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

(CASRN 75-65-0)

Supplemental Information

June 2017

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ABBREVIATIONS

AIC	Akaike's information criterion
ARCO	ARCO Chemical Company
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
BW	body weight
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary
CYP450	cytochrome P450
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EPA	U.S. Environmental Protection Agency
ETBE	ethyl *tert*-butyl ether
HBA	2-hydroxyisobutyrate
HL	human leukemia
IC_{50}	half-maximal inhibitory concentration
i.p.	intraperitoneal
i.v.	intravenous
MFO	mixed function oxidase
MPD	2-methyl-1,2-propanediol
MTBE	methyl *tert*-butyl ether
NADPH	nicotinamide adenine dinucleotide phosphate
NTP	National Toxicology Program
·OH	hydroxyl radical
PBPK	physiologically based pharmacokinetic
POD	point of departure
SD	standard deviation
TWA	time-weighted average

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) time-weighted average (TWA) for up to a 10-hour workday and a 40-hour workweek.
Occupational Safety and Health (OSHA, 2006)	Permissible Exposure Limit for general industry – 100 ppm (300 mg/m^3) TWA for an 8-hour workday.
Food and Drug Administration (FDA, 2011a, 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).

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), as *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 were carried out in conjunction with other specific endpoints (e.g., developmental). ARCO (1983) determined no 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 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).

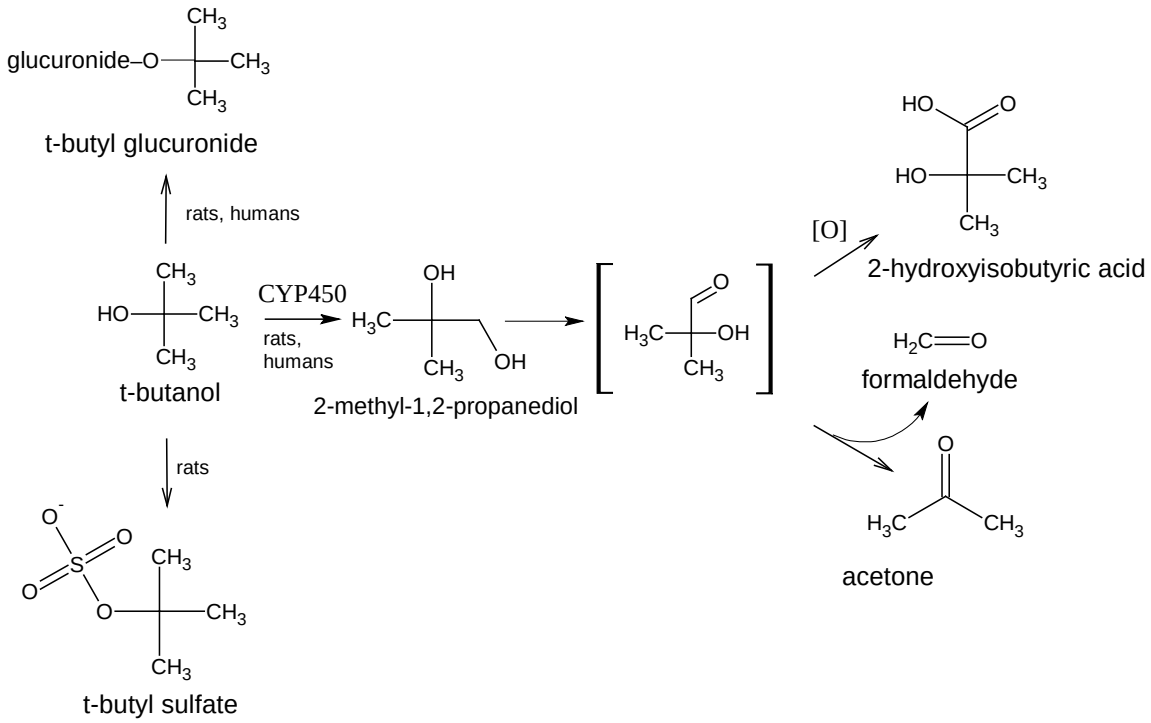
In a study aimed at determining whether *tert*-butanol (or metabolites) can bind to α_{2u}-globulin, Williams and Borghoff (2001) exposed F-344 rats to a single gavage dose of 500 mg/kg ^{14}C-*tert*-butanol and evaluated tissue levels at 12 hours. They found the radiolabel in three tissues (kidney, 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 α_{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 α_{2u}-globulin nephropathy, Borghoff et al. (2001) found similar results after F-344 rats were exposed to 0, 250, 450, or 1750 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, while 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.

No studies identified specific enzymes responsible for biotransforming *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 nicotinamide adenine dinucleotide phosphate (NADPH)-generating system resulted in formaldehyde production at a rate of approximately 25 nmoles/mg protein/30 min. According to study authors, the amount of formaldehyde generated by *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 min 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 K_m and V_{max} values of 30 mM and 5.5 nmol/min/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-radical (·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).



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

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

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 ($O_2\cdot^-$). 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 is 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:

$$H_2O_2 + Fe^{2+}\text{-chelate} \rightarrow \cdot OH + OH^- + Fe^{3+}\text{-chelate}$$

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 remain to be elucidated even though an NADPH-dependent electron transfer or $O_2^{\cdot -}$ 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; 1998b). 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/hr-kg with MTBE exposure (average of 25- and 50-ppm MTBE doses); the renal clearance rate was 0.80 ± 0.34 mL/hr-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 urine. At an ETBE level of 170 mg/m^3, *tert*-butanol displayed 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. Similar to 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 1500-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, 1750, or 5,000 ppm ^{14}C-ETBE for six hours. Besides recovery of total radioactivity in urine, feces, and expired air, 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 males. In rats pre-exposed to 5,000 ppm ETBE for 13 days, most of the excretion was in urine even at 5,000 ppm. For rats pre-exposed to 500 ppm ETBE, there also was a shift from exhalation to urinary excretion compared with naïve rats, but to a lesser degree than that elicited by the 5,000-ppm pre-exposure group.

Like rats, the fraction of radiolabel in exhaled volatiles increased with exposure level in mice 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 TBA 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

Three physiologically based pharmacokinetic (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; 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 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 pattern of drinking 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. During the active dark period (12 hours/day), it was assumed that 80% of total daily ingestion occurs (45-minute bouts with alternating 45-minute periods of other activity). During the relatively inactive light period (12 hours/day), it was assumed that the remaining 20% of daily ingestion occurs; during this time, 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).

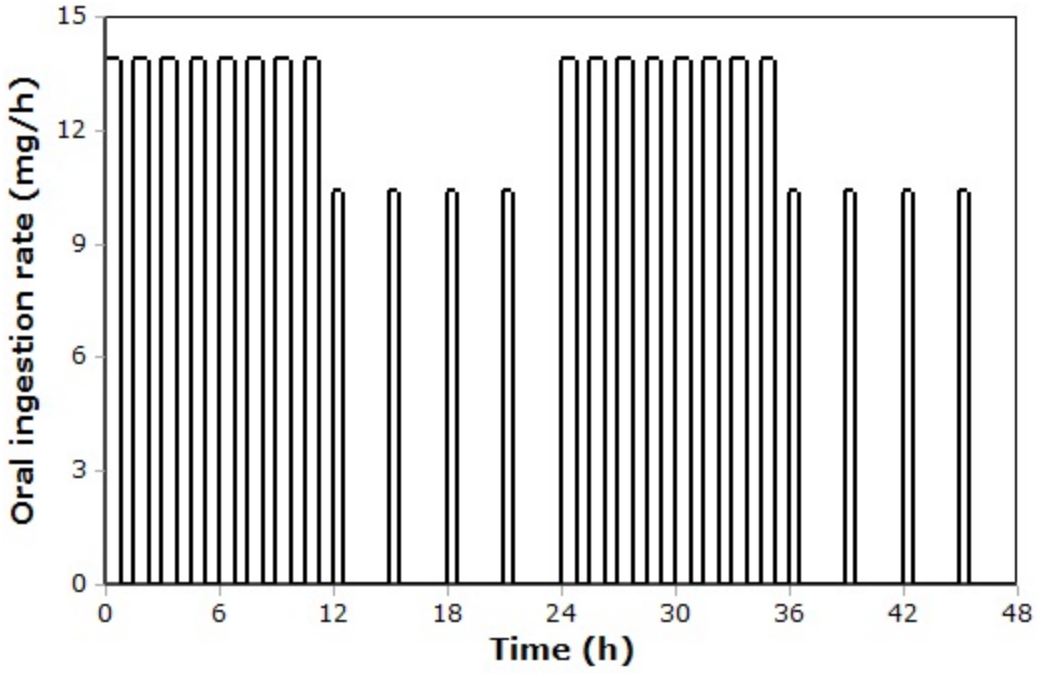


Figure B-2. Example oral ingestion pattern for rats exposed via drinking water.

PBPK modeling was also used to evaluate a variety of internal dose metrics (daily average TBA blood concentration, daily amount of TBA 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 TBA (Salazar et al., 2015). Administering ETBE either orally or via inhalation achieved similar or higher levels of TBA blood concentrations or TBA metabolic rates as those induced by direct TBA administration. 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 TBA blood concentration as the dose metric for both ETBE and TBA studies. For kidney and liver tumors, however, a consistent dose-response pattern was not obtained using any dose metric. These data are consistent with TBA 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.

B.1.6. 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.2. OTHER PERTINENT TOXICITY INFORMATION

B.2.1. Other Toxicological Effects

B.2.1.1. *Synthesis of Other Effects*

Effects other than those related to kidney, thyroid, reproductive, developmental, and neurodevelopmental effects 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, oral 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 was 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, 1980; Thurman et al., 1980; McComb and Goldstein, 1979a, b; 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-3 and Figure B-4 for oral and inhalation studies, respectively.

Kidney effects

Absolute and relative kidney weight numerical data are presented in Table B-1.

Liver effects

Liver weight and body weight were demonstrated to be proportional, and liver weight normalized to body weight was concluded 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 database. Increases in relative liver weight with *tert*-butanol exposure were observed, but the results pertaining to histopathologic changes were inconsistent (Table B-2 and Table B-3). 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) with 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 histopathological changes were seen in the subchronic mouse study (NTP, 1995). No treatment-related effects in liver histopathology were observed in rats or mice of the NTP (1997) subchronic inhalation study.

Urinary bladder effects

Subchronic studies reported effects in the urinary bladder (Table B-4), 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 incidence of minimal to mild inflammation in the urinary bladder after both subchronic and chronic exposures, with a greater incidence in males compared with females.

B.2.1.2. *Mechanistic Evidence*

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

B.2.1.3. *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-1. Changes in kidney weight in animals following exposure to *tert*-butanol

Reference and study design	Results
Kidney weight (percent change as compared to control)	
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d Males: 9 weeks beginning 4 weeks prior to mating Females: ≅ 10 weeks (4 weeks prior to mating through PND21)	**Males** Dose (mg/kg-d) / Left absolute weight / Left relative weight / Right absolute weight / Right relative weight 0 / 0 / 0 / 0 / 0 64 / 6 / 8 / 6 / 8 160 / 9 / 14* / 6 / 11* 400 / 12* / 14* / 14* / 17* 1,000 / 18* / 28* / 20* / 31* **Females** Dose (mg/kg-d) / Left absolute weight / Left relative weight / Right absolute weight / Right relative weight 0 / 0 / 0 / 0 / 0 64 / −1 / −2 / 2 / 0 160 / 0 / 0 / 1 / 0 400 / 3 / 2 / 4 / 2 1,000 / 4 / 0 / 7 / 2
NTP (1995) F344/N rat; 10/sex/treatment Drinking water 0, 2.5, 5, 10, 20, 40 mg/mL M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	**Males** / **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*

Reference and study design	Results					
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	**Males**			**Females**		
	Dose (mg/kg-d)	Absolute weight	Relative weight	Dose (mg/kg-d)	Absolute weight	Relative weight
	0	0	0	0	0	0
	350	1	1	500	0	−3
	640	3	2	820	−3	−1
	1,590	2	8	1,660	1	0
	3,940	6	22*	6,430	6	15*
	8,210	0	48*	11,620	12*	35*
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	**Males**			**Females**		
	Dose (mg/kg-d)	Absolute weight	Relative weight	Dose (mg/kg-d)	Absolute weight	Relative weight
	0	0	0	0	0	0
	90	4	8	180	8*	14*
	200	11	15*	330	18*	21*
	420	7	20*	650	22*	42*
	Only rats sacrificed at 15 months 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 hr/d, 5 d/wk 13 weeks 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*	

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 hr/d, 5 d/wk 13 weeks 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*

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

Table B-2. Changes in liver weight in animals following exposure to *tert*-butanol

Reference and study design	Results
Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	No significant treatment-related effects (results were only provided in a figure)
Lyondell Chemical Co. (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d Males: 9 weeks beginning 4 weeks prior to mating Females: 4 weeks prior to mating through PND21	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / - / - 64 / −1 / 0 160 / −3 / 1 400 / −2 / −1 1,000 / 8 / 16* **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / - / - 64 / −4 / −4 160 / −7 / −5 400 / 2 / 1 1,000 / 8 / 3
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / - / - 230 / −2 / 4 490 / 1 / 8* 840 / 5 / 20* 1,520 / 8 / 31* 3,610 / All dead / All dead **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / - / - 290 / 11* / 9* 590 / 10* / 9* 850 / 12* / 11* 1,560 / 15* / 16* 3,620 / 9* / 41*
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / - / - 350 / 2 / 3 640 / −1 / −2 1,590 / −1 / 5 3,940 / 0 / 14* 8,210 / −16 / 22* **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / - / - 500 / −1 / −4 820 / −5 / −3 1,660 / −8 / −9* 6,430 / −2 / 6 11,620 / −6 / 13*

NTP (1995)
F344/N rat; 60/sex/treatment
(10/sex/treatment evaluated at 15 months)
Drinking water (0, 1.25, 2.5, 5 or 10 mg/mL)
M: 0, 90, 200, or 420[a] mg/kg-d
F: 0, 180, 330, or 650[a] mg/kg-d
2 years

Percent change compared to control:

Males			Females		
Dose (mg/kg-d)	Absolute weight	Relative weight	Dose (mg/kg-d)	Absolute weight	Relative weight
0	-	-	0	-	-
90	2	7	180	−14*	−8
200	8	11	330	−3	−1
420	1	14*	650	−6	9*

Only animals sacrificed at 15 months were evaluated for organ weights. Organ weights were not measured in the 2-year mouse study

NTP (1997)
F344/N rat; 10/sex/treatment
Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole body chamber)
6 hr/d, 5 d/wk
13 weeks
Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported

Percent 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*

NTP (1997)
B6C3F$_1$ mouse; 10/sex/treatment
Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole body chamber)
6 hr/d, 5 d/wk
13 weeks
Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported

Percent 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*

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

Table B-3. 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, 575 mg/kg-d 10 weeks	↑ liver glycogen (~ 7 fold)* ↑incidence of centrilobular necrosis, vacuolation of hepatocytes, loss of hepatocyte architecture, peripheral proliferation, and lymphocyte infiltration (incidences and results of statistical tests not reported)
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	No treatment-related effects observed.
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	No treatment-related effects observed.
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	No treatment-related effects observed.
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	**Males** / **Females** Dose (mg/kg-d) / Incidence of fatty change / Dose (mg/kg-d) / Incidence of fatty change 0 / 12/59 / 0 / 11/60 540 / 5/60 / 510 / 8/60 1,040 / 8/59 / 1,020 / 8/60 2,070 / 29/59* / 2,110 / 6/60
NTP (1997) F344/N rat; 10/sex/treatment Inhalation analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) (dynamic whole body chamber) 6 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	No treatment-related effects observed in the high dose group (only treatment group with liver endpoints evaluated).

