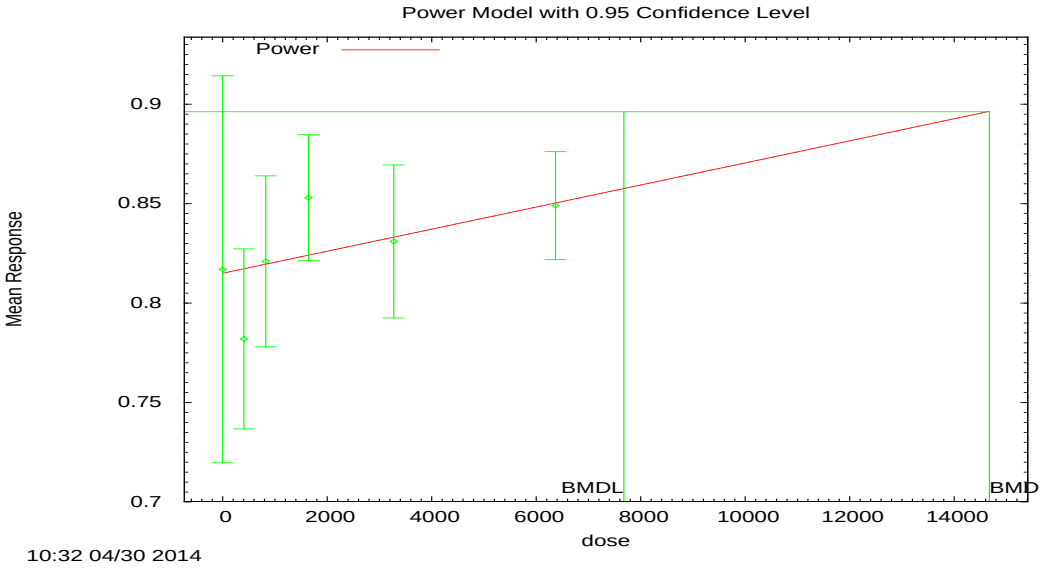

Figure C-5. Plot of mean response by concentration, with fitted curve for Power model for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hours/day, 5 days/week for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m³.

C.1.2. Cancer Endpoints

Data Sets

The cancer data sets selected for dose-response modeling are summarized in Table C-6. In all cases, administered exposure was used in modeling the response data. Because of the significant difference in survival in the high-dose male mice compared with the concurrent control, the Poly-3 procedure (Bailer and Portier, 1988) for adjusting tumor incidence rates for intercurrent mortality was used. The procedure is based on the observation that the cumulative incidence of tumors tends to increase with time raised to the second through the fourth powers for a large proportion of cases. In the Poly-3 procedure, for a study of T weeks' duration, an animal that is removed from the study after t weeks ($t < T$) without a specified type of tumor of interest is given a weight of $(t/T)^3$. An animal that survives until the terminal sacrifice at T weeks is assigned a weight of $(T/T)^3 = 1$. An animal that develops the specific type of tumor of interest obviously lived long enough to develop the tumor and is assigned a weight of 1. The Poly-3 tumor incidence, adjusted for intercurrent mortality up to time T, is the number of animals in a dose group with the specified type of tumor divided by the sum of the weights (the effective number of animals at risk). The tumor incidences, adjusted using this procedure, also are provided in Table C-6.

Model Fit

The Multistage model was fit to the cancer data sets. Model coefficients were restricted to be nonnegative (beta values ≥ 0) to estimate a monotonically increasing function. Each model was fit to the data using the maximum likelihood method and was tested for goodness of fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.05[1] indicates lack of fit). Other factors were used to assess model fit, including scaled residuals, visual fit, and adequacy of fit in the low dose region and near the BMR.

For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. For the NTP (1995) and Hard et al. (2011) data, models were run with all doses included, as well as with the high dose dropped. Dropping the high dose resulted in a better fit to the data. Including the high dose caused the model to overestimate the control.

[1]A significance level of 0.05 is used for selecting cancer models because the model family (Multistage) is selected a priori (U.S. EPA, 2000).

Table C-6. Cancer endpoints selected for dose-response modeling for *tert*-butanol

Endpoint/study	Species/sex	Doses and effect data				
Thyroid						
Thyroid follicular cell adenoma NTP (1995)	$B6C3F_1$ mice/female	Dose (mg/kg-d)	0	510	1,020	2,110
		Incidence/total	2/58	3/60	2/59	9/59
Thyroid follicular cell adenoma NTP (1995)	$B6C3F_1$ mice/male	Dose (mg/kg-d)	0	540	1,040	2,070
		Incidence/total	1/60	0/59	4/59	2/60
		Incidence/Poly-3 adjusted total	1/50	0/50	4/51	2/35
Kidney[a]						
Renal tubule adenoma or carcinoma NTP (1995)	Rat (F344)/Male	Dose (mg/kg-d)	0	90	200	420
		Incidence/total	8/50	13/50	19/50	13/50
Renal tubule adenoma or carcinoma NTP (1995)	Rat (F344)/Male	Incidence/total	8/50	13/50	19/50	13/50
Renal tubule adenoma or carcinoma NTP (1995)	Rat (F344)/Male	Incidence/total	8/50	13/50	19/50	13/50
Renal tubule adenoma or carcinoma; hard reanalysis NTP (1995);Hard et al. (2011)	Rat (F344)/Male	Dose (mg/kg-d)	0	90	200	420
		Incidence/total	4/50	13/50	18/50	12/50
Renal tubule adenoma or carcinoma; hard reanalysis NTP (1995);Hard et al. (2011)	Rat (F344)/Male	Incidence/total	4/50	13/50	18/50	12/50
Renal tubule adenoma or carcinoma; hard reanalysis NTP (1995);Hard et al. (2011)	Rat (F344)/Male	Incidence/total	4/50	13/50	18/50	12/50

[a]Endpoint presented if kidney tumors are acceptable for quantitation.

Table C-7. Summary of the oral slope factor derivations

Tumor	Species/sex	Selected model	BMR (%)	BMD mg/kg-d	POD = BMDL mg/kg-d	$BMDL_{HED}$[a] mg/kg-d	Slope factor[b] $mg/kg\text{-}d^{-1}$
Thyroid							
Thyroid follicular cell adenoma	Female $B6C3F_1$ mouse	3° Multistage	10	2,002	1,437	201	5×10^{-4}
Kidney[c]							
Renal tubule adenoma or carcinoma	Male F344 rat; dose as administered	1° multistage (high dose dropped)	10	70	42	10.1	1×10^{-2}
Renal tubule adenoma or carcinoma [Hard et al. (2011) reanalysis]	Male F344 rat; dose as administered	1° multistage (high dose dropped)	10	54	36	8.88	1×10^{-2}

HED = human equivalent dose.
[a]HED PODs were calculated using $BW^{3/4}$ scaling (U.S. EPA, 2011).
[b]Human equivalent slope factor = $0.1/BMDL_{10HED}$.
[c]Alternative endpoint if kidney tumors are acceptable for quantitation.

Modeling Results

Table C-8. Summary of BMD modeling results for thyroid follicular cell adenomas in female B6C3F$_1$ mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10}%[c] mg/kg-d	$BMDL_{10}$%[c] mg/kg-d	Basis for model selection
	p-value	AIC[b]			
Three	**0.75**	**113.665**	**2,002**	**1,437**	Multistage 3° was selected on the basis of the lowest AIC with all BMDL values for fitting models being sufficiently close (BMDLs differed by less than threefold).
Two	0.36	115.402	2,186	1,217	
One	0.63	114.115	1,987	1,378	

[a]Selected (best-fitting) model shown in boldface type.
[b]AIC = Akaike information criterion.
[c]Confidence level = 0.95.

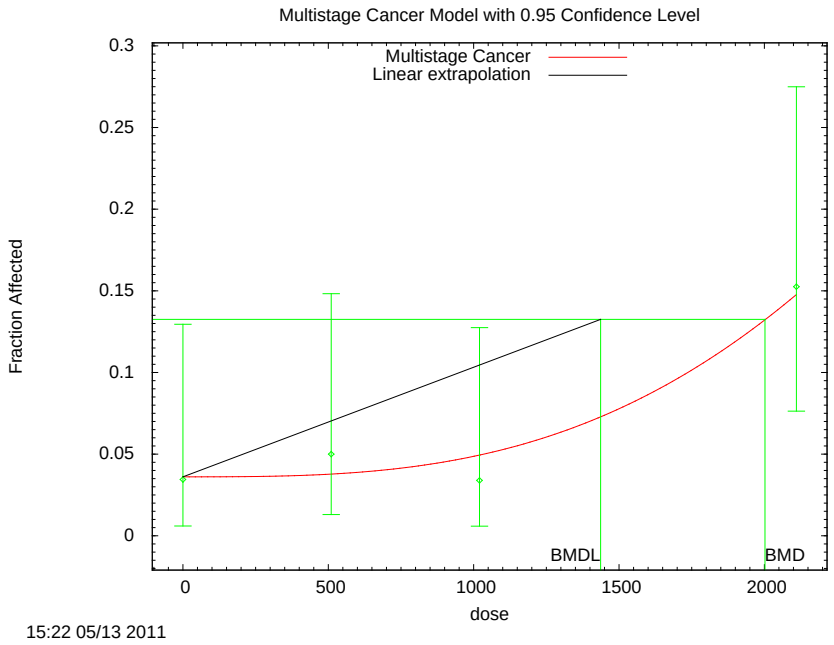

Figure C-6. Plot of incidence by dose, with fitted curve for Multistage 3° model for thyroid follicular cell adenomas in female B6C3F$_1$ mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-day.

```
================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input  Data  File:  M:\NCEA  t-Butanol\BMD  modeling\BMDS  Output\29  NTP  1995b_Thyroid
follicular cell adenoma, female mice_MultiCanc3_10.(d)
        Gnuplot Plotting File: M:\NCEA t-Butanol\BMD modeling\BMDS Output\29 NTP 1995b_Thyroid
follicular cell adenoma, female mice_MultiCanc3_10.plt
                                                  Fri May 13 15:22:18 2011
 ================================================================

 [notes]
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Incidence
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0347373
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 1.36917e-011


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(3)

Background            1        -0.53

   Beta(3)        -0.53            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0361209           *               *                   *
        Beta(1)                0           *               *                   *
        Beta(2)                0           *               *                   *
        Beta(3)     1.31301e-011           *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table
```

