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

(CASRN 75-65-0)

Supplemental Information – *tert*-Butyl Alcohol

April 2016

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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 work week
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) exposures 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 was observed between mice on a repeated dosing regimen and control 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 was 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 preference for fatty tissues (Nihlén et al., 1995).

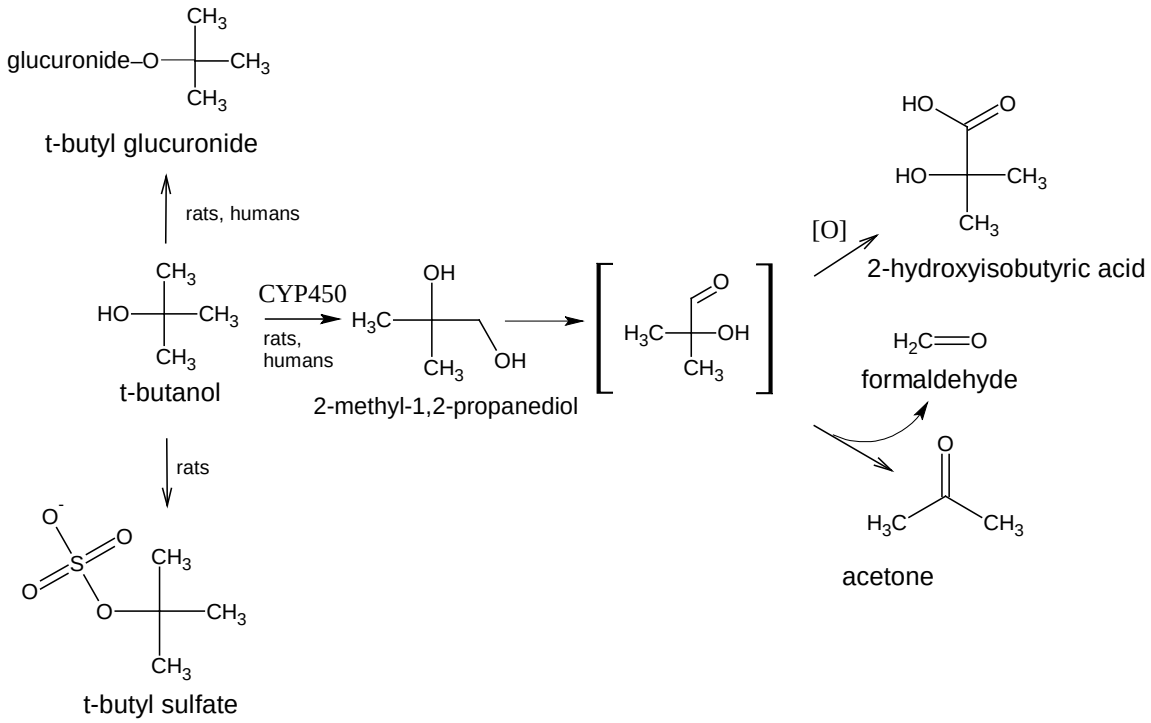
In a study aimed at determining whether *tert*-butanol (or metabolites) can bind to α_{2u}-globulin, Williams and Borghoff (2001) exposed F-344 rats to a single gavage dose of 500 mg/kg ^{14}C-*tert*-butanol. They found the radiolabel in three tissues (kidney, liver, and blood) in both sexes, but male 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 10 consecutive days. 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 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 (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, 2-hydroxyisobutyrate, 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 the 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).



Source: 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 acetone formation, in addition to formaldehyde, also 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, b). Eight or ten male human volunteers were exposed to 5, 25, or 50 ppm MTBE (18.0, 90.1, 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, 2-hydroxyisobutyrate (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).

B.1.5. Physiologically Based Pharmacokinetic Models

No physiologically based pharmacokinetic (PBPK) models have been developed specifically for administration of *tert*-butanol. Some models have been used to study *tert*-butanol as the primary metabolite after oral or inhalation exposure to MTBE or ETBE. The most recent models for MTBE oral and inhalation exposure include a component for the binding of *tert*-butanol to α_{2u}-globulin (Borghoff et al., 2010; Leavens and Borghoff, 2009).