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 hr/d, 5 d/wk 13 weeks Generation method (Sonimist Ultrasonic spray nozzle nebulizer), analytical concentration and method were reported	Authors stated that there were no treatment-related microscopic changes, but data were not provided.

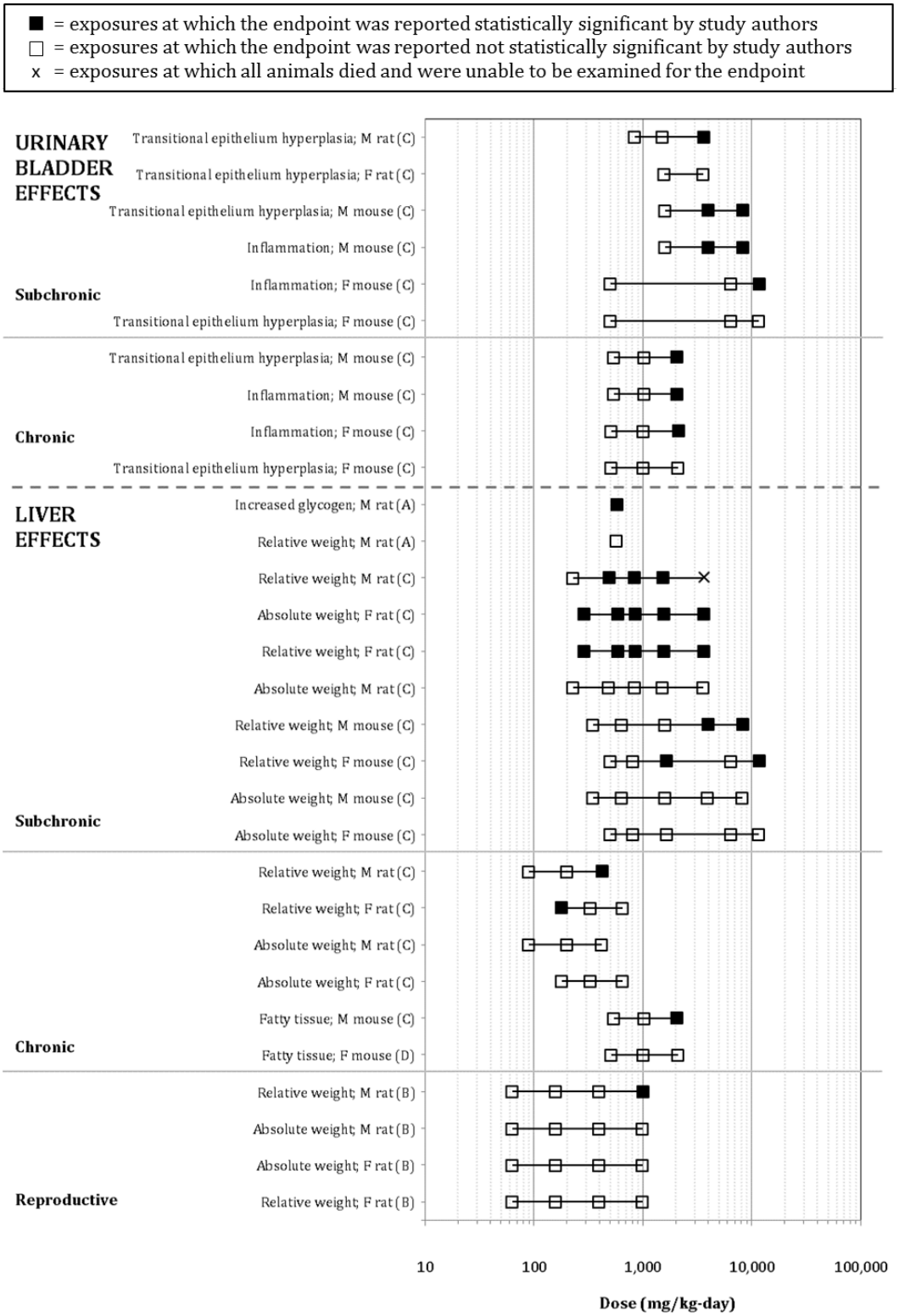
[a]The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.

Table B-4. Changes in urinary bladder histopathology in animals following oral exposure to *tert*-butanol

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

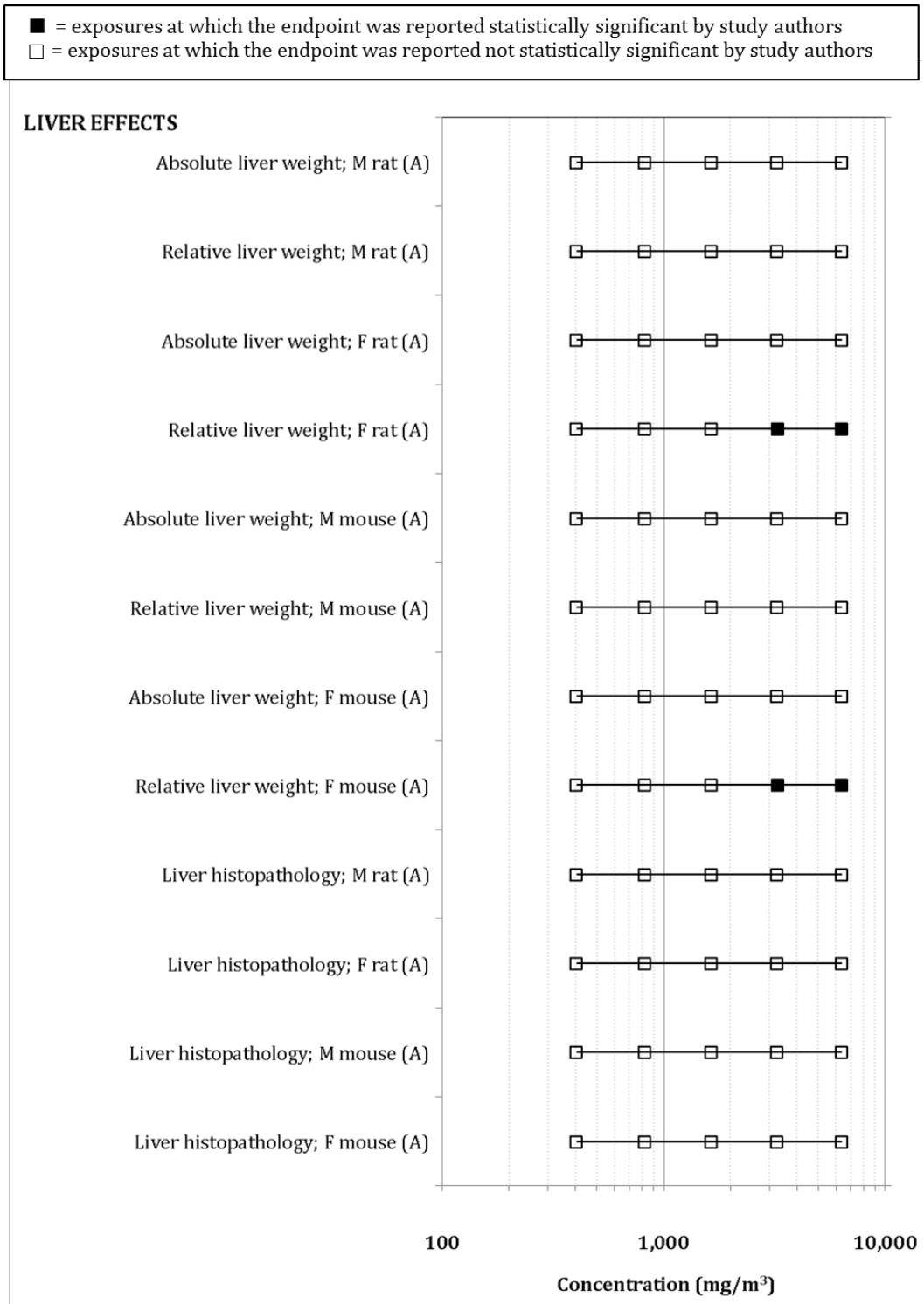
Reference and study design	Results
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, 2,070[a] mg/kg-d F: 0, 510, 1,020, 2,110 mg/kg-d 2 years	Incidence (severity): **Males** Dose (mg/kg-d) / Transitional epithelial hyperplasia / Inflammation 0 / 1/59 (2.0) / 0/59 540 / 3/59 (1.7) / 3/59 (1.7) 1,040 / 1/58 (1.0) / 1/58 (1.0) 2,070 / 17/59* (1.8) / 37/59* (2.0) **Females** Dose (mg/kg-d) / Transitional epithelial hyperplasia / Inflammation 0 / 0/59 / 0/59 510 / 0/60 / 0/60 1,020 / 0/59 / 0/59 2,110 / 3/57 (1.0) / 4/57* (2.0) Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

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



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

Figure B-3. Exposure-response array of other effects following oral exposure to *tert*-butanol.



Source: (A) NTP (1997)

Figure B-4. Exposure-response array of other effects following inhalation exposure to *tert*-butanol.

B.2.2. Genotoxicity

The genotoxic potential of *tert*-butanol has been studied using a variety of genotoxicity 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-5.

B.2.2.1. *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 (Jimenez 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 (Jimenez et al., 1988).

B.2.2.2. *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 locus (tk) in the L5178Y tk+/- 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+/− 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, percent 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).

B.2.2.3. *In Vivo Mammalian Studies*

Few in vivo studies are available to understand the role of *tert*-butanol on genotoxicity. The National Toxicology Program studied the effect of *tert*-butanol in a 13-week toxicity study (NTP, 1995). Peripheral blood samples were obtained from male and female B6CF1 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 (8 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 *tert*-butanol was found, for the first time, to form DNA adducts in mouse liver, lung, and kidney. Because this is a single and first-time study, further validation of this study will provide certainty in understanding the mechanism of *tert*-butanol-induced DNA adducts.

Table B-5. Summary of genotoxicity (both in vitro and in vivo) studies of *tert*-butanol

Test system	Dose/Conc.	Results[a]		Comments	Reference
Bacterial Systems					
		-S9	+S9		
Reverse Mutation Assay *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	100, 333, 1,000, 3333, 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)	1000–4000 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)	1.75mol/L	-	-	Eighty four percent 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	Jimenez et al. (1988)
In vitro Systems					
Gene Mutation Assay, Mouse lymphoma cells L5178Y $TK^{+/-}$	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 days 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)
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 effect of cell cycle control genes and mechanism of action for TBA	Sgambato et al. (2009)

Test system	Dose/Conc.	Results[a]		Comments	Reference
DNA damage, (comet assay), HL-60 leukemia cells	1, 5, 10, 30 mmol/L	+	ND	Exposure duration – 1h	Tang et al. (1997)
In vivo Animal Studies					
Micronucleus induction , B6C3F1 mouse peripheral blood cells	3,000, 5,000, 10,000, 20,000, 40,000 ppm	-		13-week, subchronic, drinking water study	NTP (1995)
Micronucleus induction, male rats, bone marrow cells	39, 78, 156, 312, 625, 1250	-		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)

[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. Other species such as *Neurospora crassa* did not produce reverse mutations due to exposure to *tert*-butanol.

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 $TK^{+/-}$). 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, however, 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 observed. 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 then is 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 the National Toxicology Program.

Overall, a limited database is available for understanding the role of *tert*-butanol-induced genotoxicity for mode of action and carcinogenicity. The database 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 database in terms of the methodology used, number of studies in the overall database, 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 thus 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 cancer and noncancer parts 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

C.1.1.1. *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.

C.1.1.2. *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 log-logistic model, restrict slope ≥1; for gamma and Weibull models, restrict power ≥1; and for 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, such as 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 Hill, power, and exponential models, restrict power ≥1. Model fit was assessed by a series of tests. For each model, first the homogeneity of the variances was tested 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, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and near the BMR.