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-54.5437	4			
Fitted model	-54.8326	2	0.577881	2	0.7491
Reduced model	-58.5048	1	7.92235	3	0.04764

AIC: 113.665

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0361	2.095	2.000	58	-0.067
510.0000	0.0378	2.268	3.000	60	0.496
1020.0000	0.0495	2.918	2.000	59	-0.551
2110.0000	0.1480	8.730	9.000	59	0.099

Chi^2 = 0.56 d.f. = 2 P-value = 0.7544

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2002.03

BMDL = 1436.69

BMDU = 3802.47

Taken together, (1436.69, 3802.47) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 6.96043e-005

Table C-9. Summary of BMD modeling results for thyroid follicular cell adenomas or carcinomas in male B6C3F$_1$ mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 5% extra risk

Model[a]	Goodness of fit		BMD_5% mg/kg-d	$BMDL_5$%[c] mg/kg-d	Basis for model selection
	p-value	AIC[b]			
One, two, three	**0.202**	**61.6**	**1,788**	**787**	Multistage 1° was selected. Only form of multistage that resulted; fit adequate.

[a]Selected (best-fitting) model shown in boldface type.
[b]AIC = Akaike information criterion.
[c]Confidence level = 0.95.

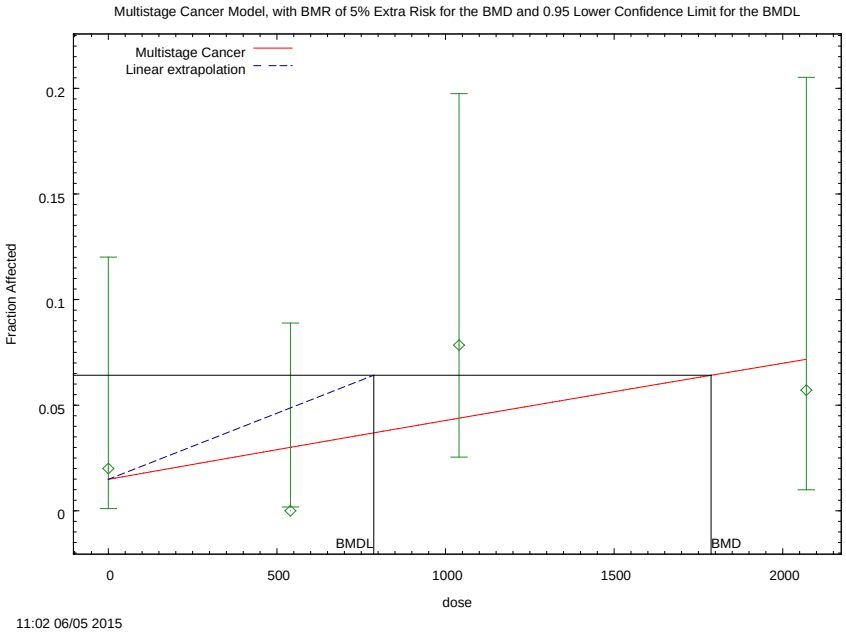

Figure C-7. Plot of incidence by dose, with fitted curve for Multistage 1° model for thyroid follicular cell adenomas or carcinomas in male B6C3F$_1$ mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 5% extra risk; dose shown in mg/kg-day.

```
 ================================================================
         Multistage Model. (Version: 3.4;  Date: 05/02/2014)
         Input  Data  File:  C:/Users/KHOGAN/BMDS/BMDS260/Data/msc_TBA  NTP1995  MMthyroid  tumors
poly3_Msc1-BMR05.(d)
         Gnuplot  Plotting  File:    C:/Users/KHOGAN/BMDS/BMDS260/Data/msc_TBA  NTP1995  MMthyroid
tumors poly3_Msc1-BMR05.plt
                                                  Fri Jun 05 11:02:14 2015
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =    0.0164855
                        Beta(1) = 2.58163e-005


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.56

   Beta(1)        -0.56            1



                               Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background        0.0149284        0.0144833          -0.0134584           0.0433151
        Beta(1)     2.86952e-005     1.99013e-005       -1.03105e-005         6.7701e-005



                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -26.5891         4
   Fitted model          -28.808         2       4.43785      2          0.1087
  Reduced model         -29.8255         1       6.47273      3         0.09074

          AIC:           61.616



                                  Goodness  of  Fit
```

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0149	0.746	1.000	50.000	0.296
540.0000	0.0301	1.504	0.000	50.000	-1.245
1040.0000	0.0439	2.238	4.000	51.000	1.204
2070.0000	0.0717	2.511	2.000	35.000	-0.335

Chi^2 = 3.20 d.f. = 2 P-value = 0.2019

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1787.52

BMDL = 787.153

BMDU did not converge for BMR = 0.050000
BMDU calculation failed
BMDU = Inf

Table C-10. Summary of BMD modeling results for thyroid follicular cell adenomas or carcinomas in male B6C3F$_1$ mice exposed to *tert*-butanol in drinking water for 2 years, high dose omitted (NTP, 1995); BMR = 5% extra risk

Model[a]	Goodness of fit		BMD_5% mg/kg-d	$BMDL_5$%[c] mg/kg-d	Basis for model selection
	p-value	AIC[b]			
One stage	0.105	46.0	1,341	538	Multistage 2° was selected based on lowest AIC.
Two stage	**0.174**	**44.9**	**1,028**	**644**	

[a]Selected (best-fitting) model shown in boldface type.
[b]AIC = Akaike information criterion.
[c]Confidence level = 0.95.

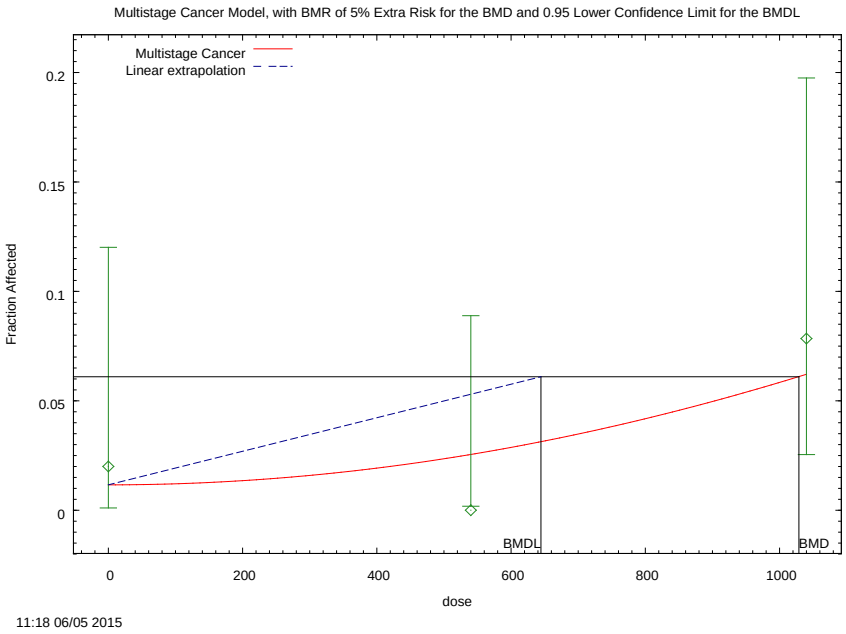

Figure C-8. Plot of incidence by dose, with fitted curve for Multistage 2° model for thyroid follicular cell adenomas or carcinomas in male B6C3F$_1$ mice exposed to *tert*-butanol in drinking water for 2 years, high dose omitted (NTP, 1995); BMR = 5% extra risk; dose shown in mg/kg-day.

```
================================================================
         Multistage Model. (Version: 3.4;  Date: 05/02/2014)
         Input Data File: C:/Users/KHOGAN/BMDS/BMDS260/Data/msc_TBA NTP1995 MMthyroid tumors poly3
-h_Msc2-BMR05.(d)
         Gnuplot  Plotting  File:    C:/Users/KHOGAN/BMDS/BMDS260/Data/msc_TBA  NTP1995  MMthyroid
tumors poly3 -h_Msc2-BMR05.plt
                                                  Fri Jun 05 11:18:05 2015
 ================================================================


 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =   0.00347268
                        Beta(1) =            0
                        Beta(2) = 6.65923e-008


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(2)

Background            1        -0.34

   Beta(2)        -0.34            1



                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background         0.011558        0.0114911          -0.010964           0.0340801
        Beta(1)                0              NA
        Beta(2)     4.84624e-008     3.15009e-008       -1.32781e-008        1.10203e-007

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



                         Analysis of Deviance Table
```

```
      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -18.9229         3
   Fitted model        -20.4481         2       3.05031      1         0.08072
  Reduced model        -21.9555         1        6.0651      2         0.04819

           AIC:         44.8962


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0116         0.578     1.000      50.000        0.558
  540.0000     0.0254         1.271     0.000      50.000       -1.142
 1040.0000     0.0620         3.164     4.000      51.000        0.485

 Chi^2 = 1.85      d.f. = 1        P-value = 0.1735


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1028.79

            BMDL =        644.475


BMDU did not converge for BMR = 0.050000
BMDU calculation failed
            BMDU =        14661.6

Cancer Slope Factor =  7.75825e-005
```

Table C-11. Summary of BMD modeling results for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and including all dose groups (NTP, 1995); BMR = 10% extra risk

Model[a]	Goodness of fit			BMD_{10Pct} mg/kg-d	$BMDL_{10Pct}$ mg/kg-d	Basis for model selection
	p-value	Scaled residuals	AIC			
Three Two	**0.0806**	**−0.989, 0.288, 1.719, and −1.010**	**233.94**	**294**	**118**	Multistage 2° is selected as the most parsimonious model of adequate fit.
One	0.0806	−0.989, 0.288, 1.719, and −1.010	233.94	294	Error[b]	

[a]Selected model in bold.
[b]BMD or BMDL computation failed for this model.