C.1.1.3. *Model Selection*

For each endpoint, the BMDL estimate (95% lower confidence limit on the 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 three-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

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)/Male	Dose (mg/kg-d)	0	90	200	420	
		Incidence/Total	25/50	32/50	36/50	40/50	
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)/Male	Dose (mg/kg-d)	0	90	200	420	
		Mean ± SD (n)	1.78 ± 0.18 (10)	1.85 ± 0.17 (10)	1.99 ± 0.18 (10)	1.9 ± 0.23 (10)	
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)/Male	Concentration (mg/m^3)	0	406	825	1643	3274	6369
		Mean ± SD (n)	1.21 ± 0.082 (10)	1.21 ± 0.096 (9)	1.18 ± 0.079 (10)	1.25 ± 0.111 (10)	1.34 ± 0.054 (10)	1.32 ± 0.089 (10)
Increased absolute kidney weight	Rat (F344)/Female	Concentration (mg/m^3)	0	406	825	1643	3274	6369

Endpoint/Study	Species/ Sex	Doses and effect data						
NTP (1997)		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)

C.1.1.4. *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 male 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			
Log-logistic	**0.976**	**248.0**	**30**	**16**	Log-logistic model selected as best-fitting model based on lowest AIC with all BMDL values sufficiently close (BMDLs differed by slightly more than 3-fold).
Gamma	0.784	248.5	46	29	
Logistic	0.661	248.8	58	41	
Log-probit	0.539	249.2	84	53	
Multistage, 3°	0.784	248.5	46	29	
Probit	0.633	248.9	60	43	
Weibull	0.784	248.5	46	29	
Dichotomous-Hill	0.968	250.0	25	15	

[a] Scaled residuals for selected model for doses 0, 90, 200, and 420 mg/kg-d were –0.076, 0.147, 0.046, and –0.137, respectively.

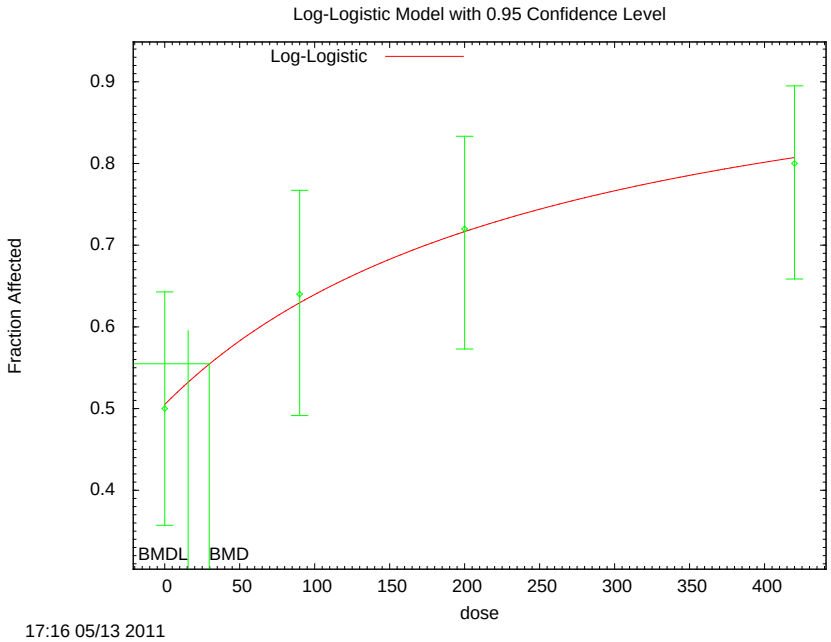


Figure C-1. Plot of incidence by dose, with fitted curve for LogLogistic model for kidney transitional epithelial hyperplasia in male F344 rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d.

```
 ================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File: M:\NCEA t-Butanol\BMD modeling\BMDS Output\17 NTP 1995b_Kidney
transitional epithelial hyperplasia, male rats_LogLogistic_10.(d)
        Gnuplot Plotting File: M:\NCEA t-Butanol\BMD modeling\BMDS Output\17 NTP
1995b_Kidney transitional epithelial hyperplasia, male rats_LogLogistic_10.plt
                                                  Fri May 13 17:16:25 2011
 ================================================================

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

  The form of the probability function is:

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


  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 Parameter Values
          background =     0.5
          intercept =   -5.54788
```

```
              slope =      1


      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    -0.71

 intercept    -0.71      1



                Parameter Estimates

                            95.0% Wald Confidence Interval
    Variable     Estimate    Std. Err.   Lower Conf. Limit  Upper Conf. Limit
   background     0.505366      *         *          *
   intercept     -5.58826      *         *          *
     slope        1      *         *          *

* - Indicates that this value is not calculated.


            Analysis of Deviance Table

    Model   Log(likelihood) # Param's Deviance Test d.f.  P-value
   Full model    -121.996     4
  Fitted model     -122.02     2   0.048148   2     0.9762
 Reduced model    -127.533     1    11.0732   3     0.01134

      AIC:     248.04


                Goodness of Fit
                                Scaled
   Dose   Est._Prob.  Expected  Observed   Size    Residual
 ---------------------------------------------------------------------
  0.0000   0.5054    25.268  25.000     50    -0.076
  90.0000   0.6300    31.498  32.000     50    0.147
 200.0000   0.7171    35.854  36.000     50    0.046
 420.0000   0.8076    40.382  40.000     50    -0.137

 Chi^2 = 0.05   d.f. = 2    P-value = 0.9762


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

Confidence level =      0.95

       BMD =    29.6967

      BMDL =    15.6252
```

Table C-3. 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 3-fold).
Logistic	0.50	92.81	461	393	
LogLogistic	0.79	91.57	414	333	
LogProbit	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.

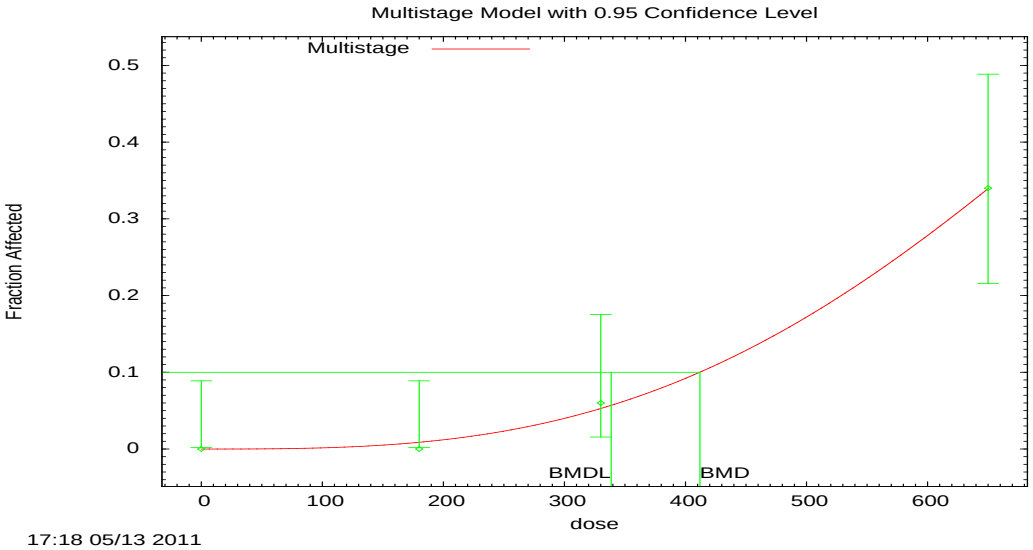


Figure C-2. Plot of incidence by dose, with fitted curve for Multistage 3° model for kidney transitional epithelial hyperplasia in female F344 rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d.

```
================================================================
        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]
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

         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-4. Summary of BMD modeling results for absolute kidney weight in male F344 rats exposed to *tert*-butanol in drinking water for 15 months (NTP, 1995); BMR = 10% rel. dev. 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)[b]	0.123	−86.757	661	307	Of the models that provided an adequate fit and a valid BMDL estimate, the linear model was selected based on lowest AIC.
Exponential (M3)[c]	0.123	−86.757	661	307	
Exponential (M4)	0.167	−87.041	error[d]	0	
Exponential (M5)	N/A[e]	−85.880	error[d]	0	
Hill	0.301	−87.880	error[d]	error[d]	
Power[f] **Polynomial 3°[g]** **Polynomial 2°[h]** **Linear**	**0.126**	**−86.804**	**657**	**296**	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.777), selected model in bold; scaled residuals for selected model for doses 0, 90, 200, and 420 mg/kg-d were −0.78, −0.11, 1.65, −0.76, respectively.
[b]The Exponential (M2) model can appear equivalent to the Exponential (M3) model, however differences exist in digits not displayed in the table.
[c]The Exponential (M3) model can appear equivalent to the Exponential (M2) model, however differences exist in digits not displayed in the table.
[d]BMD or BMDL computation failed for this model.
[e]No available degrees of freedom to calculate a goodness-of-fit value.
[f]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[g]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.
[h]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.

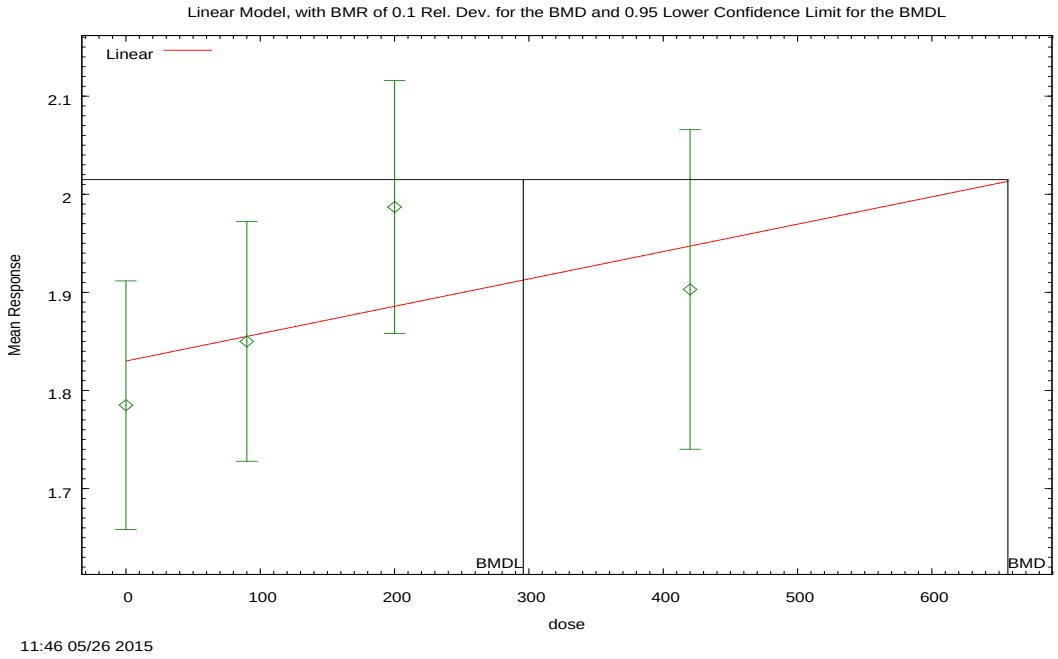


Figure C-3. Plot of mean response by dose, with fitted curve for Linear model with constant variance for absolute kidney weight in male F344 rats exposed to *tert*-butanol in drinking water for 15 months (NTP, 1995); BMR = 10% rel. dev. from control mean; dose shown in mg/kg-d.

Polynomial Model. (Version: 2.20; Date: 10/22/2014)

The form of the response function is: Y[dose] = beta_0 + beta_1*dose.

A constant variance model is fit.

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 656.583

BMDL at the 95% confidence level = 295.826

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	0.0361494	0.0362125
rho	n/a	0
beta_0	1.83173	1.83173
beta_1	0.000278979	0.000278979

1 **Table of Data and Estimated Values of Interest**

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	10	1.78	1.83	0.18	0.19	−0.777
90	10	1.85	1.86	0.17	0.19	−0.114
200	10	1.99	1.89	0.18	0.19	1.65
420	10	1.9	1.95	0.23	0.19	−0.763

2 **Likelihoods of Interest**

Model	Log(likelihood)	# Param's	AIC
A1	48.474229	5	−86.948457
A2	49.025188	8	−82.050377
A3	48.474229	5	−86.948457
fitted	46.401914	3	−86.803828
R	45.368971	2	−86.737942

3 **Tests of Interest**

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	7.31243	6	0.2929
Test 2	1.10192	3	0.7766
Test 3	1.10192	3	0.7766
Test 4	4.14463	2	0.1259

4

Table C-5. 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% rel. dev. 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.

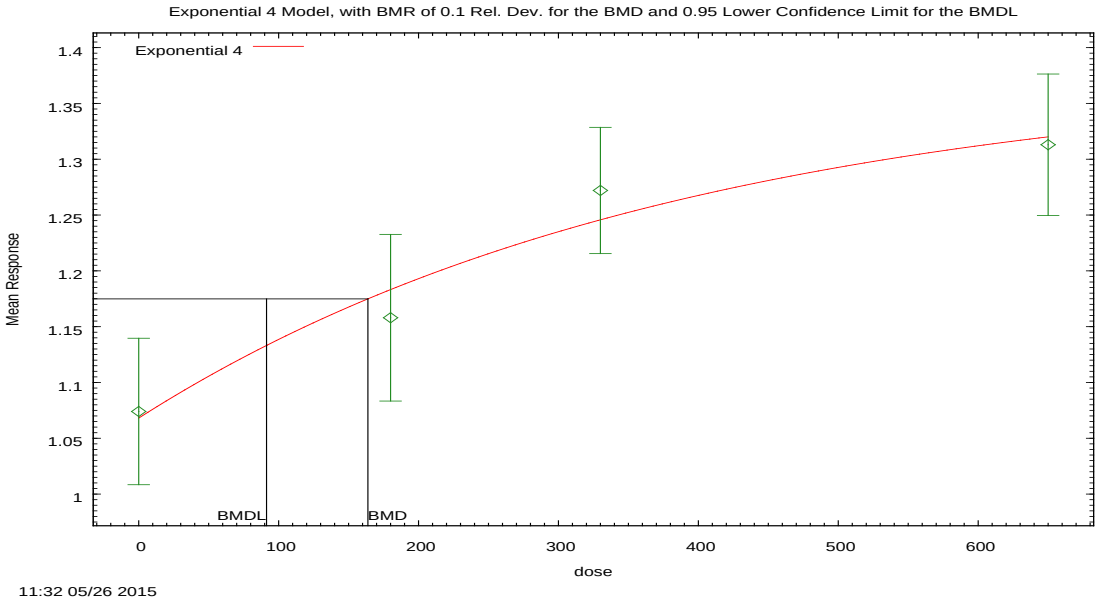


Figure C-4. Plot of mean response by dose, with fitted curve for Exponential (M4) model with constant variance for absolute kidney weight in female F344 rats exposed to *tert*-butanol in drinking water for 15 months (NTP, 1995); BMR = 10% rel. dev. from control mean; dose shown in mg/kg-d.

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

1 **Table of Data and Estimated Values of Interest**

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
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

2 **Likelihoods of Interest**

Model	Log(likelihood)	# Param's	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

3 **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

4

Table C-6. 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 3-fold).
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.

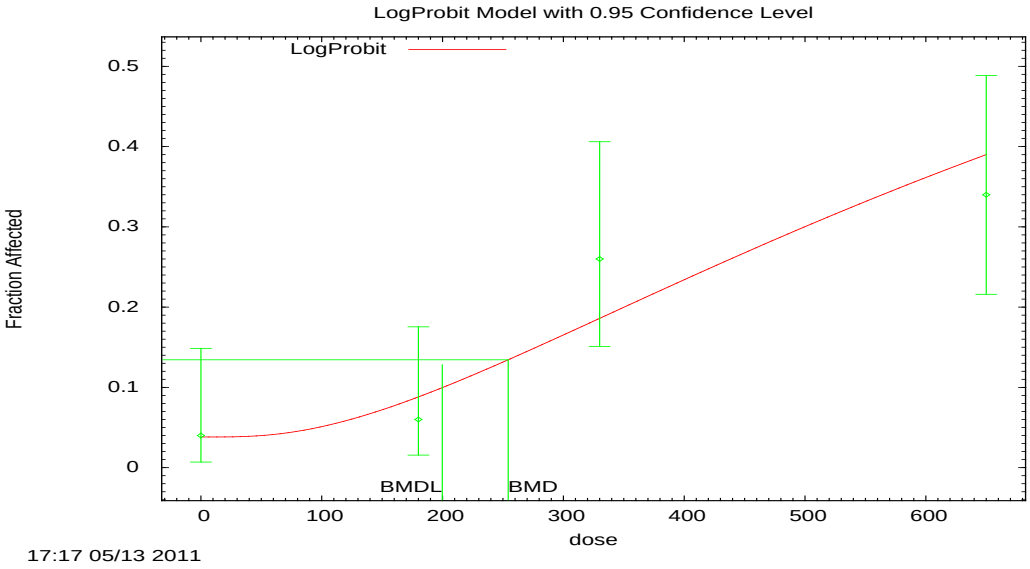


Figure C-5. Plot of incidence by dose, with fitted curve for LogpPobit model for kidney inflammation in female rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d.