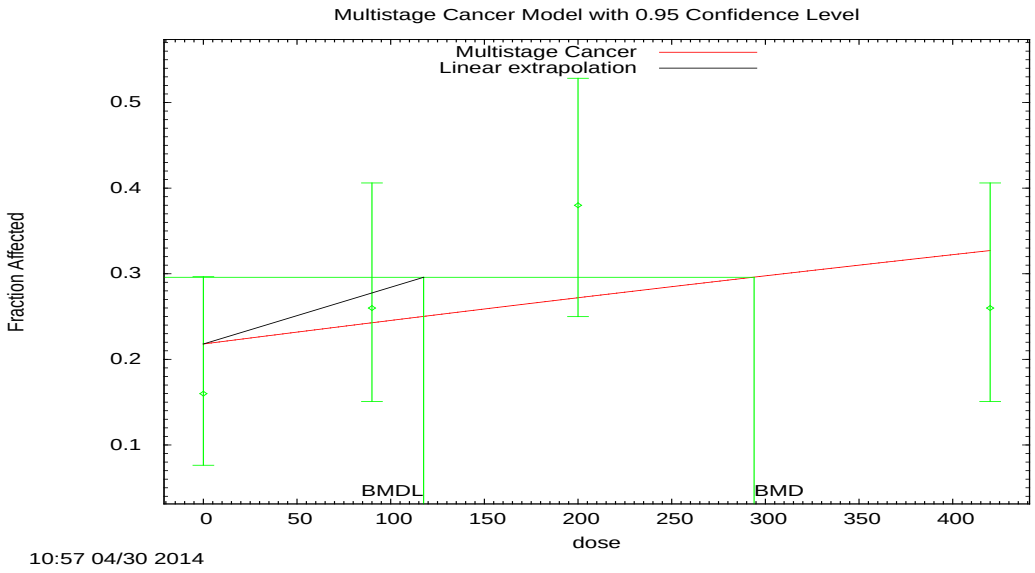

Figure C-9. Plot of incidence by dose, with fitted curve for Multistage 2° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and including all dose groups (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-day.

Multistage Cancer Model. (Version: 1.9; Date: 05/26/2010)

The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]
The parameter betas are restricted to be positive

Benchmark Dose Computation.

BMR = 10% Extra risk
BMD = 293.978
BMDL at the 95% confidence level = 117.584
BMDU at the 95% confidence level = 543384000
Taken together, (117.584, 543384000) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.000850453

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.217704	0.2335
Beta(1)	0.000358397	0.000268894
Beta(2)	0	0

Analysis of Deviance Table

Model	Log(likelihood)	# Parameters	Deviance	Test df	*p*-value
Full model	−112.492	4			
Fitted model	−114.97	2	4.95502	2	0.08395
Reduced model	−115.644	1	6.30404	3	0.09772

AIC = 233.94

Goodness-of-Fit Table

Dose	Estimated Probability	Expected	Observed	Size	Scaled residuals
0	0.2177	10.885	8	50	−0.989
90	0.2425	12.127	13	50	0.288
200	0.2718	13.591	19	50	1.719
420	0.327	16.351	13	50	−1.01

$\chi^2 = 5.04$

df = 2

p-value = 0.0806

Table C-12. Summary of BMD modeling results for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ mg/kg-d	Basis for model selection
	p-value	Scaled residuals	AIC			
Two	N/A[b]	0.000, −0.000, and −0.000	173.68	75.6	41.6	Multistage 1° was selected as the only adequately-fitting model available
One	**0.924**	**0.031, −0.078, and 0.045**	**171.69**	**70.1**	**41.6**	

[a]Selected model in bold.
[b]No available degrees of freedom to calculate a goodness-of-fit value.

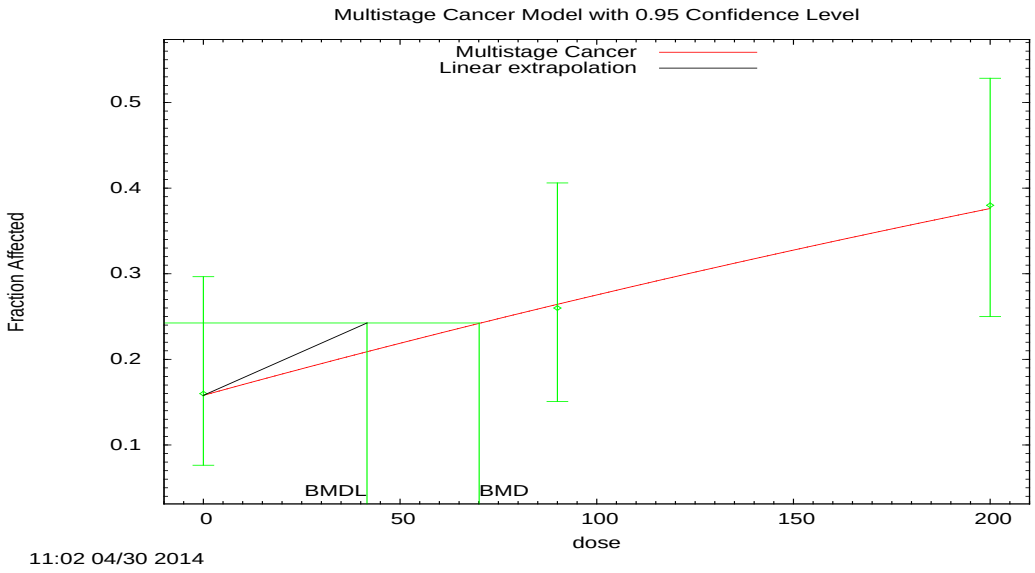

Figure C-10. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk.; dose shown in mg/kg-day.

Multistage Cancer Model. (Version: 1.9; Date: 05/26/2010)

The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]
The parameter betas are restricted to be positive

Benchmark Dose Computation.

BMR = 10% Extra risk
BMD = 70.1068
BMDL at the 95% confidence level = 41.5902
BMDU at the 95% confidence level = 203.311
Taken together, (41.5902, 203.311) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.00240441

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.158399	0.156954
Beta(1)	0.00150286	0.0015217

Analysis of Deviance Table

Model	Log(likelihood)	# Parameters	Deviance	Test df	*p*-value
Full model	−83.8395	3			
Fitted model	−83.8441	2	0.00913685	1	0.9238
Reduced model	−86.9873	1	6.29546	2	0.04295

AIC = 171.688

Goodness-of-Fit Table

Dose	Estimated Probability	Expected	Observed	Size	Scaled residuals
0	0.1584	7.92	8	50	0.031
90	0.2649	13.243	13	50	−0.078
200	0.3769	18.844	19	50	0.045

$\chi^2 = 0.01$

df = 1

p-value = 0.9239

Table C-13. Summary of BMD modeling results for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and including all dose groups; reanalyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk

Model[a]	Goodness of fit			BMD_{10Pct} mg/kg-d	$BMDL_{10Pct}$ mg/kg-d	Basis for model selection
	p-value	Scaled residuals	AIC			
Three Two One	0.0117	−1.476, 1.100, 1.855, and −1.435	218.68	184	94.8	No model fit the data.

[a]No model was selected as a best-fitting model.

Table C-14. Summary of BMD modeling results for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group; reanalyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk

Model[a]	Goodness of fit			BMD_{10Pct} mg/kg-d	$BMDL_{10Pct}$ mg/kg-d	Basis for model selection
	p-value	Scaled residuals	AIC			
Two One	**0.572**	**−0.141, 0.461, and −0.296**	**154.84**	**54.2**	**36.3**	Multistage 1° was selected as the most parsimonious model of adequate fit.

[a]Selected model in bold.