```
        ================================================================
                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]
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

          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-7. Summary of BMD modeling results for absolute kidney weight in male F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean

Model[a]	Goodness of fit		BMC_{10RD} (mg/m^3)	$BMCL_{10RD}$ (mg/m^3)	Basis for model selection
	p-value	AIC			
Exponential (M2)	<0.0001	−205.06	error[b]	error[b]	Although the Hill model was the only adequately fitting model (p>0.1), the resulting fit was essentially a step-function that does not support interpolation between the well-fit observations.
Exponential (M3)	<0.0001	−203.06	9.2E+07	7094	
Exponential (M4)	<0.0001	−203.06	error[b]	0	
Exponential (M5)	<0.0001	−201.06	error[b]	0	
Hill	**0.763**	**−226.82**	**1931**	**1705**	
Power[c] Linear	0.0607	−220.97	5364	3800	
Polynomial 5°[d] Polynomial 4°[e] Polynomial 3°	1.44E−04	−207.06	−9999	error[f]	
Polynomial 2°	1.44E−04	−207.06	−9999	18436	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.390), selected model in bold; scaled residuals for selected model for doses 0, 406, 825, 1,643, 3,274, and 6,369 mg/m^3 were 0.395, 0.374, −0.75, −1.96e−006, 0.381, and −0.381, respectively.
[b]BMC or BMCL computation failed for this model.
[c]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[d]For the Polynomial 5° model, the b5 and b4 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Polynomial 3° model.
[e]For the Polynomial 4° model, the b4 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 3° model.
[f]BMC or BMCL computation failed for this model.

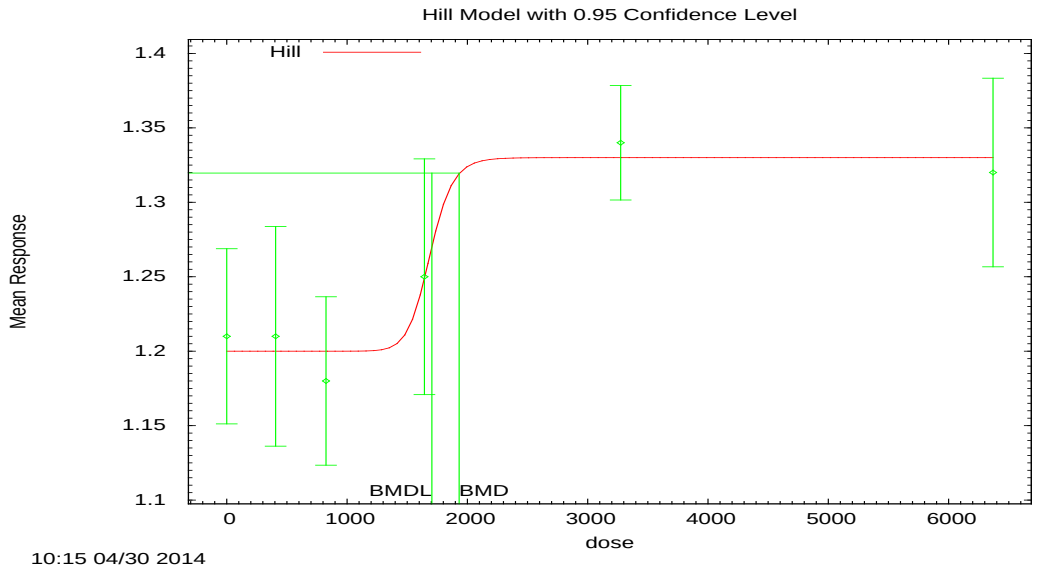


Figure C-6. Plot of mean response by concentration, with fitted curve for Hill model for absolute kidney weight in male F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m³.

Hill Model. (Version: 2.15; Date: 10/28/2009)

The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n).

A constant variance model is fit.

Benchmark Dose Computation.

BMR = 10% Relative risk

BMD = 1931.35

BMDL at the 95% confidence level = 1704.82

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	0.00687349	0.00750263
rho	n/a	0
intercept	1.19966	1.21
v	0.130345	0.13
n	18	18
k	1685.82	4451.94

1 **Table of Data and Estimated Values of Interest**

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	10	1.21	1.2	0.0822	0.0829	0.395
406	9	1.21	1.2	0.096	0.0829	0.374
825	10	1.18	1.2	0.0791	0.0829	−0.75
1643	10	1.25	1.25	0.111	0.0829	−0.00000196
3274	10	1.34	1.33	0.0538	0.0829	0.381
6369	10	1.32	1.33	0.0885	0.0829	−0.381

2 **Likelihoods of Interest**

Model	Log(likelihood)	# Param's	AIC
A1	117.992549	7	−221.985098
A2	120.600135	12	−217.20027
A3	117.992549	7	−221.985098
fitted	117.41244	4	−226.82488
R	105.528775	2	−207.05755

3 **Tests of Interest**

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	30.1427	10	0.0008118
Test 2	5.21517	5	0.3902
Test 3	5.21517	5	0.3902
Test 4	1.16022	3	0.7626

4

Table C-8. Summary of BMD modeling results for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean

Model[a]	Goodness of fit		BMC_{10RD} (mg/m^3)	$BMCL_{10RD}$ (mg/m^3)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0378	−261.52	14500	7713	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	14673	7678	
Polynomial 3°[d] Polynomial 2°[e] Linear	0.0274	−261.61	14673	7678	
Polynomial 5°	0.0274	−261.61	14673	7569	
Polynomial 4°	0.0274	−261.61	14673	7674	

[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.

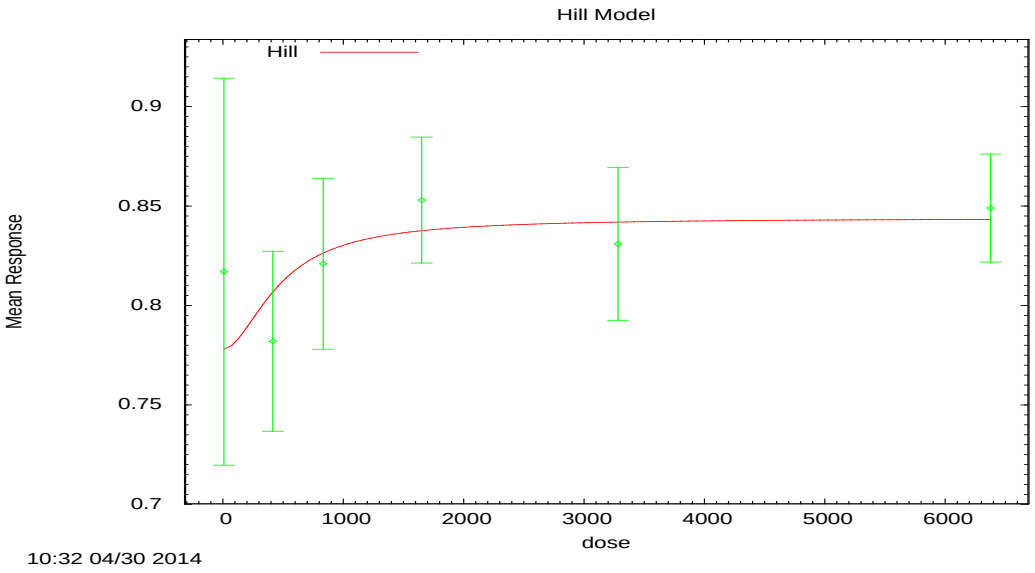


Figure C-7. Plot of mean response by concentration, with fitted curve for Hill model for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m³.

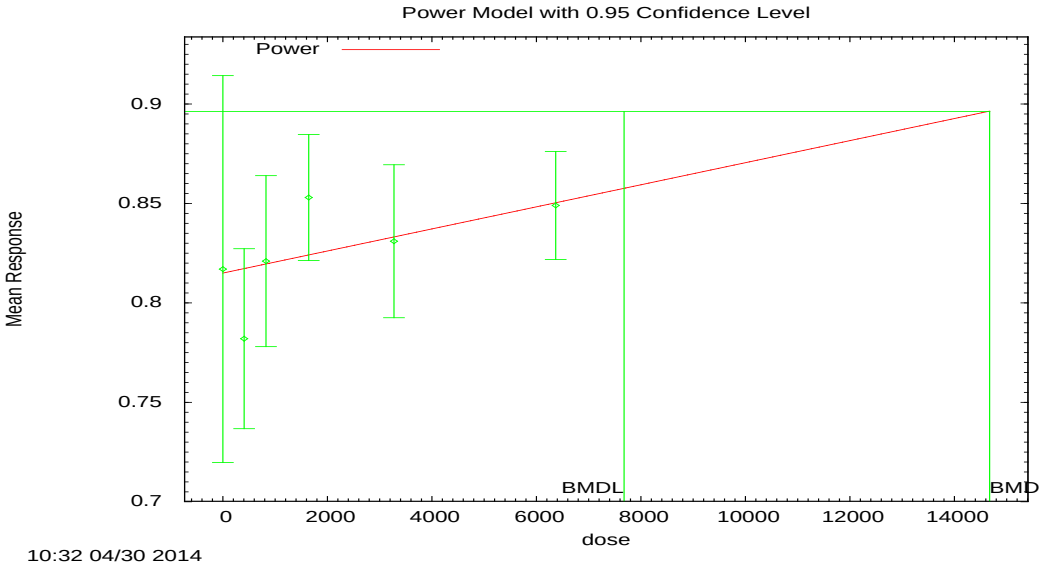


Figure C-8. Plot of mean response by concentration, with fitted curve for Power model for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m³.

C.1.2. Cancer Endpoints

C.1.2.1. *Data Sets*

The cancer data sets selected for dose-response modeling are summarized in Table C-9. In all cases, administered exposure was used in modeling the response data. Due to 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-9.

C.1.2.2. *Model Fit*

The multistage model was fit to the cancer data sets. Model coefficients were restricted to be non-negative (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, such as 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-9. 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-10. Summary of the oral slope factor derivations

Tumor	Species/Sex	Selected Model	BMR	BMD (mg/kg-d)	POD= BMDL (mg/kg-d)	$BMDL_{HED}$[a] (mg/kg-d)	Slope factor[b] $(mg/kg\text{-}day)^{-1}$
Thyroid							
Thyroid follicular cell adenoma	Female B6C3F1 mouse	3° Multistage	10%	2002	1437	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}

[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.