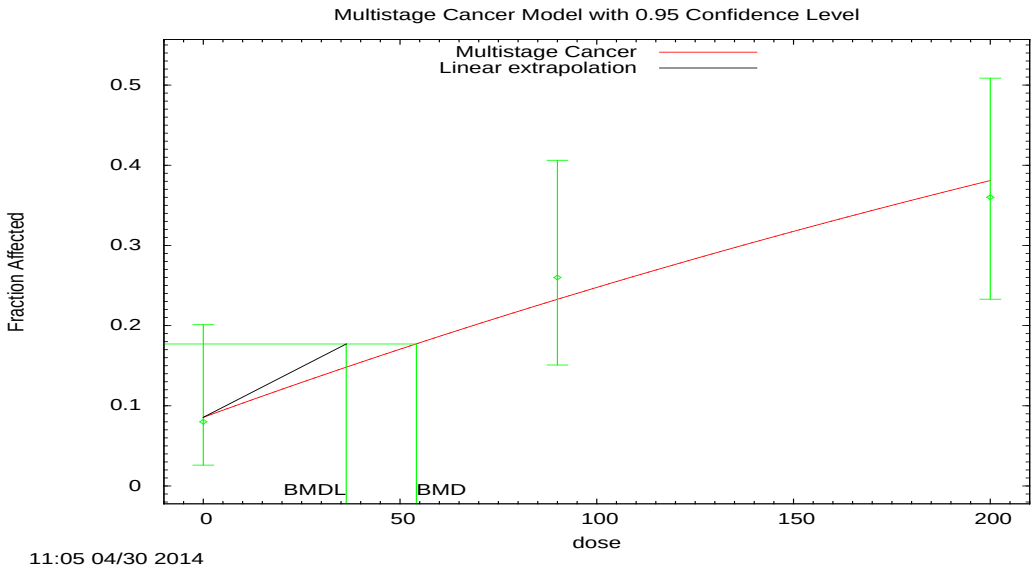

Figure C-11. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group; reanalyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-day.

Multistage Cancer Model. (Version: 1.9; Date: 05/26/2010)

The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]
The parameter betas are restricted to be positive

Benchmark Dose Computation.

BMR = 10% Extra risk
BMD = 54.1642
BMDL at the 95% confidence level = 36.3321
BMDU at the 95% confidence level = 101.125
Taken together, (36.3321, 101.125) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.00275239

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0855815	0.0981146
Beta(1)	0.00194521	0.00179645

Analysis of Deviance Table

Model	Log(likelihood)	# Parameters	Deviance	Test df	*p*-value
Full model	−75.2622	3			
Fitted model	−75.4201	2	0.315716	1	0.5742
Reduced model	−81.4909	1	12.4574	2	0.001972

AIC = 154.84

Goodness-of-Fit Table

Dose	Estimated probability	Expected	Observed	Size	Scaled residuals
0	0.0856	4.279	4	50	−0.141
90	0.2324	11.622	13	50	0.461
200	0.3803	19.015	18	50	−0.296

$\chi^2 = 0.32$
df = 1
p-value = 0.5715

APPENDIX D. PATHOLOGY CONSULT FOR ETBE AND *TERT*-BUTANOL

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

November 28, 2018

To: John Bucher, NTP

From: Kristina Thayer, NCEA-IRIS

Subject: Pathology consult for ETBE and tBA

Purpose

The purpose of this memo is to request a consult for pathology-related issues discussed in the ethyl tertiary butyl ether (ETBE) and tert-butyl alcohol (tBA) draft IRIS assessments. This request is being conducted under the existing MOU between EPA NCEA and the National Toxicology Program (NTP) that covers cooperation and communication in the development of human health toxicological assessments.

Background

The draft IRIS assessments identify kidney effects as a potential human hazard of ETBE and its metabolite tBA, primarily based on evidence in rats (ETBE and tBA Sections 1.2.1, 1.3.1). EPA evaluated the evidence, including the role of α2u – globulin (in accordance with EPA guidance [U.S. EPA, 1991]) and chronic progressive nephropathy (CPN; for which no formal guidance is available). tBA was determined to induce α2u -globulin mediated nephrotoxicity, however, for ETBE, although increased hyaline droplets of α2u -globulin were observed, data were insufficient to conclude that ETBE induces α2u-globulin nephropathy (only one of the five steps in the pathological sequence, linear mineralization, was consistently observed). Both chemicals show dose-related exacerbation of CPN (increased incidence and/or severity), as well as lesions that are not specifically defined as CPN (increased urothelial hyperplasia of the renal pelvis and suppurative inflammation) but are reported to be associated with late stages of CPN (Frazier et al., 2012). Thus, EPA selected urothelial hyperplasia/transitional epithelial hyperplasia of the renal pelvis as the basis for the reference values for both ETBE and tBA.

The SAB committee reviewing ETBE and tBA was unable to reach a consensus with respect to how the EPA interpreted the ETBE and tBA databases for noncancer kidney effects. There was disagreement within the SAB as to whether any noncancer kidney effects for ETBE and tBA should be considered a hazard relevant to humans. Specifically, the difference in opinion was related to the extent of confidence in the roles that CPN and/or α2u-globulin-based mechanisms played in the development of the renal effects seen with tBA and ETBE.

Charge Questions

In this pathology consult, IRIS is seeking additional input on the role that α2u-globulin and CPN play in the observed kidney toxicity. Please consider the following questions and provide references, as applicable, with your responses. Please also comment on any sex-related aspects that are pertinent to these questions.

- Is the etiology of CPN in rats known?
- Are urothelial hyperplasia of the renal pelvis and transitional epithelial hyperplasia of the renal pelvis considered to be the same lesion?
- Suppurative inflammation and urothelial hyperplasia have been reported to be associated with advanced stages of CPN (Frazier et al 2012). Does NTP agree with this conclusion? Are these lesions also associated with α2u -globulin nephropathy?
- CPN exacerbation has been reported in some chemicals that NTP identified as candidates for acting via the α2u-globulin pathway (Travlos et al., 2011). A theory has been proposed that CPN exacerbation seen in male animals with ETBE and tBA exposure is caused by α2u-globulin related processes. Please comment on the strength of the above proposition.
- It has been hypothesized that there is no analog to the CPN process in the aging human kidney. Does this position reflect the consensus in the field of pathology?
- Given what is known about the biology of CPN development in rodents, is it plausible a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. diabetic nephropathy, glomerulonephritis, interstitial nephritis)?

References

Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B, Bube A. 2012 Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol. 40(4 Suppl):14S-86S.

Travlos GS, Hard GC, Betz LJ, Kissling GE. 2011. Chronic progressive nephropathy in male F344 rats in 90-day toxicity studies: its occurrence and association with renal tubule tumors in subsequent 2-year bioassays. Toxicol Pathol. 39(2):381-9.

U.S. EPA 1991. Alpha-2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. EPA/625/391/019F.

Attachments

JPEC (Japan Petroleum Energy Center). (2010a). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Drinking water study). (Study No: 0691).

JPEC (Japan Petroleum Energy Center). (2010b). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Inhalation study). (Study No: 0686).

Kristina Thayer

Kristina Thayer, Ph.D.
Director, NCEA-IRIS

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

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Environmental Health Sciences
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Research Triangle Park, NC 27709
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February 13, 2019

Kristina Thayer, Ph.D.
Director, NCEA-IRIS
U.S. Environmental Protection Agency
109 T.W. Alexander Drive, MD B243-01
Research Triangle Park, NC 27709

Dear Dr. Thayer,

With respect to your November 28, 2018 request for a pathology consult under the NTP/NCEA Memorandum of Understanding, I asked Dr. Robert Sills, Chief, Cellular and Molecular Pathology Branch to provide responses reflecting the current NTP perspective on the issues you raise. Dr. Sills worked with John Curtis Seely, DVM Diplomate, ACVP Senior Pathologist Experimental Pathology Laboratories, Inc, an internationally recognized expert in rodent renal pathology, to provide answers to your questions.

1. Is the etiology of CPN known?

   The etiology of CPN is unknown (Peter et al., 1986; Hard and Khan, 2004; Hard et al., 2013). Although several theories have been postulated to be the etiology of CPN none have been recognized as the absolute cause of CPN. Factors which have been suggested to be associated with the etiology of CPN include genetics, increased glomerular permeability and dysfunction due to hyperfiltration and functional overload, high renal protein levels, and hemodynamic changes. All of these may influence the progression of CPN but do not appear to initiate renal CPN disease (Baylis, 1984; Barthold, 1998; Abrass, 2000; Hard and Khan, 2004). CPN is a spontaneous and complex degenerative/regenerative disease process influenced by age (incidence and severity increases with age), sex (males affected more than females), and strain (in order of highest to lowest CPN incidence: Sprague-Dawley →Fischer 344 →Wistar rats). It can be modified by diet (increased protein and high caloric intake), hormones (testosterone, estrogen), and many other factors (Seely et al., 2018).

2. Are urothelial hyperplasia of the renal pelvis and transitional epithelial hyperplasia of the renal pelvis considered to be the same lesion?

   Yes, the older terminology of "transitional epithelium hyperplasia, renal pelvis" is being updated and replaced by the newer terminology of "urothelial hyperplasia, renal pelvis". Urothelium is recognized as the correct terminology of the epithelium lining the renal pelvis, ureter, urinary bladder and a portion of the urethra (Frazier and Seely, 2018). However, in advanced stages of CPN a type of epithelial proliferation/hyperplasia may be observed along the epithelial lining of the

renal papilla which in some older studies was designated as "urothelial hyperplasia". Recently, the epithelial lining of the renal papilla has been unequivocally demonstrated to represent a type of epithelium different from the urothelium lining the renal pelvis. The difference between urothelium (uroplakin positive) and the epithelium lining the renal papilla (uroplakin negative) was confirmed by immunostaining for uroplakin (a distinct cell marker for urothelium) (Souza et al., 2018).

3. Suppurative inflammation and urothelial hyperplasia have been reported to be associated with advanced stages of CPN (Frazier et al., 2012). Does NTP agree with this conclusion? Are these lesions also associated with α2u-globulin nephropathy?

   Renal inflammation is not uncommon in the laboratory rat and can be observed throughout all portions of the kidney. Within the pelvis, inflammation tends to result in a reactive hyperplasia of the urothelium (Seely et at., 2018). Most cases of suppurative inflammation and urothelial hyperplasia are observed as spontaneous changes of undetermined origin. Interstitial mononuclear cell infiltrates are commonly observed in advanced stages of CPN (Frazier and Seely, 2018). However, suppurative inflammation and urothelial hyperplasia are typically unrelated to CPN or, at most, occasionally noted as an uncommon secondary change to CPN. Therefore, CPN does not directly result in suppurative inflammation or urothelial hyperplasia of the renal pelvis in its advanced stages. Cases of suppurative inflammation and urothelial hyperplasia are more likely to be associated with the presence of renal pelvic mineralization, pelvic calculi, or from an ascending bacterial infection or pyelonephritis (Seely et al., 2018). Furthermore, mineralization has been reported to be associated with an increased incidence and severity of spontaneous inflammation and urothelial hyperplasia in the renal pelvis of female rats (Tomonari et al., 2016). In addition, there is no information that appears to support that suppurative inflammation and pelvic urothelial hyperplasia are directly associated with the spectrum of morphological changes associated with α2u-globulin nephropathy (Frazier et al., 2012; Frazier and Seely, 2018).

4. CPN exacerbation has been reported in some chemicals that NTP identified as candidates for acting via the α2u-globulin pathway (Travlos et al., 2011). A theory has been proposed that CPN exacerbation seen in male animals with ETBE and tBA exposure is caused by α2-globulin related processes. Please comment on the strength of the above proposition

   According to the IARC Scientific Publication No. 147 (1999), chemicals which cause α2u-globulin nephropathy are often associated with an accelerated onset and severity (exacerbation) of the cortical changes typical of chronic progressive nephropathy seen in older male rats (Alden et al., 1984; Swenberg and Lehman-McKeenan, 1999; Travlos et al., 2011; Frazier et al., 2012). However, studies on 2-ethoxy-2 methylpropene (ethyl tertiary butyl ether; inhalation and drinking water studies) confirmed the presence of exacerbated CPN in both male and female rats at the highest dose levels (Japan Industrial Safety and Health Association/Japan Bioassay Research Center, 2010[a] 2010[b]). Because of "urothelial hyperplasia" and linear pelvic (papillary) mineralization noted in the male rats from these studies, it was proposed that α2u-globulin nephropathy contributed to the exacerbation of CPN in the males although no pathogenesis of the exacerbated CPN in females was given. Additionally, in these studies, "urothelial hyperplasia" was apparently and according to its description more likely to represent a proliferation of the papillary lining epithelium and not representative of true "urothelial hyperplasia". This proliferative epithelial finding is often observed

as part of advanced cases of rat CPN and has no similarity to any human renal papillary finding (Seely et al., 2018; Souza et al., 2018). Long term exposures to methyl tertiary -butyl ether also resulted in an α2u-globulin nephropathy and exacerbated CPN in both male and female rats (Cruzan et al., 2007). The etiology of exacerbated CPN in females is not known since α2u-globulin nephropathy is regarded as a male only condition. Therefore, although α2u-globulin nephropathy may account for cases of chemically exacerbated CPN, other undetermined factors contributing to CPN exacerbation cannot be discounted (Doi et al., 2007).

5. It has been hypothesized that there is no analog to the CPN process in the aging human kidney. Does this position reflect the consensus in the field of pathology.

   Yes, the publication by Hard, Johnson, and Cohen makes a very strong case that the renal development, biological behavior, and morphological spectrum of CPN have no analog in the human kidney and that CPN is a distinct entity in the rat. (Hard et al., 2009). Overall, CPN has prominent protein filled dilated tubules, no vascular changes, no immunological or autoimmune basis, and little inflammation which distinguishes CPN from most human nephropathies (Hard et al., 2009). There appears to be nothing in the literature that counters this assumption.

6. Given what is known about the biology of CPN development in rodents, is it plausible a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. diabetic nephropathy, glomerulonephritis, interstitial nephritis)?

   The etiology of CPN is unknown and represents a complex disease process in rats. Given the fact that there is no definitive pathogenesis for this multifactorial disease process, it cannot be fully ruled out that chemicals which exacerbate CPN in rats may have the potential to exacerbate disease processes in the human kidney.

   Please let me know if you have additional questions or wish further clarification of any of these responses.

   Sincerely,

   John Bucher

   John Bucher, Ph.D.
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APPENDIX E. SUMMARY OF SCIENCE ADVISORY BOARD (SAB) PEER-REVIEW COMMENTS AND EPA'S DISPOSITION

The Toxicological Review of *tert*-butyl alcohol (*tert*-butanol; tBA), dated June 2017, underwent a formal external peer review in accordance with U.S. Environmental Protection Agency (EPA) guidance on peer review (U.S EPA, 2015). This peer review was conducted by the Chemical Assessment Advisory Committee (CAAC) Augmented for Review of the Draft IRIS *tert*-butanol Assessment (SAB-CAAC *tert*-butanol panel) of EPA's Science Advisory Board (SAB). An external peer review workshop was held on August 15–17, 2017. Public teleconferences of the SAB-CAAC *tert*-butanol panel were held on July 11, 2017, March 22, 2018, March 27, 2018, and June 6, 2018. The Chartered SAB held a public meeting on September 26, 2018 to conduct a quality review of the draft SAB-CAAC peer review report.[1] The final report of the SAB was released on February 27, 2019.

The SAB-CAAC was tasked with providing feedback in response to charge questions that addressed scientific issues related to the hazard identification and dose-response assessment of *tert*-butanol. Key recommendations of the SAB[2] and EPA's responses to these recommendations, organized by charge question, follow. Editorial changes and factual corrections offered by SAB were incorporated in the document as appropriate and are not discussed further.

1. Literature Search/Study Selection and Evaluation – Systematic Review Methods

Charge Question 1. Please comment on the strategy for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

Key Recommendation: The SAB recommended EPA should provide clarification on the rationales for several decisions that impacted how the literature search was conducted. This includes (a) the rationale for the selection of some synonyms of *tert*-butanol as key search words and not others; (b) the rationale for imposing limitations on sources in the first stage of the scientific literature

[1]During the quality review by the Chartered SAB, 2 of the 44 members provided dissenting comments related to the cancer weight of evidence descriptors and the quantitative cancer risk estimates for ETBE and tBA. These comments were included as an appendix to the final SAB report and are summarized and addressed following the disposition of the SAB-CAAC recommendations below.

[2]The SAB provided tiered recommendations: Tier 1 (key recommendations), Tier 2 (suggestions), and Tier 3 (future considerations).

search (i.e., PubMed, Web of Science); and (c) the rationale for limiting the search for additional citations to only some of the publications available in peer-reviewed literature and secondary sources, but not others.

Response: The literature search was developed and executed in consultation with information specialists and librarians through EPA's Health and Environmental Research Online (HERO) database. This includes developing, testing, and implementing a comprehensive literature search strategy in an iterative and collaborative manner. (a) The most common synonyms and trade names were used as the keywords in the literature search. This included the preferred IUPAC name of 2-Methylpropan-2-ol. Clarification has been added in the Literature Search Strategy/Study Selection and Evaluation Section. (b) PubMed, Web of Science, and Toxline are the core sources that IRIS uses for published studies. Prior experience has also demonstrated that searching of PubMed, Web of Science and Toxline provides sufficient coverage for literature pertinent to human health assessments. TSCATS2 database was included to capture submissions of health and safety data submitted to the EPA either as required or voluntarily under certain sections of TSCA. Based on the attributes of the chemical, along with input from HERO, EPA did not include supplemental databases (e.g., databases for pesticides, U.S. Department of Agriculture (USDA)-related compounds or inhalation values). Clarification has been added in the Literature Search Strategy/Study Selection and Evaluation Section. (c) To ensure no key studies were missed, a manual search of citations was performed on published reviews and studies identified from public comments, as well as reviews previously conducted by other international and federal health agencies. Table LS-2 lists the approach used and the sources used in the manual searching of citations.

Key Recommendation: The SAB recommended EPA should provide a rationale for the exclusion of studies of dermal contact as a relevant route of exposure in light of the occurrence of *tert*-butanol in many consumer products such as perfumes and cosmetics.

Response: Studies evaluating dermal exposure were not excluded (see Table LS-3 on inclusion-exclusion criteria). Several studies were identified that examined acute dermal exposures; however, as stated in the Literature Search Strategy/Study Selection and Evaluation Section, studies investigating the effects of acute dermal chemical exposures are generally less pertinent for characterizing health hazards associated with chronic exposure, and therefore were not considered as primary evidence. These studies were considered as sources of supporting health effects data (see Figure LS-1).

Key Recommendation: The SAB recommended EPA should provide a justification for the complete exclusion of studies with non-mammalian species, which affects the completeness of the hazard identification.

Response: As described in Table LS-3, the populations of interest are humans and animals. Non-mammalian species were not included because studies in mammalian model systems are available. However, these studies were considered supplemental and retained as secondary literature and sources of contextual information as shown in Figure LS-1.

Key Recommendation: The SAB recommended EPA should provide more transparent documentation of the process of application of inclusion and exclusion criteria and the quality evaluation of studies, in order to support decision making by the EPA. This could be done through the HERO database.