C.1.2.1. *Modeling Results*

Table C-11. Summary of BMD modeling results for thyroid follicular cell adenomas in female B6C3F1 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**	**2002**	**1437**	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 3-fold).
Two	0.36	115.402	2186	1217	
One	0.63	114.115	1987	1378	

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

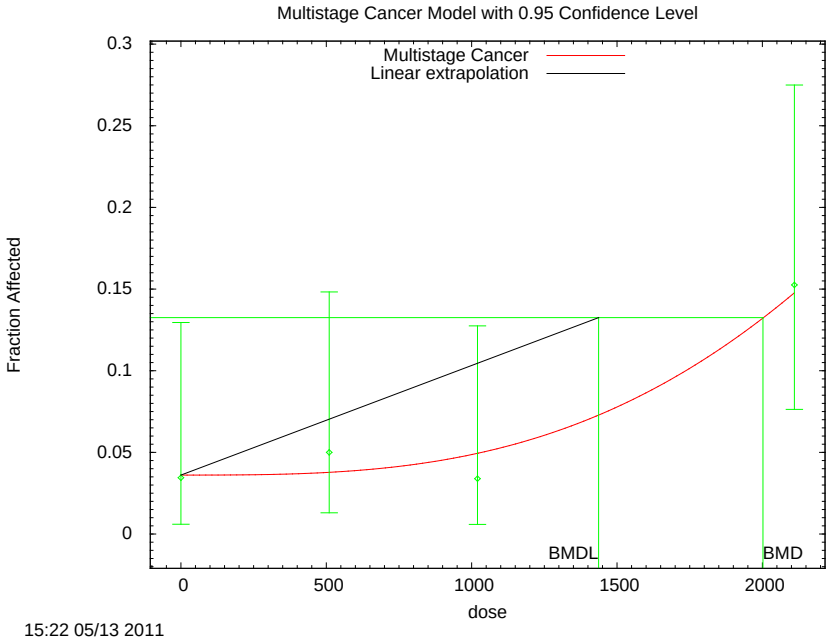


Figure C-9. Plot of incidence by dose, with fitted curve for Multistage 3° model for thyroid follicular cell adenomas in female B6C3F1 mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d.

```
        ================================================================
                 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
folluclar cell andenoma, female mice_MultiCanc3_10.(d)
                 Gnuplot Plotting File: M:\NCEA t-Butanol\BMD modeling\BMDS Output\29 NTP
1995b_Thyroid folluclar cell andenoma, 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-12. Summary of BMD modeling results for thyroid follicular cell adenomas or carcinomas in male B6C3F1 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**	**1788**	**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.

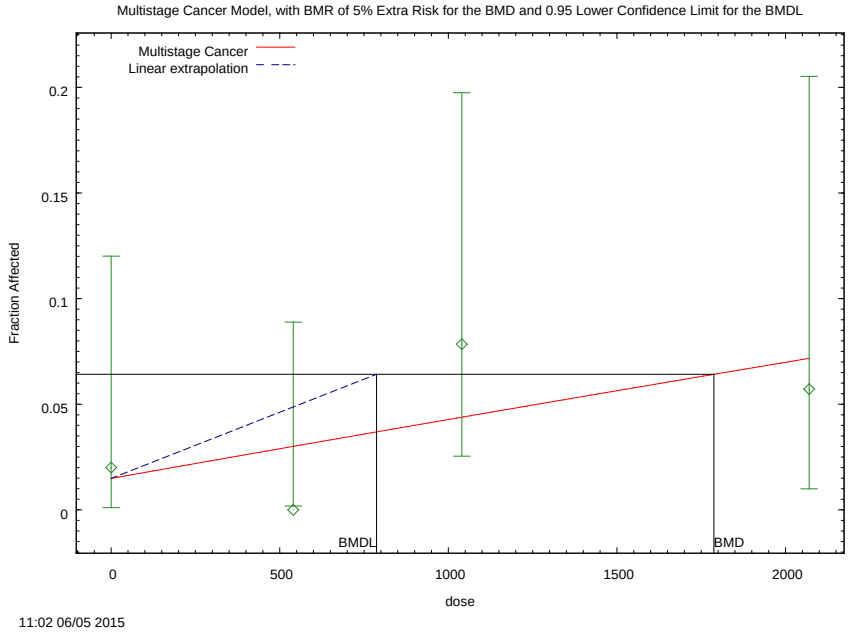


Figure C-10. Plot of incidence by dose, with fitted curve for Multistage 1° model for thyroid follicular cell adenomas or carcinomas in male B6C3F1 mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 5% extra risk; dose shown in mg/kg-d.

```
 ======================================================================
         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
                                                                      Scaled
      Dose     Est._Prob.    Expected    Observed     Size       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-13. Summary of BMD modeling results for thyroid follicular cell adenomas or carcinomas in male B6C3F1 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	1341	538	Multistage 2° was selected based on lowest AIC.
Two stage	**0.174**	**44.9**	**1028**	**644**	