Response: This assessment was conducted prior to the implementation of systematic review tools in the IRIS program, like HAWC, that could be used to document the systematic review process. Database evaluation is described in the Literature Search Strategy/Study Selection and Evaluation Section. As stated in the section, information on study evaluation is reported in evidence tables and documented in the synthesis of evidence. Study strengths and limitations are also included in the text, where relevant.

2. Hazard Identification-Chemical Properties and Toxicokinetics

Charge Question 2a.-Chemical Properties- Is the information on chemical properties accurate?

Key Recommendations: The SAB recommended EPA make improvements to the chemical properties table by focusing on increasing confidence and transparency in the values presented. The SAB also recommended the use of a template focusing on the chemical properties most relevant to the chemical and the assessment. Several recommendations focused on a preference for the citation of chemical properties from primary sources, for vetting the data in cases in which more than one value is published, and for presenting rationales for the selected values.

Response: In response to SAB comments, EPA has revised the *tert*-butanol chemical properties table (Table 1-1) to present average experimental and predicted chemical properties from high quality databases as curated by EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard). EPA's CompTox Chemicals Dashboard aggregates and presents both experimental and predicted chemical property data, with links to the source and/or model data. The experimental data are sourced from publicly available databases as well PHYSPROP downloadable files (see Mansouri et al. (2016)). Predicted chemical property data are curated from EPISuite, OPERA models (see Mansouri et al. (2016)), NICEATM models (see Zang et al. (2017)), Toxicity Estimation Software Tool (TEST) Models, and the Open PHACTS project (as predicted by ACD/LabsEXIT). A key benefit of this aggregation of chemical properties over reporting an individual measurement is a more robust point estimate than is possible from the

measure derived from any individual study, with each study reporting measurements that are expected to have some degree of error. For more information on EPA's CompTox Chemicals Dashboard see Williams et al. (2017).

Charge Question 2b.-Toxicokinetic modeling- Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of tert-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

Key Recommendations: The SAB recommended the model code should be revised to describe metabolism as a function of the free liver concentration, CVL, and metabolic parameters (e.g., Km or first order rate constants) should be re-estimated. Metabolism based upon total liver concentration, CL, is not scientifically correct.

Response: Model code has been revised to describe metabolism as a function of the free liver concentration and metabolic parameters have been re-estimated. The new final code is available in HERO (https://hero.epa.gov/hero/index.cfm/project/page/project_id/1543/usage_id/2896).

Key Recommendations: The SAB recommended evaluation of *tert*-butanol dose metrics for kidney toxicity should be compared for ETBE and *tert*-butanol exposures (similar to Figure 6 in Salazar et al. (2015)).

Response: Change in absolute kidney weight in female rats as a function of estimated *tert*-butanol blood concentration and *tert*-butanol rate of metabolism for both ETBE and *tert*-butanol exposures has been added to Appendix B of the Supplemental Information (see Figure B-3).

Key Recommendations: The SAB recommended the overall presentation of the PBPK modeling should be cohesive, clear, and transparent, and should provide essential information, assumptions, results and conclusions. The text in Section 1.1.3 of the draft *tert*-butanol assessment and text in Appendix should be reworded. The SAB suggests that the material in U.S. EPA (2017) be included in Appendix B or as a separate appendix and a conclusion section added to it.

Response: Text has been added and revised in Section 1.1.3 and Appendix B to increase cohesion and to ensure essential information, assumptions, results, and conclusions are clear and transparent. Text describing PBPK model evaluation for the IRIS assessments of ethyl tertiary butyl ether and *tert*-butanol (U.S. EPA, 2017) has been added as a new appendix to the toxicological review (Appendix B.1.7.). Text in Section 1.1.3 and Appendix B have been revised to clarify that no

models of *tert*-butanol have been created independently of other chemicals from which it arises as a metabolite.

Charge Question 2c.-Choice of dose metric- Is the average concentration of tert-butanol in blood an appropriate choice for the dose metric?

Key Recommendation: The SAB recommended EPA state in the draft *tert*-butanol assessment how the average concentration of *tert*-butanol in blood was calculated given that the SAB agrees with use of this dose metric. The SAB also agreed with the use of an oral to inhalation extrapolation for *tert*-butanol.

Response: For non-continuous exposures the PBPK model was run for a number of days or weeks such that the predicted time course of *tert*-butanol in blood did not change with further days or weeks simulated. The average blood concentration of *tert*-butanol was calculated during the final periodic exposure. For uniformity, all scripts now calculate the average from episodic exposures on the basis of the final week of exposure (regardless of whether exposure is once per day or 5 times per week, since either exposure profile will be fully captured by averaging a 1-week time period). Details on how the average concentration of *tert*-butanol in blood was calculated has been added to Section 2.2.2, under subsection *PODs from oral studies- use of PBPK model for route-to-route extrapolation*.

3. Hazard Identification and Dose-Response Assessment- Noncancer

Charge Question 3a.-Noncancer kidney toxicity (Sections 1.2.1, 1.3.1) identifies kidney effects as a potential human hazard of tert- butanol. EPA evaluated the evidence, including the role of alpha 2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991a). Please comment on whether this conclusion is scientifically supported and clearly described.

Key Recommendation: The SAB recommended EPA should provide a more thorough explanation for considering the enhancement of CPN as a kidney effect relevant to human hazard assessment. The SAB was unable to reach consensus on whether noncancer kidney effects should be considered a hazard relevant to humans based on the available evidence.

Response: In response to SAB comments, EPA consulted with pathologists at the National Toxicology Program (NTP) on the applicability of alpha 2u-globulin and the components of CPN in the evaluation of the human relevance of kidney effects (see Appendix D of the Supplemental Information). In consideration of the expert opinions of the pathologists, the assessment was revised to strengthen the explanation for considering the enhancement of CPN as a kidney effect

relevant to human hazard (see Section 1.2.1). Briefly, following *tert*-butanol exposure, dose related increases in kidney weight and exacerbation of CPN were observed in both male and female rats. While tert-butanol exposure has been shown to act through an alpha-2u-globulin mechanism in male rats (which can exacerbate CPN, see Section 1.2.1), the dose related exacerbation of kidney effects in female rats cannot be explained by α2u-globulin. The NTP consultation (NIEHS, 2019) acknowledged existing literature and concluded that no analog to CPN occurs in humans (Hard et al., 2009) and that the etiology of CPN is unknown (Hard et al., 2013; Hard and Khan, 2004; Peter et al., 1986). However, many of the lesions observed in CPN are also observed in chronic kidney disease in humans (NIEHS, 2019; Lusco et al., 2016; Zoja et al., 2015; Frazier et al., 2012; Satirapoj et al., 2012). As summarized in the consultation, NTP concluded that due to the unknown etiology and lack of a clear pathogenesis "it cannot be ruled out that chemicals which exacerbate CPN in rats may have the potential to exacerbate disease processes in the human kidney". A more thorough explanation for considering the enhancement of CPN as a kidney effect relevant to human hazard assessment has been added to Section 1.2.1.

Charge Question 3b.-Noncancer at other sites (Sections 1.2.3-6, and 1.3.1) finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are scientifically supported and clearly described. If there are* publicly *available studies to associate other health outcomes with tert-butanol exposure, please identify them and outline the rationale for including them in the assessment.

Key Recommendation: The SAB recommended EPA include contact dermatitis (Edwards and Edwards, 1982) in hazard identification as dermal exposure is a relevant route of exposure.

Response: Studies investigating the effects of acute dermal exposures are generally less pertinent for characterizing health hazards associated with chronic exposure. However, these studies were considered as sources of supporting health effects data (see Figure LS-1).

Key Recommendation: The SAB recommended EPA change the description to "minimal effects at otherwise toxic dose levels," rather than "inadequate information to assess," since the SAB believes there is an adequate amount of information, and only minimal effects have been shown, even at toxic dose levels.

Response: The description of noncancer effects was revised to be responsive to the SAB's suggested language in Sections 1.2.3–1.2.6 and 1.3.1.

Charge Question 3c.-Oral reference dose for noncancer kidney outcomes- Section 2.1 presents an oral reference dose of 4x10−1 mg/kg–day, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is

scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

Key Recommendation: The SAB recommended EPA carefully reexamine the validity and applicability of the endpoints chosen and analyzed for the oral RfD for *tert*-butanol, including the potential for CPN and/or alpha- 2u-globulin to serve as mechanism(s) of the kidney effects of *tert*-butanol, in light of SAB advice regarding consideration of the criteria for definition of CPN. The SAB was unable to reach a consensus as to whether the selection of nephropathy effects was appropriate. The SAB states that if EPA determines that increases in severity of nephropathy in female rats following *tert*-butanol in drinking water exposure remains the basis of the oral RfD, then the "SAB considers the derivation of the oral reference dose to be scientifically supported and its derivation clearly described".

Response: As recommended by SAB, EPA carefully reexamined the kidney endpoints analyzed for the RfD with consideration of CPN and alpha 2u-globulin. EPA also consulted with pathologists at the National Toxicology Program (NTP) on the applicability of alpha 2u-globulin and the components of CPN in the evaluation of the human relevance of kidney effects (see Appendix D of the Supplemental Information). With this additional expert consultation, the assessment has been revised to clarify which effects were considered and to strengthen the justification regarding the human relevance of the observed kidney effects. For example, the assessment was revised to clarify that the kidney endpoints in males were not considered and the endpoints in females not confounded by alpha-2u-globin were considered (Section 1.2.1). See *Integration of Kidney Effects* in Section 1.2.1 of the Toxicological Review. See also response to Charge Question 3a.