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

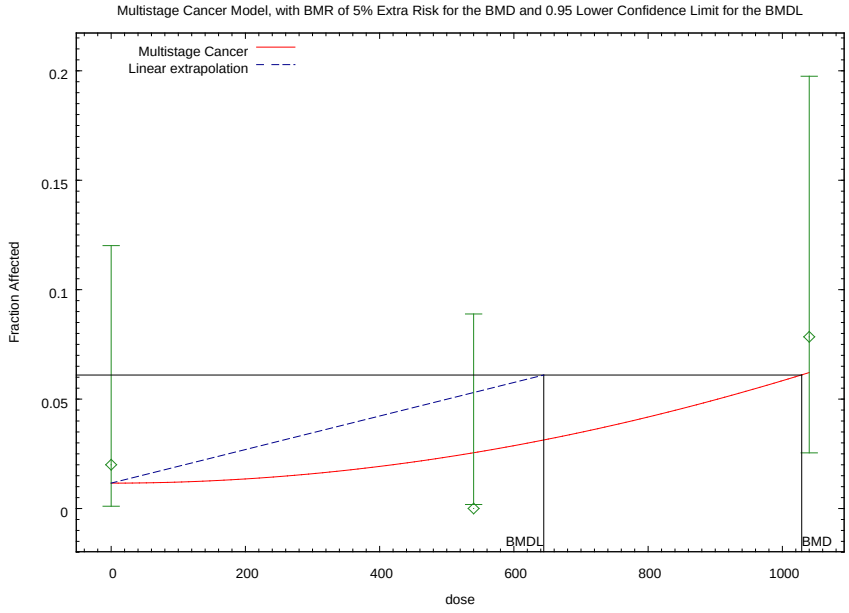


Figure C-11. Plot of incidence by dose, with fitted curve for Multistage 2° model for thyroid follicular cell adenomas or carcinomas in male B6C3F1 mice exposed to *tert*-butanol in drinking water for 2 years, high dose omitted (NTP, 1995); BMR = 5% extra risk; dose shown in mg/kg-d.

```
================================================================
         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-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 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.

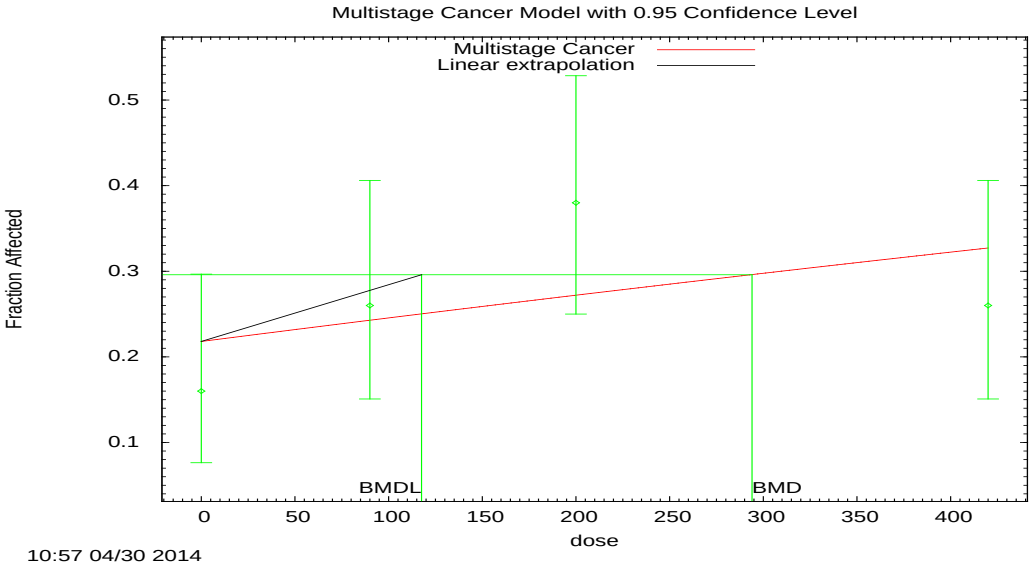


Figure C-12. Plot of incidence by dose, with fitted curve for Multistage 2° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and including all dose groups (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d.

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)	# Param's	Deviance	Test d.f.	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	Est. Prob.	Expected	Observed	Size	Scaled Resid
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 d.f = 2 P-value = 0.0806

Table C-15. 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.

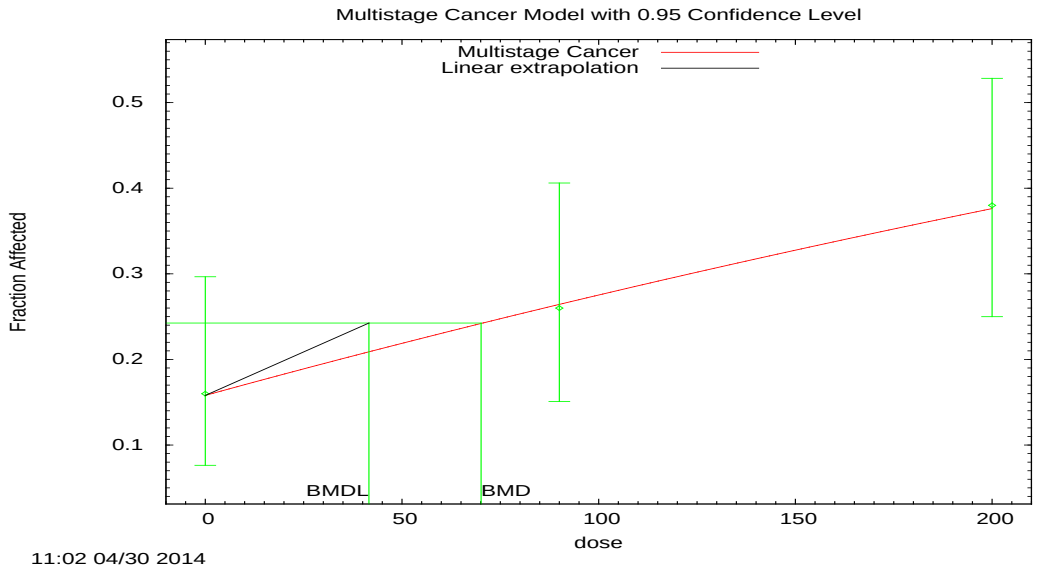


Figure C-13. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk.; dose shown in mg/kg-d.

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)	# Param's	Deviance	Test d.f.	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	Est. Prob.	Expected	Observed	Size	Scaled Resid
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 d.f = 1 P-value = 0.9239

Table C-16. 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-17. 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; re-analyzed 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.

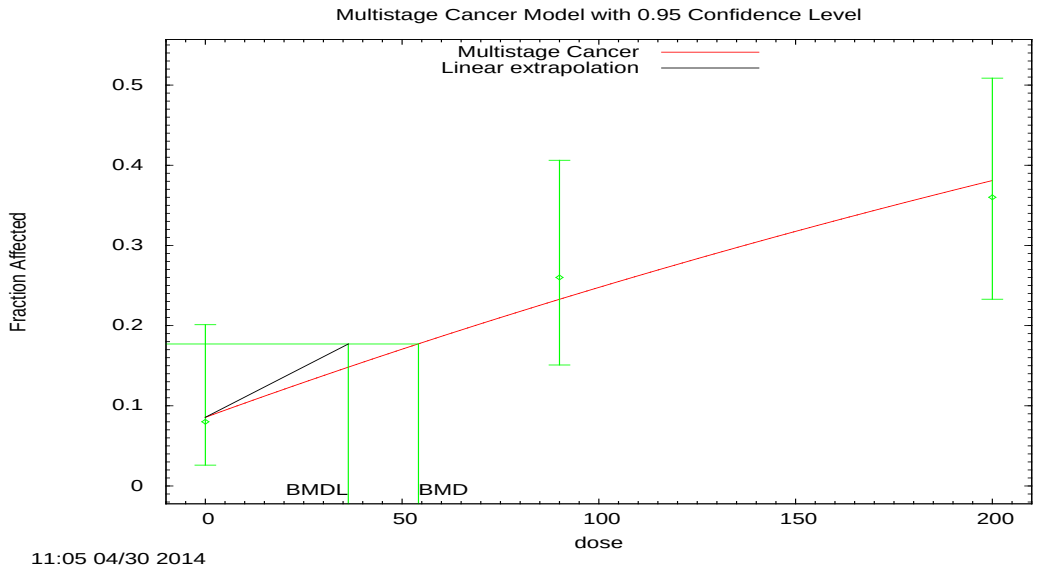


Figure C-14. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to tert-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group; re-analyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d.

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)	# Param's	Deviance	Test d.f.	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	Est. Prob.	Expected	Observed	Size	Scaled Resid
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 d.f = 1 P-value = 0.5715

APPENDIX D. SUMMARY OF PUBLIC COMMENTS AND EPA's DISPOSITION

The Toxicological Review of *tert*-Butyl alcohol (*tert*-Butanol) was released for a 60-day public comment period on May 16, 2016. Public comments on the assessment were submitted to EPA by:

- Japan Petroleum Energy Center (posted June 24, 2016);
- Exponent, Inc. on behalf of LyondellBasell (posted June 24, 2016);
- Samuel M. Cohen (posted July 7, 2016);
- Lawrence H. Lash (posted June 24, 2016);
- LyondellBasell (posted June 24, 2016 and July 19, 2016);
- American Chemistry Council (posted July 7, 2016);
- Tox-Logic Consulting on behalf of ExxonMobil Biomedical Sciences (posted July 19, 2016);
- Tox Strategies on behalf of LyondellBasell (posted June 24, 2016); and
- American Petroleum Institute (posted July 19, 2016).

A summary of major public comments provided in these submissions and EPA's response to these comments are provided in the sections that follow. The comments have been synthesized and paraphrased. Because several commenters often covered the same topic, the comment summaries are organized by topic. Editorial changes and factual corrections offered by public commenters were incorporated in the document as appropriate and are not discussed further. All public comments provided were taken into consideration in revising the draft assessment prior to releasing for external peer review.

Comments Related to the Preface, Preamble and Executive Summary

Comment [LyondellBasell]: The Salazar et al. (2015) model should be verified. This includes the model structure, code, and data sets used.

EPA Response: This peer-review draft uses a PBPK model based on Borghoff et al. (2016).

Comment [LyondellBasell]: The Executive Summary does not adequately capture:

1) The key uncertainties and high degree of conservatism associated with selecting rat kidney transitional epithelial hyperplasia as the key response for the RfD derivation in that this response is a recognized element of CPN and thus not relevant to human risk;

2) Uncertainties regarding the excessively high dose(s) used in the mouse thyroid tumor assessment, which exceed both the EPA and OECD test guidance for selection of a Limit Dose and other EPA and OECD guidance addressing the limitations of toxicity responses observed at dose levels saturating metabolic saturation with resulting nonlinear toxicokinetics of TBA; and

3) An acknowledgment that the oral SF should be clearly annotated with the conclusion that the overall "suggestive evidence" of TBA carcinogenicity does not allow for its use in quantitative human risk analyses.

EPA Response: The previous Executive Summary did not include the commenter's points because they had not been conclusions of the public-comment draft. The Executive Summary has been revised to reflect the conclusions of this peer-review draft, and the IRIS program has proposed a charge question for the SAB/CAAC to comment on whether the Executive Summary appropriately presents the major conclusions of the assessment.

Comments Related to the Literature Search and Study Quality

Comment [LyondellBasell]: Despite statements in preamble, there is no evidence that toxicity data from TBAc, MTBE or ETBE was robustly searched, despite clear toxicokinetic bridging from these studies to TBA.

EPA Response: The literature search was focused on tert-butanol as the primary chemical of interest. Toxicity reported on chemicals extensively metabolized to *tert*-butanol (i.e., TBAc, MTBE, and ETBE) are summarized in 1.1.4, and cross-compound comparisons for non-cancer and cancer effects are discussed in Sections 1.3.1 and 1.3.2, respectively.

Comment [LyondellBasell]: Clarity is needed regarding how the primary references were selected for "Sources of Health Effects Data" versus "Supporting Studies" and consistent application of decision criteria.

EPA Response: Table LS-3, in the row labeled "Outcome" provides a list of the health effects that cause a study to be considered a "source of health effects data." Other pertinent studies, including

mechanistic studies, are considered "supporting studies."

Comments Related to Data Presented in Evidence Tables

Comment [LyondellBasell]: Clarification is needed on what determined the endpoints selected for inclusion in evidence tables. Also, the exclusion of mechanistic key events (e.g., hyaline droplet accumulation) is not consistent with the intent of the EPA IRIS program and does not lead to the development of useful hazard evaluation.

EPA Response: Primary health effects information are included in evidence tables. Hyaline droplets do not constitute primary health effects information, but it is included in a table of mechanistic events used to evaluate the α2u-globulin mode of action (Table 1-4).

Comments Related to Kidney Effects

Comment [Dr. Bogen on behalf of American Petroleum Institute]: *tert*-Butanol induced male rat kidney tumors are not relevant to humans because *tert*-butanol-associated male rat kidney tumors were exacerbated by a CPN mode of action that is specific to rats. CPN has no human counterpart and is not considered relevant for human health risk assessment.

EPA Response: CPN is a common and well-established constellation of age-related lesions in the kidney of rats, and there is no known counterpart to CPN in aging humans. However, CPN is not a specific diagnosis on its own. These individual lesions or processes (tubular degeneration/regeneration and dilatation, glomerular sclerosis and atrophy, interstitial fibrosis and inflammation, etc.) could certainly occur in a human kidney. Because they happen to occur as a group in the aged rat kidney does not necessarily make them rat-specific individually if there is a treatment effect for one or more of them. In addition, exacerbation of one or more of these processes likely reflects some type of cell injury/cytotoxicity, which is relevant to the human kidney. One potential confounder is the alpha-2u globulin nephropathy in males which could also exacerbate CPN but would not be considered relevant to human risk.

In a recent draft proposal for public comment (2015), FDA used CPN in their calculation of PDEs for MIBK (http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm467089.pdf). Similarly, EPA considered the rat kidney tumors to be relevant to human health risk assessment.

Comment [Dr. Cohen, Dr. Hard, and LyondellBasell]: All the kidney changes identified in the assessment associated with tert-butanol exposure are associated with α2u-globulin nephropathy or/and CPN, except for the cortical-medullary calcification that is common in F344 rats. Cortical-

medullary calcification also is not relevant to humans based on a long series of articles published over the past 30 years or more. They asserted that transitional epithelial hyperplasia is not a valid endpoint for dose response because the lesion is a component of CPN, and that *tert*-butanol male rat renal tumors are adequately explained by alpha2u-globulin nephropathy combined with advanced CPN.

EPA Response: Section 1.2.1 shows that, although renal tumors are correlated with CPN in male rats, this correlation is weak in female rats and so the renal tumors cannot be attributed to solely to CPN. The peer-review draft does not consider cortical-medullary calcification relevant to humans, nor does it use TEH as an endpoint for dose-response assessment.

Comment [LyondellBasell]: Hyaline droplet accumulation was not given adequate importance and more discussion of the hyaline droplet pathology should be included.

EPA Response: Additional text describing hyaline droplets would not affect overall conclusion of the MOA nor would it serve to increase the clarity of the decision.

Comment [LyondellBasell]: An increase in kidney weights is a non-specific endpoint and should not be a candidate for potential use in BMD modeling.

EPA Response: Changes in kidney weights are a sensitive, non-specific endpoint that is often used for dose-response modeling. Although CPN and α2u-globulin nephropathy are occurring and can influence kidney weights, MOA analysis determined that they are only partially responsible for the observed kidney effects. Therefore, kidney weight changes are relevant for human health risk assessment.

Comment [LyondellBasell]: Suppurative inflammation in the rat kidney is a result of bacterial infection, and therefore this lesion cannot be used as an indicator of chemically-induced renal toxicity for the purposes of characterizing human hazard associated with *tert*-butanol. The suppurative inflammation would have been associated with either advanced CPN or an ascending infection probably related to urinary tract calculi, or both processes.

EPA Response: Suppurative inflammation is often but not always associated with bacterial infection. The bacteria may not be apparent on H&E staining though; culture and/or special staining are often needed. Suppurative inflammation may be part of the spectrum of CPN lesions and additional analysis indicates that suppurative inflammation is correlated with CPN in females. According to p39S of INHAND document: “Solitary proximal tubules affected with microabscessation often occur in advanced stages of CPN, in which setting they need not be

diagnosed separately (Frazier et al., 2012)." The text in the Executive Summary, Section 1.2.1, and 1.3.1 of the assessment has been clarified to reflect this information.

Comments Related to Thyroid Effects

Regarding the potential for high dose effects

Comment [Dr. Bus on behalf of LyondellBasell]: The dose levels at which thyroid tumors were identified in male and female mice was a major concern, and discussion of the toxicity and carcinogenicity findings at these high exposure concentrations would be informative, proposing that the MOA most likely operates under nonlinear kinetics. If the NTP (1995) *tert*-butanol bioassay was designed according to current dose selection guidance of EPA and OECD, mouse thyroid tumors likely would not have emerged as a significant cancer concern. In addition to referencing passages from OECD Guidance Document 116 (2012) describing the importance of considering rodent toxicokinetic or ADME data, and suggesting that the presence of toxicokinetic inflection point could be used as a surrogate for traditional target organ effects, the EPA Cancer Guidelines (2005) were referenced, noting that changes in toxicokinetics with increasing dose may result "…in important differences between high and low dose levels in disposition of the agent or generation of its active forms. These studies play an important role in providing a rationale for dose selection in carcinogenicity studies."

EPA Response: The discussion of the thyroid follicular cell tumors (adenomas and carcinoma) as well as the follicular cell hyperplasias, considered by both NTP and EPA to be pre-neoplastic lesions and thus not suitable candidates for non-cancer reference value derivation, is presented in Section 1.2.2, while considerations and uncertainties pertaining to dose-response evaluation are discussed in Sections 2.3.2 and 2.3.4, respectively. As discussed in Section 1.2.2, incidence of thyroid follicular cell hyperplasia was significantly elevated in all male mice treatment groups (i.e. doses of 540, 1040 or 2070 mg/kg-d), in both mid and high-dose female mice groups (i.e. 1020 and 2110 mg/kg-d), and was increased in low-dose female mice as well (510 mg/kg-d). As the hyperplasia was considered to be a pre-neoplastic lesion, and would be a key precursor step in the progression of initiated thyroid follicular cells towards neoplasia, the presence of increased hyperplasia and/or neoplasia incidence in all treatment groups in both sexes of mice does not support the assertion that a kinetic non-linearity exists which is responsible for a tumor-relevant response within the experimental treatment range (i.e. 510 – 2110 mg/kg-d). Furthermore, no MOA was identified for thyroid tumorigenesis, and no mouse PBPK model is available; as such, the available information appears insufficient to clearly describe the kinetics of mouse thyroid tumorigenesis following *tert*-butanol exposure. Therefore, there is insufficient information to predict with confidence what exposure level, if any, may result in metabolic saturation of *tert*-butanol oxidative metabolism in $B6C3F_1$ mice, especially considering the highly inducible nature of the cytochrome p450 system following repeated substrate exposure. The mouse follicular cell thyroid tumors were determined

to be qualitatively and quantitatively relevant to human cancer hazard characterization following recommendations from the EPA guidance on assessment of thyroid follicular cell tumors (EPA, 1998) and the EPA Cancer Guidelines (2005).

Comment [Dr. Bus and Dr. Borghoff on behalf of LyondellBasell]: Dose selection in these studies did not follow recommendations of EPA and OECD testing guidelines, including long-established limit dose guidelines of 1000 mg/kg bw/day and more recent considerations of saturated metabolism and nonlinear toxicokinetics in selection of appropriate doses for carcinogenicity and other test bioassays. Both male and female top dose groups reported in the NTP (1995) bioassay (2,070 and 2,100 mg/kg-d, respectively) exceeded the Limit Dose by 2-fold, questioning dose-relevance of thyroid tumor findings. Regarding the limit dose of 1,000 mg/kg-d, statements from a OECD dose selection guidance were provided "A limit of 1000 mg/kg body weight/day may apply except when human exposure indicates the need for a higher dose level to be used" (OECD, 2009), along with a 1998 guideline document from the EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS): "The highest dose tested need not exceed 1,000 mg/kg/day." (EPA 870.4300, Combined Chronic Toxicity/Carcinogenicity, 1998).

EPA Response: The 1998 guideline document referenced from the EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS), informs the design of chronic animal bioassays in the context of chemical testing and assessment prioritization, but does not provide instructions regarding the evaluation or interpretation of similar bioassays already planned or conducted, such as the NTP rodent bioassay reporting thyroid effects in mice following 2 years of oral *tert*-butanol exposure (NTP, 1995), and so referencing any specific statement from the document without noting the larger context for which it was intended can be misleading. Furthermore, the public comment appears to interpret "need not" as "shall not", whereas another section from the same EPA guidance document suggests that this was not the intended interpretation, and that bioassays evaluating doses higher than 1,000 mg/kg-d could be informative: "If a test at one dose level of at least 1,000 mg/kg body weight (expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects or if toxic effects would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary." Lastly, the paragraph immediately preceding the sentence from EPA OPPTS guidance quoted in the public comment describes the various considerations informative to selecting dose levels for a chronic rodent bioassay, which are analogous to the process that NTP employs. Notably, NTP does not appear to observe an arbitrary high-dose level limit, such as 1,000 mg/kg-d, as part of recent 2-year rodent bioassay study design; 5 NTP 2-year oral exposure technical reports (TR) published since 2010 have evaluated one or more dose levels > 1,000 mg/kg-d in mice and rats (TR-578, TR-567, TR-565, TR-562 and TR-556).