Key Recommendation: The SAB recommended that the units need to be added to the tables in this section for completeness and interpretability. It would be useful to attempt a more integrated presentation of the current text, tables and graphs (i.e., the EPA should present key and related information/graphics on concurrent pages as much as possible). As currently laid out, the reader is forced to engage in a lot of page flipping in order to read the draft *tert*-butanol assessment, making it difficult to track information.

Response: Units have been added to the tables were missing, however, endpoints which display changes as "% change relative to control" are unitless. A more integrated presentation of text, table and figures is being implemented in future IRIS assessment templates.

Key Recommendation: The SAB recommended EPA include the outcomes of statistical analyses and their rationale in study selection choice in the draft *tert*-butanol assessment.

Response: Statistical significance as reported by the study authors was included in the appropriate evidence tables in Section 1.2.1. Key toxicological effects in the kidney were reported in eight studies derived from five references following oral exposure (Section 1.2.1). However, all kidney outcomes considered for dose response were derived from a single study (NTP, 1995). As described in Section 2.1.1, NTP, 1995 was identified as the most suitable for dose-response assessment considering the study duration, comprehensive reporting of outcomes, and multiple doses tested. Section 1.2.1 has been edited for clarity.

Charge Question 3d.-Inhalation reference concentration for noncancer kidney outcomes- Section 2.2 presents an inhalation reference concentration of 5x100 mg/m3, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995), converted for inhalation exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

Key Recommendation: The SAB recommended EPA provide more detailed information about the specific application of the Borghoff et al. (2016)/U.S. EPA (2017) PBPK model used for route-to-route extrapolation to derive the inhalation RfC.

Response: Detailed information has been provided in Section 2.2 under *PODs from oral studies- use of PBPK model for route-to-route extrapolation*. This includes the choice of internal dose metric and uncertainty inherent in the use of a PBPK model for route-to route extrapolation. Section 2.2.4 provides an explanation for why preference was given to an RfC derived from the route-to-route extrapolated POD based on the chronic oral study over a POD from the subchronic inhalation study.

Key Recommendation: The SAB recommended EPA provide more reporting of statistical analysis of individual studies to help clarify the appropriateness of inclusion/exclusion and use of studies.

Response: Statistical significance as reported by the study authors has been included in the appropriate evidence tables for each hazard section. The rationale for study selection and endpoint inclusion is discussed in Section 2.1.1.

4. Hazard Identification and Dose-Response Assessment- Cancer

Charge Question 4a.-Cancer modes of action

(i) Cancer* modes *of action in the kidney- As described in section 1.2.1, kidney tumors were observed in male rats following tert-butanol exposure, and a mode-of-action involving alpha

2u-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991b), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically supported.

Key Recommendation: The SAB recommended EPA provide additional justification for the assumption that kidney tumors in male rats exposed to *tert*-butanol are relevant to humans.

Response: Based on EPA (U.S. EPA, 1991a) and IARC criteria (Capen et al., 1999) alpha 2u-globulin may contribute to kidney tumor formation in male rats exposed to *tert*-butanol (See section 1.2.1). However, evidence indicative of the alpha 2u-globulin process was not consistently observed across all studies. This observation suggests that *tert*-butanol may be a weak inducer of alpha 2u-globulin and its associated nephropathy. These inconsistencies are discussed in detail in Section 1.2.1. Although renal tubule hyperplasia and renal tumors are poorly correlated following *tert*-butanol exposure (NTP, 1995), there is a moderate correlation between CPN and renal tumor incidence in male rats suggesting a role for CPN in renal tumorigenesis. EPA requested an independent pathology consultation on the applicability of alpha 2u-globulin and CPN on kidney effects ((NIEHS, 2019); see Appendix D). NTP (NIEHS, 2019) concluded that the unknown etiology and poorly understood pathogenesis of CPN suggest that chemicals that exacerbate CPN may potentially induce kidney effects in humans. CPN also was exacerbated in female rats in the absence of renal tumor formation; therefore, it is also possible that other unknown mechanisms relevant to humans contribute to renal tumor formation in male rats. Additional justification for the human relevance of kidney tumors in male rats exposed to *tert*-butanol has been added in Section 1.2.1.

(ii) Cancer modes of action in the thyroid- As described in Section 1.2.2, thyroid tumors were observed in male and female mice following tert-butanol exposure, and an anti-thyroid mode-of-action was evaluated. The analysis, conducted in accordance with EPA's guidance on thyroid follicular cell tumors in rodents (U.S. EPA, 1998), found the information inadequate to determine whether an anti-thyroid mode-of-action was operating and considered the thyroid follicular cell tumors in male and female mice to be relevant to humans. Please comment on whether this conclusion is scientifically supported.

Key Recommendations: The SAB concurred with EPA's determination that the mode of action for follicular tumors is unknown in male and female mice following *tert*- butanol exposure and should be considered relevant to humans in accordance with EPA policy. The SAB had no specific recommendations.

Response: No response needed.

Charge Question 4b- Cancer characterization- As described in Sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is* suggestive *evidence of carcinogenic potential for tert-butanol, based on thyroid follicular cell tumors in male and female B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported. Please comment on whether the "suggestive evidence" cancer descriptor is scientifically supported for all routes of exposure. If another cancer descriptor should be selected, please outline how it might be supported.

Key Recommendation: The SAB agrees that there is scientific support for EPA's conclusion that there is suggestive evidence of carcinogenic potential for *tert*-butanol for all routes of exposure. The SAB recommended EPA expand the scope and breadth of its discussion of potential modes and sites of action of *tert*-butanol on the thyroid.

Response: Discussion of modes of action for thyroid tumor formation was expanded in Section 1.2.2 and Appendix B to include studies that evaluate the mutagenic mode of action as recommended by EPA's guidance on the assessment of thyroid cell tumors (U.S. EPA, 1998) (See section 1.2.2 and Appendix B). Information on other potential MOAs for *tert*-butanol in the thyroid is not currently available.

Charge Question 4c- Cancer toxicity values- Section 3 of EPA's cancer guidelines (U.S. EPA, 2005) states:
"When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the data generally would not support one, however, when the evidence includes a well-*conducted *study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence." Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for including a quantitative analysis given the "suggestive evidence" descriptor. Also comment whether the NTP (1995) study is a suitable basis for this quantitative analysis.

Key Recommendation: The SAB recommended EPA provide a rationale for performing a quantitative analysis of thyroid tumors in Section 2.3 and suggested EPA consider potential worker and consumer exposures as a rationale. The SAB thought the dose-response modeling of thyroid tumors may not be useful because tumors were only observed at the highest dose; however, several committee members supported conducting a quantitative analysis to provide some sense of magnitude of potential carcinogenic risk. Therefore, the SAB recommended EPA refrain from

conducting a quantitative analysis for *tert*-butanol carcinogenicity or explain the limitations of the analysis and clearly state the intended purpose is to simply provide some sense of the magnitude of potential risks.

Response: A rationale for performing a quantitative analysis of thyroid tumors has been added to Section 2.3, including potential worker and consumer exposures. One possible limitation in the interpretation of thyroid tumors that has been added to the discussion in the assessment is the increased incidence of thyroid tumors observed at highest dose tested and the possibility of nonlinear kinetics at the high dose. However, at the high dose level no increase in mortality was observed in female mice suggesting that the incidence of thyroid tumors was neither confounded by increased mortality nor exceeded the MTD (see added text in Section 1.2.2). Text was added to clearly state the intended purpose is to simply provide some sense of the magnitude of potential risks (See added text in Section 2.3).

Charge Question 4d- Oral slope factor for cancer- Section 2.3 presents an oral slope factor of 5 x 10–4 per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

Key Recommendation: The SAB had no specific recommendations. The SAB was unable to reach a consensus on the suitability of the NTP (1995) drinking water study for developing an oral slope factor. Some reviewers were concerned about the potential lack of biological relevance due to the magnitude of the high dose and the possibility of non-linear kinetics at the high dose at which follicular tumors were observed. However, other members concluded that EPA's choice for the oral slope factor for *tert*-butanol was scientifically supported.

Response: Justification (including strengths and limitations) for the derivation of an oral slope factor using thyroid tumors in mice was added in Section 1.3.2, as well as Sections 1.2.2 and 2.3 (see response to Question 4c).

Charge Question 4e- Inhalation unit risk for cancer- Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of alpha 2u - globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation unit risk estimate, please outline how it might be developed.

Key Recommendation: The SAB had no specific recommendations.

Response: The SAB concurred with EPA's decision to not develop an inhalation unit risk for *tert*-butanol.

Charge Question 5- Susceptible Populations and Lifestages- As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this conclusion is* scientifically *supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

Key Recommendation: The SAB recommended EPA correct the actual body weight for the treated group in Table 1-12 of the EPA's draft *tert*-butanol assessment.

Response: Body weight is presented as percent change in Table 1-12. No errors were identified in the tables.

Charge Question 6- Question on the Executive Summary- The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.

Key Recommendations: The SAB recommended EPA highlight the consequences of alternative choices for the final assessment in the Executive Summary, especially when these hinge on decisions made about the interpretation and relevance of key toxicity endpoints that have been contested (based on the history of public comment on the draft assessment).
Response: Text has been added to the Executive Summary to more clearly highlight the context around the interpretation and relevance of key endpoints such as the human relevance of the observed kidney effects (see *Key Issues Addressed in Assessment*).
Key Recommendations: The SAB recommended EPA provide clarification for the Reference HSDB (HSDB, 2007) cited on page xiii. Reference HSDB (2007) is cited for *tert*-butanol in human milk. The two articles cited by HSDB (2007) do not provide evidence for the presence of *tert*-butanol in milk.