Comment [Dr. Borghoff on behalf of LyondellBasell and Dr. Fowles on behalf of Exxon Mobil Biomedical Sciences]: The fact that rats did not exhibit thyroid effects may likely be due to fact that rats were administered substantially lower TBA doses relative to mice (i.e. high doses of 650 mg/kg-d in female rats versus 2,110 mg/kg-d in female mice). Reliability of the NTP (1995) bioassay is questionable because the dose levels used for rats were significantly lower than used for mice.

EPA Response: The assertion is not entirely correct: treatment-related pre-neoplastic and/or neoplastic thyroid lesions were observed in mouse treatment groups exposed to 510 – 2,110 mg/kg-d, an exposure range which overlaps with the upper end of rat treatment doses (90 – 650 mg/kg-d). The lowest doses administered to male and female B6C3F$_1$ mice (540 and 510 mg/kg-d, respectively) are comparable to the highest doses administered to male and female F344 rats (420 and 650 mg/kg-d, respectively). Therefore, the presence of treatment-associated thyroid toxicity in mice, and the absence of thyroid toxicity in rats, is not due to rats being administered lower doses: if rats were at least similarly sensitive, which is the general assumption (Section 1.2.2; EPA, 1998), then thyroid effects should have been observed in the high dose rat groups.

It is unclear how the reliability of a rodent bioassay is directly related to the dose levels employed; however, uncertainties in dose-response evaluations are discussed in Section 2.3.4. As dose levels in NTP chronic rodent bioassays are selected based upon toxicity findings from subchronic and acute range-finding studies, and renal toxicity from these studies was found to be dose-limiting in rats but not mice, the higher dose-levels evaluated for mice versus rats is not unexpected, and does not represent any flaw in study design or methodology pertaining to study reliability.

Regarding the possible Mode of Action

Comment [Dr. Fowles on behalf of Exxon Mobil Biomedical Sciences and Dr. Bogen on behalf of American Petroleum Institute]: *tert*-butanol is a weak CYP and SULT liver enzyme inducer in female B6C3F$_1$ mice, sharing some PB- and CAR- like induction elements (Blanck et al., 2010). Furthermore, observed thyroid effects are likely secondary to a high dose hepatic enzyme induction effect on thyroid hormone elimination and homeostasis in mice. While the magnitude of effects were generally similar after 14 days in both the 2 and 20 mg/mL treatment groups (receiving 418 and 1616 mg/kg-d, respectively), early events such as the selective induction of SULT1A1, an important enzyme regulating mouse thyroid hormone clearance rates, were consistent with the slight reductions in T3/T4 as reported in the 14-day study, and would be expected to magnify with time at the high dose. While agreeing that no liver pathology was reported in the NTP study in rats or mice, as described in the *tert*-butanol draft, a statistically significant increase in relative liver weight was seen in high dose male and female mice, and that a fatty change was observed in male

high dose mice (but not females), the commenters concluded that TBA is not a general liver enzyme inducer but selectively induces specific CYPs and SULT1A1, and therefore evidence of histopathology associated with generalized liver enzyme induction would not be necessarily expected.

The decreases in T3 and T4 levels reported by Blanck et al., (2010), along with increases in liver enzyme levels and mRNA induction, particularly in CYP2B10, demonstrates effects which were "virtually certain to have been associated with CAR activation". CAR-activation mediated anti-thyroid MOA is not relevant to humans due to differences in T3/T4 hormone half-lives in humans versus rodents.

EPA Response: As discussed in Section 1.2.2, based upon recommendations from the EPA guidance document regarding the assessment of rodent thyroid follicular cell tumors (EPA, 1998), the available evidence was found to be inadequate to determine if any anti-thyroid MOA was operative in mice, including the suggested mechanism of nuclear-receptor stimulated induction of hepatic enzyme expression and increased thyroid hormone metabolism. The conclusion of a high dose effect is inconsistent both with the results reported in the single mechanistic study available (Blanck et al., 2010), which noted similar decreases in female B6C3F1 mouse serum T3 and T4 levels following 14 days of exposure to either 418 or 1616 mg/kg-d, as well as with the single chronic mouse bioassay available (NTP, 1995), which reported increased incidence of thyroid follicular cell hyperplasia and/or neoplasia in male and female B6C3F1 mice following 2 years of exposure to 540 – 2110 mg/kg-d. The effects on thyroid hormones in mice following short-term exposure to ≥ 418 mg/kg-d, and on thyroid histology following chronic exposure to ≥ 540 mg/kg-d, suggests that *tert*-butanol induces thyroid effects at the lowest doses evaluated in both studies. Inter-species differences in thyroid hormone metabolism is discussed in the EPA thyroid follicular cell tumor guidance (EPA, 1998), which concludes that despite these and other uncertainties, rodent thyroid tumors should be considered relevant to human cancer hazard characterization.

As discussed in the thyroid cancer MOA analysis in Section 1.2.2, if the decreases in T3/T4 levels observed following 14 days of exposure to 418 or 1616 mg/kg-d *tert*-butanol would be expected to magnify with time, then the sustained liver enzyme induction should have resulted in some treatment-associated increase in liver histopathology (such as centrilobular hyprotrophy) after subchronic or chronic exposures to comparable doses, i.e. 510 – 2110 mg/kg-d. However, as noted in the public comments, no such liver effects were reported in male or female $B6C3F_1$ mice. Furthermore, while it is unclear whether or not a 15 or 22% increase in liver SULT1A1 mRNA levels reported following 14 days to 418 or 1616 mg/kg-d exposure (Blanck et al., 2010) is sufficient to increase T3/T4 catabolism, and therefore cause the decrease in serum T3/T4 levels as reported at 14 days, it does provide further support to the identification of thyroid effects associated with thyroid carcinogenesis following exposure to low and high doses.

Regarding liver effects in mice following subchronic or longer exposure, the comment is assumed to reference the increased mouse relative liver weight in the 13 week subchronic component of the bioassay reported by NTP (1995) since a 15 month sacrifice was not collected from mice due to higher than anticipated early mortality, and organ weights were not reported at the terminal harvest (i.e. 2 years). Indeed, after 13 weeks of oral exposure, relative liver weight was induced in the two highest dose groups of male B6C3F1 mice (8210 and 3240 mg/kg-d), and in the highest dose group of female B6C3F1 mice (11620 mg/kg-d). However, in the groups receiving administered doses similar to those evaluated by Blanck et al. (2010), i.e. 418 and 1616 mg/kg-d, there was no significant change in relative liver weights in male mice exposed to 640 and 1590 mg/kg-d, or in female mice exposed to 820 mg/kg-d, while the relative liver weights in female mice exposed to 1660 mg/kg-d were significantly decreased by treatment, not increased. Notably, the relative liver weights of male and female F344 rats were increased following 13 weeks of exposure to 290 – 3620 mg/kg-d, and were significantly increased after 15 months of exposure in the high dose males and females administered 420 and 650 mg/kg-d, respectively. However, there were no liver or thyroid histopathological effects associated with tert-butanol exposure in rats, so changes in relative liver weight does not appear to be linked to either liver or thyroid pathology in either rats or mice following *tert*-butanol exposure.

The fatty change noted by the public comment was observed in the liver of only high dose male mice after 2 years of exposure, but no such effect was present in the livers of female mice, which were more sensitive to the thyroid toxicity induced by chronic *tert*-butanol exposure (80% of high dose females had thyroid lesions versus 30% of high dose males; NTP, 1995). Also, thyroid hyperplasia was induced in male mice in all treatment groups, despite fatty liver being only induced in the high dose group. Because of this, the induction of fatty change in the livers of males was considered to not be related to thyroid toxicity, as discussed in Section 1.2.2.

Comment [Dr. Fowles on behalf of Exxon Mobil Biomedical Sciences]: The lack of a statistically significant elevation in TSH in the short-term study does not invalidate the MOA, as TSH is a notoriously variable parameter, strongly influenced by stress and diurnal factors, and that low magnitude decreases in thyroid hormones in rodents may or may not trigger a measurable increase in circulating TSH. After such a short-term exposure to TBA and mild reductions in T3/T4, TSH levels would not be expected to be induced, as even the positive control phenobarbitol failed to induce an increase in TSH (Blanck et al., 2010).

EPA Response: While detecting small, treatment-associated changes in thyroid hormone and related pituitary hormone levels such as TSH maybe be experimentally difficult, complicated by several factors including diurnal variation in background levels, inter-animal differences, and analytical variability as pointed out by the public comment, the presence of such complications does not in and of itself constitute positive evidence supporting the effect. To address these

challenges, alternative endpoints (e.g. pituitary TSH subunit mRNA expression) have been evaluated in studies of other compounds as an alternative measure to blood TSH levels. As discussed in Section 1.2.2, changes in TSH levels was one of numerous factors evaluated in the analysis of support for an anti-thyroid MOA, as described in the EPA guidance on rodent thyroid follicular cell tumorigenesis (EPA, 1998).

Comments Related to Reproductive, Developmental and Neurotoxic Effects

Comment [LyondellBasell]: The assessment should draw a conclusion regarding reproductive toxicity that indicates that there is a low concern for TBA reproductive toxicity and there is no need for further reproductive testing. This conclusion is supported by studies in MTBE (Bevan et al, 1997) and ETBE (CIT, 2004; JPEC 2008; Fujii et al 2010; de Peyster, 2010)

EPA Response: The reproductive toxicity studies for MTBE and ETBE provide evidence for some reproductive effects at higher doses, but are not consistent across studies or doses. For example, a one-generation reproductive study in rats given 0, 100, 300, or 1,000 mg/kg-day ETBE for 16-17 weeks reported a 13.6% total incidence of total litter loss in the high dose group (control data for whole litter loss ranged from 0-4.8%, mean value 0.7%), but was confounded by evidence of systemic toxicity in two of the dams. There was also a slight but significant prolongation of gestation in the high dose group. In another study, ETBE administration at 0, 250, 500, or 1,000 mg/kg-day during the pre-mating, mating, gestation, and lactation periods, and no effects on reproductive endpoints were reported.

Data for maternal body weight gain after exposure to ETBE were also inconsistent across studies. In Asano et al. (2001) and JPEC (2008i), New Zealand White rabbits were exposed to 0, 100, 300, or 1,000 mg/kg-day ETBE and showed a significant decrease in maternal weight gain at the highest dose (although interpretation of maternal weight in rabbits should reviewed with caution due to the high variability in weight in rabbits during pregnancy). Similarly, decreased maternal weight gain was also reported by Gaoua (2004a) in Sprague Dawley rats exposed to ETBE at 1,000 mg/kg-day. In contrast, ETBE induced an increase in maternal weight the same dose and rat strain in another study (Fujii et al 2010 and JPEC (2008e). In other studies with similar dose administrations, maternal weight was not affected (Aso et al. 2014; Asano et al 2011; Fujii et al 2010; Gaoua, 2004b). Taken together, these studies do not provide compelling evidence of no effect in animals, therefore we cannot draw conclusions about the reproductive toxicity of ETBE. Further, the inconsistent findings across these studies do not strengthen the TBA database to the level that would allow a different conclusion to be drawn.

Comment [LyondellBasell]: The assessment should include a separate section on neurotoxicity that includes relevant data from well-conducted TBA studies as well as available neurotoxicity and

developmental neurotoxicity studies of structurally related chemicals such as other butanol isomers and chemicals that are metabolized to TBA.

EPA Response: While we appreciate the comment about including other chemicals, this assessment is focused on the toxicity resulting from TBA exposure as the parent compound.

Comment [LyondellBasell]: Contrary to the statement in the draft assessment on page 1-53 line 19-22, Nelson et al. (1991) provided detailed information on exposure methods and results to indicate the studies were conducted similarly. Taken together, the data from these two studies indicate that no pattern of developmental neurotoxicity was observed in studies at very high dose levels. These results also support the current statement in Section 2.1.3 on database uncertainty factor that further studies are unlikely to lead to identification of a more sensitive endpoint or a lower point of departure than those selected by EPA for oral and inhalation exposures. This is further supported by a series of studies conducted on other butanols and short-chain aliphatic alcohols that should be considered relevant read-across data (Nelson et al. 1989, 1990)(note this Nelson et al., 1989 is a different publication from that reported in the draft assessment, the citation for this present reference is found in our reference list to this document).

EPA Response: The statement that the studies were not conducted similarly was removed. However, as noted by the authors, there were still limitations in the study design and the groups were exposed nonconcurrently which prevented direct comparisons between the two concentrations of TBA. In regard to the read-across for other butanols, while it is true that some studies point toward little to no evidence for neurotoxicity (e.g. Nelson et al, 1989) still other more recent publications have concluded that there is evidence of neurotoxicity (Bale et al., 2016). Therefore, it is difficult to make the conclusion that there is compelling evidence of no neurotoxicity or developmental neurotoxicity due to exposure to TBA.

Comment [LyondellBasell]: "…conclusion fails to mention that the developmental toxicity observed occurred only in the presence of significant maternal toxicity and it is not possible to determine if the maternal toxicity observed played a role in the developmental toxicity." In addition, "there is no examination or consideration of the dose levels used in several of the studies presented as causing developmental toxicity". Also, "While developmental effects should not be "discounted" because of the maternal toxicity observed, it is the responsibility of the document to inform the reader of all possible explanations for the observed effects. Inclusion of the maternal toxicity endpoints, in the same level of detail and accuracy as the developmental toxicity endpoints is necessary for this assessment to be complete." Finally, "In considering the fetal and maternal toxicity data following tert-butanol exposure, the severity of the maternal effects were minimal and therefore the developmental effects in the fetuses should not be discounted (U.S. EPA, 1991b)."

EPA Response: Although this was mentioned in the current draft we have strengthened the language in Sections 1.2.3 and 1.2.4 to clearly address this issue. The draft now calls out the higher doses at which both maternal toxicity and developmental effects are observed. The draft contains maternal toxicity in the developmental evidence tables. The maternal data, if collected, is presented in similar fashion to that of the other data. This language has been altered and the draft no longer states that the maternal effects were minimal.

Comment [LyondellBasell]: The draft assessment is incorrect in concluding that the data provide inadequate information to draw conclusions regarding neurodevelopmental toxicity of TBA. The Nelson et al. (1991) study is a comprehensive developmental neurotoxicity study that included multiple tests of motor activity, motor coordination, and cognitive behavior including schedule controlled operant behavior.

EPA Response: We appreciate the commenter's position on the neurodevelopmental toxicity of TBA. However, as noted by the authors, there were still limitations in the Nelson study including not running the two concentrations of TBA concurrently and often only reporting data for the significant changes. As noted by the commenters and in the draft, there are limitations with the Daniel and Evans study as well. Taken together, it is difficult to make the strong conclusions about the evidence of neurotoxicity or developmental neurotoxicity due to exposure to TBA. For this reason, EPA concludes that the available evidence is inadequate.

Comments Related to Cancer Weight of Evidence

Comment [LyondellBasell]: The descriptor *suggestive evidence* represents a highly conservative assessment. The overall weight of evidence indicates that *tert*-butanol induced rat renal cancer is qualitatively not relevant to humans based on robust mode of action evidence (α2u-globulin and CPN). In addition, the mouse thyroid tumors are not quantitatively relevant to humans due the observation that the high-dose used in mouse oral bioassay was substantially above EPA guidance recommendations for a Limit Dose, as well as being above the dose at which *tert*-butanol metabolism was saturated with associated onset of nonlinear toxicokinetics.

EPA Response: Although the evidence suggests that *tert*-butanol induces α_{2u}-globulin nephropathy, the data indicate that *tert*-butanol is a weak inducer of α_{2u}-globulin and that this process is not solely responsible for the renal tubule nephropathy and carcinogenicity observed in male rats. The lack of compensatory cell proliferation in male rats and evidence of nephrotoxicity in female rats suggest that other processes, in addition to the α_{2u}-globulin process, are operating. Furthermore, the accumulation of hyaline droplets and the induction of renal tubule hyperplasia were affected at higher doses compared to those inducing renal tubule tumors. EPA conducted a MOA analysis

under EPA's cancer guidelines using the proposed criteria from Hard and Khan (2004) and Hard et al. (2013). This analysis is presented in 1.2.1. under mode of action analysis- kidney effects. In summary, considering discrepant patterns in the dose-response relationships for CPN, hyperplasia, and renal tubule tumors and the lack of relationships between CPN grades and renal tubule tumors in female rats, together with the lack of a generally accepted MOA for CPN, the renal tubule tumors in rats cannot be attributed to CPN. Regarding the relevance of the mouse thyroid follicular cell tumors, please see the EPA response to a similar comment above in the "Comments Related to Thyroid Effects" Section.

Comments Related to Dose-Response

Comment [ACC]: The following analogy was not clear: "A 10% relative change from control was used as a BMR for absolute kidney weight by analogy with a 10% change in body weight as an indicator of toxicity." Is a 10% change in absolute kidney weight known to be adverse? How would a 10% extra risk calculation compare?

EPA Response: Extra risk and relative deviation are not alternatives; extra risk is for dichotomous data and relative deviation is for continuous data. Whatever is selected as the BMR (including a 10% relative deviation in kidney weight) doesn't have to be adverse per se, since the point of POD selection is to identify an exposure level without adverse effects (minimally biologically significant effects).

Comment [LyondellBasell]: While correct for female mice, a BMR of 5% was selected for males "to represent the observed response for low-dose extrapolation", likely because the responses for the control and 3 treatment groups were 2, 0, 7 and 2 percent, respectively. The use of the 5% BMR, for apparent statistical reasons only, should be clearly indicated in Table 2-9 as an additional source of uncertainty in the derivation of the SF. In addition, it should be noted that NTP guidance for statistical evaluation of tumors responses with a high background incidence should be evaluated against a $p<0.01$ to reduce the potential for false positive tumor determinations.

EPA Response: The text in the Toxicological Review has been clarified on page 1-42. The trend test that was used tested for a linear trend in the mortality-adjusted incidences, so the apparent non-monotonicity mostly reflects noisy data (i.e., insufficient to conclude that the responses in the two highest groups differ). The statement that the data were non-monotonic is an over-interpretation, given the reduced effective size of the high dose group. Contrary to the comment, follicular cell thyroid adenomas and carcinomas overall are not common (3.4% in females for years 1984-1994) and NTP does not rely solely on p-value cut-offs for interpreting whether there is a positive response. The NTP specifically noted that the adenomas were uncommon, that related hyperplasia

was increased in higher dose groups, and that the only carcinoma occurred in the highest dose group. Support also comes from the concordance with female mice. Although none of the NTP's reported trend tests had $p<0.05$, the trend test EPA applied was developed by NTP after this NTP report was issued.

Comments Related to the Physiologically-Based Pharmacokinetic Model and Toxicokinetics

Comment [LyondellBasell]: Given that kidney toxicity is being considered following inhalation and oral exposure, a more appropriate dose metric to evaluate would be the AUC for *tert*-butanol in the kidney.

EPA Response: There are effects of *tert*-butanol in both male and female rats, showing these effects are not attributable to a2u-globulin binding alone. For the females the *tert*-butanol blood concentrations are very representative of kidney concentrations because the blood:kidney partition coefficient is 1.1. For the exposure levels evaluated for route-to-route extrapolation, the ratio of the AUC of the *free tert*-butanol concentration in kidney to blood AUC is 0.828 in female and male rats (slight variation in 4th decimal place) for oral exposures. For inhalation exposures, at steady state the ratio of free TBA in kidney to blood ranged from 0.832-0.834 in both male and female rats, but the ratio differed by no more than 0.04% for any exposure level. Thus, use of AUC in the kidney vs. blood would result in less than a 0.1% change in the resulting HECs. Given that there are many more data points to inform blood AUC, there is greater confidence in the model's ability to predict blood AUC, so it was selected as a dose metric.

Comment [LyondellBasell]: A variety of questions and concerns are related to specific aspects of PBPK modeling as implemented in the Salazar et al. (2015) model.

EPA Response: EPA has adopted the newly available Borghoff et al. (2016) model.

Comment [LyondellBasell]: Prior to using a model for extrapolation to derive an RfC, the model and data sets used to develop and verify the model need to be confirmed. There is a lack of identification of data sets from unpublished reports used for model development and review of the model code is needed before its use for deriving an RfC.

EPA Response: A table has been included in the PBPK evaluation (U.S. EPA 2017), which replaces most of Appendix B, summarizing all of the data sets used in the modeling, with details on specific data sets provided. All citations should now be correct. All of the sources are available in HERO.

Comment [LyondellBasell]: The differential responses in the 13 week inhalation studies versus the oral studies could be attributed to the discontinuous nature of the inhalation exposures. Thus, use of "similar blood TBA" is only appropriate if AUC values are the basis of the route comparisons.

EPA Response: Using a 6 h/d, 5 d/w exposure pattern, the average blood concentrations predicted by the rat model are 84 and 190 mg/L for the two highest inhalation exposure levels. The corresponding levels from the oral NTP bioassay range from 74 to 1900 mg/L, with the 2nd lowest dose predicted to yield 200 mg/L in male rats. So when accounting for the pulsatile pattern, the highest inhalation exposures are predicted to yield blood levels comparable to the two lowest oral doses. So the internal dose ranges for the two overlap, though are much lower overall for inhalation.
AUCs are the basis being used for route comparisons. But tissue levels will track with blood levels and even using a model with alpha-2-u binding, when exposures are selected to match blood AUCs, the free concentrations of TBA in kidney will also be similar for males exposed by DW vs. inhalation, and likewise females exposed by DW vs. inhalation. So incorporation of this metric and/or using alternate internal dose metrics is not likely to change the conclusion.

Comment [LyondellBasell]: Figure 4F and FG in the Salazar et al. (2015) were produced by assuming only 67% of the amount of ETBE or TBA in exhaled air was collected as a cumulative amount in exhaled breath following ETBE nose only inhalation exposure. While this improves the model fit to the data, it is not supported by experimental evidence.

EPA Response: The 67% correction was mistakenly applied to account for the difference between expired alveolar air and total expired air, which includes air that only enters the conducting airways ("dead space"). We agree that it is not appropriate and the term has been removed from the calculation.

Comment [LyondellBasell]: The ARCO (1983) study reported toxicokinetics evaluations in rats using radiolabeled TBA. Although this study provides useful information, the data collected in this study needs to be described clearly as to how it was recalculated to provide the actual concentration of *tert*-butanol in blood.

EPA Response: The values from ARCO (1983) were calculated by combining the % *tert*-butanol and *tert*-butanol equivalents from Tables 15 and 24 in the Arco report for 1 mg/kg and from Tables 37 and 59 for 500 mg/kg. A table is included in the PBPK evaluation (U.S. EPA, 2017), which replaces Appendix B, showing the calculations. A table has been included with details on all data sources used.

Comment [LyondellBasell]: The Leavens and Borghoff (2009) *tert*-butanol PBPK model does in fact represent the *tert*-butanol blood levels measured in the Poet et al. (1997) study, as shown in

the figure presented in these comments. As also noted, this PBPK model predicted *tert*-butanol concentration in tissues of male and female rats exposed to *tert*-butanol via inhalation.

EPA Response: The U.S. EPA attempted to reconstruct the *tert*-butanol submodel from the Leavens and Borghoff (2009) publication; the results of using that model to simulate the i.v. exposures of Poet et al. (1997) are shown in the PBPK evaluation (U.S. EPA, 2017), and were deemed inadequate. The Borghoff et al. (2016) model differs in several details from that of Leavens and Borghoff (2009). In particular, Leavens and Borghoff (2009) shows urinary clearance as coming from the kidney compartment, while Borghoff et al. (2016) describes it as coming from (mixed) venous blood. Hence we cannot conclude that Leavens and Borghoff (2009) fits the Poet et al. (1997) data, though it is possible that EPA's attempt to reproduce that model was erroneous. In any case, the Borghoff et al. (2016) model does fit the data.

Comment [LyondellBasell]: The metabolism of *tert*-butanol suggests that it is reasonable to predict potential high-dose specific metabolic saturation. There is reasonable toxicokinetic data indicating that the top male/female doses of greater than 2000 mg/kg bw/day, and possibly even the female/male mid-doses of 1020 and 1040 mg/kg bw/day, exceeded saturation of *tert*-butanol metabolism resulting in onset of nonlinear plasma *tert*-butanol toxicokinetics. Mice (C56BL6) administered single intraperitoneal doses of *tert*-butanol at doses of 5, 10 and 20 mmol/kg bw (370, 741 and 1482 mg/kg bw) resulted in respective AUC values of 28, 96 and 324 mmol.hrs/L (Faulkner and Hussain, 1989). Thus, a 4-fold increase in dose (5 to 20 mmol/kg bw) resulted in an 11.6-fold increase in systemic AUC; metabolic saturation may have been present even at the next lowest dose of 10 mmol/kg bw in which a 2-fold increase in dose (5 to 10 mmol/kg bw) resulted in a 3.4-fold increase in AUC.

EPA Response: Saturation of *tert*-butanol metabolism is reasonable and the PBPK model incorporated Michaelis-Menten kinetics which account for saturation. The KM used is 0.379 mM. Considering that this value is for rats rather than mice, it appears reasonably consistent with the range reported by Faulker and Hussain (1989), 0.56-0.92 mM, from fitting a one-compartment TK model separately to each dose level. What matters is that the PBPK model adequately fits the PK data across the range of exposures. Since this is a PK nonlinearity which is consistent with the (fairly standard) model structure, that may or may not indicate nonlinearity in pharmacodynamic mechanisms, the EPA does not consider it particularly useful to point it out for each data set where it occurs. One of the reasons for using a PBPK model is that it allows one to appropriately account for metabolic saturation, and the impact that has on the internal dose(s) across the entire dose range.

Comment [LyondellBasell]: Use of toxicokinetic data to provide a data-informed selection of the appropriate top dose in animal toxicity tests has recently been described as a Kinetically Derived

Maximum (KMD) dose selection strategy (Saghir et al., 2012). The KMD dose selection strategy specifically emphasizes that toxicokinetic data, when available, can and should be used as an alternative to conventional top dose selection strategies based on Maximum Tolerated Dose (MTD). tert-Butanol would have been a strong candidate for a KMD-based dose selection strategy.

EPA Response: While the KMD approach may provide better study designs in the future, the NTP studies were conducted more than 15 years prior to Saghir et al. (2012). But the U.S. EPA does not agree that toxicity data collected at exposure levels which saturate a metabolic pathway are not usable or relevant for estimating human health risk, in particular when part of a dose-response array that spans high and low exposure levels.

Comments Related to Genotoxicity

Comment [LyondellBasell]: **I**t is rather surprising that the draft assessment concluded that "…a limited database is available for understanding the role of *tert*-butanol-induced genotoxicity for mode of action and carcinogenicity." LyondellBasell commented that the statement that only limited animal studies were conducted to investigate micronucleus formation is inaccurate. Contrary to the above statement, the WoE from a large database informs that TBA does not have the potential to be an in vivo genotoxicant and a mutagenic MOA in the etiology of animal tumors can thus be excluded with a reasonable degree of certainty. Finally, the NTP conducted a total of 3 micronucleus studies, two in the mouse and one in the rat and all three studies were clearly negative.

***EPA Response*:** As indicated in the summary of the genotoxicity section, the database is rather small for both the array of genotoxicity tests conducted as well as the number of studies within the same type of test category. In addition, sometimes, the data is either conflicting or inconsistent. Since there are a few studies that are positive, *tert*-butanol cannot be considered nongenotoxic with complete certainty, therefore, the conclusion presented in Section B.3.2 of the Supplementary Materials will remain unchanged. Finally, the two mouse studies referred to by LyondellBasell are one study published both in NTP (1995) and NTP (1997).

Comment [LyondellBasell]: There are two key studies missing in Section B.3.2.3 under in vivo mammalian studies. The first study is a rat bone marrow micronucleus test reported by the NTP (1997). The second missing study is a mouse bone marrow micronucleus test reported by the NTP. The two missing, negative NTP mouse and rat bone marrow micronucleus studies that were discussed above should be included under "In vivo Animal Studies" section of this table.

EPA Response: The NTP (1997) rat study is now included in the assessment. However, both micronucleus tests referenced in the comments are the same study. With respect to the mouse study, the NTP (1995) and NTP (1997) studies are from the same set of experiments and is already present in the current assessment.

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