Response: The articles cited by the HSDB (2007) reference reported the results for 2-methyl-2-propanol, which is a synonym for *tert*-butanol; demonstrating the presence of *tert*-butanol in mother's milk. This synonym for *tert*-butanol was included in Table LS-1 for clarification.

Comments from two members of the Chartered SAB during the QA Review of the SAAB CAAC Peer Review Report

The Chartered SAB is tasked with conducting quality reviews of draft SAB reports to determine if they are ready for transmittal to the Administrator, reviewing whether the charge questions were adequately addressed by the CAAC, whether the report has technical errors or omissions, if the report is clear and logical, and if the CAAC recommendations in the report are supported by the body of the draft report. During this quality review of the draft SAB-CAAC report on the Draft IRIS assessments of ETBE and *tert*-butanol, two members of the chartered SAB (44 total members) disagreed with the CAAC regarding the recommendation for the cancer weight of evidence descriptors for ETBE and *tert*-butanol. These two members provided additional comments which were included as Appendix C of the Final SAB report. A summary and response to their comments, as they pertain to *tert*-butanol, are included below.

Comment: Two members of the chartered SAB disagreed with the SAB-CAAC's support of EPA's cancer weight of evidence descriptor of "suggestive evidence" for *tert*-butanol. They stated *tert*-butanol should be characterized as "insufficient evidence" (presumably analogous to EPA's cancer weight of evidence descriptor for "inadequate evidence") because thyroid follicular cell tumors were observed only in female mice at the highest exposure concentration in the NTP 2-year drinking water bioassay (NTP, 1995), a concentration they characterized as beyond the maximum tolerated dose (MTD) due to a 10-15% reduction in body weight. They concluded that the renal tubule adenomas are not relevant to humans because of poor survival rates in control animals in NTP 2-year drinking water bioassay (NTP, 1995), rat-specific MOA(s), and dose exceeds the MTD.

Response: The SAB-CAAC agreed with EPA's determination of 'suggestive evidence of carcinogenic potential" (See Charge Question 4b), as the database was consistent with this descriptor as illustrated in EPA's 2005 Cancer Guidelines, based on thyroid follicular cell adenomas in female mice and thyroid follicular cell adenomas and carcinomas in male mice and renal tubule adenomas in male rats, although the SAB-CAAC did not reach consensus regarding the MOA(s) by which *tert*-butanol caused renal tubule adenomas in male rats. Briefly, an increase in thyroid adenomas was observed in female mice exposed to *tert*-butanol via drinking water (primarily at the high dose) with the incidence of combined adenomas and carcinomas of 2/58, 3/60, 2/59, 9/59 at 0, 510, 1,020, and 2,110 mg/kg-d. The incidence of combined thyroid adenomas and carcinomas in male mice of 1/60, 0/59, 4/59, 2/57 at 0, 510, 1,020, and 2, 110 mg/kg-d was observed. The incidence of renal tubule adenomas and carcinomas observed by NTP in male rats was 8/50, 13/50, 19/50, 13/50 (or observed by Hard et al. (2011) in male rats was 4/50, 13/50, 18/50, 12/50) at 0, 90, 200, and 420 mg/kg-d. These thyroid and kidney tumors were statistically significantly increased by pairwise comparison (Fisher exact test, $p \leq 0.05$) and by trend test (Cochran-Armitage trend test,

$p \leq 0.05$). Taken together, this evidence supports the descriptor of "suggestive evidence of carcinogenic potential".

With regards to the comments on thyroid tumors, as discussed above, thyroid tumors were also observed in male mice. Regarding the assertion that the highest oral dose in the NTP (1995) study exceeded the MTD in mice, EPA's 2005 Cancer Guidelines discuss the determination of an "excessively high dose" and describe the process as one of expert judgment which requires that "...adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent." In the case of thyroid follicular cell adenomas, the study authors noted that water consumption by exposed female mice was similar to controls and that no overt toxicity was observed. In addition, the final average body weight reduction in female mice at the highest dose was 12% (NTP, 1995) and female mice in the high dose group had higher rates of survival than control animals (see discussion and added text in Sections 1.2.2 and 2.3.1). The final average body weight reduction in male mice at the highest dose was 5% to 10% (NTP, 1995) and water consumption by exposed males was similar to controls, but survival was reduced at the highest dose and the tumor response in male mice was adjusted for early mortality. Thus, there is no evidence of exceedance of the MTD or that this is the cause of tumor development.

With regards to the comment on renal tubule adenomas and poor survival rates in the controls, EPA's cancer guidelines (U.S. EPA, 2005) states that "the most relevant historical control data come from the same laboratory and the same supplier and are gathered within 2 or 3 years one way or the other of the study under review; other data should be used only with extreme caution." Genetic drift in the laboratory strains and differences in pathology examination at different times and in different laboratories could affect comparability of historical and concurrent control data. In this case due to the lack of suitable historical control data, it is preferred to use concurrent controls to determine statistical significance of tumor incidence. Decreased survival in controls may be due in part to the increased severity of CPN in control animals (see Section 1.2.1). However, tumor increases were statistically significant in trend testing which accounted for mortality. With regards to the additional comments on MOA, the etiology of CPN is unknown and CPN is both a spontaneous and complex disease whose processes are affected by aging and strain specificity (NIEHS, 2019). Therefore, it is difficult to separate the effects of spontaneously occurring CPN from those effects on CPN induced by chemical exposure (see response to comments under Question 4a and discussion in Section 1.2.1.). With regards to the comment related to the MTD, in the case of renal tubule adenomas, the study authors did not report exposure-related overt toxicity in male rats or any changes in toxicokinetics at the middle or high doses. Mortality increased with increasing exposure ($p = 0.001$) over the 2-year exposure period; however increased mortality does not account for the highest tumor incidence occurring at the middle dose. Furthermore, the tumor incidence at the high dose in male rats, which had a final body weight reduction of 24% was not significantly different from controls (see discussion and added text in Section 1.2.1).

Discussion regarding the cancer descriptor for all routes of exposure, the rationale for deriving the oral slope factor, and the characterization of the cancer risk estimate can be found in Sections 1.3.2 and 2.3.1, and in response to comments under Charge Questions 4b, 4c, and 4d.

APPENDIX F. QUALITY ASSURANCE (QA) FOR THE IRIS TOXICOLOGICAL REVIEW OF *TERT*-BUTYL ALCOHOL (*TERT*-BUTANOL)

This assessment is prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) Program. The IRIS Program is housed within the Office of Research and Development (ORD) in the Center for Public Health and Environmental Assessment (CPHEA). EPA has an agency-wide quality assurance (QA) policy that is outlined in the *EPA Quality Manual for Environmental Programs* (see CIO 2105-P-01.1) and follows the specifications outlined in EPA Order CIO 2105.1.

As required by CIO 2105.1, ORD maintains a Quality Management Program, which is documented in an internal Quality Management Plan (QMP). The latest version was developed in 2013 using Guidance for Developing Quality Systems for Environmental Programs (QA/G-1). An NCEA/CPHEA-specific QMP was also developed in 2013 as an appendix to the ORD QMP. Quality assurance for products developed within CPHEA is managed under the ORD QMP and applicable appendices.

The IRIS Toxicological Review of *tert*-Butanol has been designated as Influential Scientific Information (ISI) and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits. The development of IRIS assessments is done through a seven-step process. Documentation of this process is available on the IRIS website: https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#process.

Specific management of quality assurance within the IRIS Program is documented in a Programmatic Quality Assurance Project Plan (PQAPP). A PQAPP is developed using the EPA Guidance for Quality Assurance Project Plans (QA/G-5), and the latest approved version is dated March 2020. All IRIS assessments follow the IRIS PQAPP, and all assessment leads and team members are required to receive QA training on the IRIS PQAPP. During assessment development, additional QAPPs may be applied for quality assurance management. They include:

Title	Document Number	Date
Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program	L-CPAD-0030729-QP-1-3	March 2020
An Umbrella Quality Assurance Project Plan (QAPP) for PBPK Models	B-003740-QP-1-0	Feb 2018
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	B-003742-QP-1-0	Apr 2019
Contractor QAPP 1	B-IRISD-0030538	
Contractor QAPP 2	B-IRISD-0030622	

During assessment development, this project underwent two quality audits during assessment development including:

Date	Type of audit	Major findings	Actions taken
August 2019	Technical System Audit	None	None
June 2018	Technical System Audit	None	None

During Step 3 and Step 6 of the IRIS process, the IRIS toxicological review is subjected to external reviews by other federal agency partners, including the Executive Offices of the White House. Comments during these IRIS Process steps are available in the docket (Docket ID No. EPA-HQ-ORD-2013-1111) on http://www.regulations.gov.

During Step 4 of assessment development, the IRIS Toxicological Review of *tert*-Butanol underwent public comment from May 16, 2016 to Jul 15, 2016. Following this comment period, the toxicological review underwent external peer review by SAB in June 2017. The peer review report is available on the SAB website (https://yosemite.epa.gov/sab/sabproduct.nsf/0/8e4436d62da1fd2d85257e38006a3131!OpenDocument&TableRow=2.3#2.). All public and peer-review comments are available in the docket (Docket ID No. EPA-HQ-ORD-2013-1111).

Prior to release (Step 7 of the IRIS Process), the final toxicological review is submitted to management and QA clearance. During this step, the CPHEA QA Director and QA Managers review the project QA documentation and ensure that EPA QA requirements have been met.

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