Faulkner and Hussain (1989) used a one-compartment, open model with Michaelis-Menten elimination kinetics to fit *tert*-butanol blood concentrations obtained from C57BL/6J mice given i.p. injections of 5, 10, or 20 mmol/kg *tert*-butanol. Elimination was indistinguishable from first-order kinetics in the range of concentrations studied. An increase in V_{max} and decrease in apparent volume of distribution with dose are consistent with this model and suggest the existence of parallel elimination processes.

Borghoff et al. (1996) developed a PBPK model for MTBE and its metabolite *tert*-butanol in rats. Doses and blood levels were taken from several published studies. The initial model included a tissue-specific, five-compartment model using blood, liver, kidney, muscle, and fat with liver metabolism rate constants. The model predicted the accumulation of *tert*-butanol in blood, but not its clearance. A two-compartment model was better at predicting *tert*-butanol blood levels, but the volume of total body water had to be changed to obtain an adequate fit, suggesting dose-dependent changes in the kinetics of *tert*-butanol. Overall, evaluation of the *tert*-butanol models suggests that

the clearance of *tert*-butanol from the blood of rats after exposure to MTBE involves processes beyond metabolic elimination.

Nihlén and Johanson (1999) developed a PBPK model for evaluation of inhalation exposure in humans to the gasoline additive ETBE. Model compartments for ETBE included lungs (with arterial blood), liver, fat, rapidly perfused tissues, resting muscles, and working muscles. The same set of compartments and an additional urinary excretion compartment were used for the metabolite, *tert*-butanol. First-order metabolism was assumed in the model, and tissue/blood partition coefficients were determined by in vitro methods (Nihlén et al., 1995). Estimates of individual metabolite parameters of eight subjects were obtained by fitting the PBPK model to experimental data from humans (5, 25, or 50 ppm ETBE; 2-hour exposure) (Nihlén et al., 1998a). This model primarily was applied to predict levels of the biomarkers ETBE and *tert*-butanol in blood, urine, and exhaled air after various scenarios, such as prolonged exposure, fluctuating exposure, and exposure during physical activity (Nihlén and Johanson, 1999).

Rao and Ginsberg (1997) developed a PBPK model for MTBE and its principal metabolite, *tert*-butanol, based on the Borghoff et al. (1996) model. The modified model included a skin compartment to simulate dermal absorption of MTBE during bathing or showering. A brain compartment was added as a target organ for MTBE-induced neurological responses. MTBE metabolism to *tert*-butanol was assumed to occur in the liver through two saturable pathways. The *tert*-butanol portion of the model included further metabolism of *tert*-butanol in the liver, exhalation in the lungs, and renal excretion (in the human model only). The model was validated against published human and rat data and was used to help determine the contribution of *tert*-butanol to the acute central nervous system effects observed after MTBE dosing.

The Rao and Ginsberg (1997) model used peak concentrations of MTBE and *tert*-butanol in the blood and brain for interspecies, route-to-route, and low-/high-dose extrapolations. The MTBE/*tert*-butanol PBPK model was adapted to humans by adjusting physiology according to literature values, incorporating the blood/air partition coefficient for humans reported by Johanson et al. (1995), and allometrically scaling the metabolic rate based on body weight. A renal elimination component was added to account for the small percentage of MTBE disposition that occurs in humans via urinary excretion of *tert*-butanol. *tert*-Butanol concentrations in human blood during and after MTBE exposure (25 or 50 ppm for 2 hours) were accurately predicted by the human model (Johanson et al., 1995).

Kim et al. (2007) expanded the Borghoff et al. (1996) model to develop a multi-exposure route model for MTBE and its primary metabolite, *tert*-butanol, in humans. The significant features and advantages of the Kim et al. (2007) model are that parameters used for quantifying the pharmacokinetic behavior of MTBE and *tert*-butanol are calibrated using time-series measurements from controlled-exposure experiments in humans as reported by Prah et al. (2004). MTBE partition coefficient values described in the Licata et al. (2001) model and skin compartment parameters from the Rao and Ginsberg (1997) model were incorporated. The PBPK model for

MTBE consists of nine primary compartments representing the lungs, skin, fat, kidney, stomach, intestine, liver, rapidly perfused tissue, and slowly perfused tissue. The *tert*-butanol model consists of three compartments representing blood, liver, and other tissue.

Leavens and Borghoff (2009) developed a PBPK model for inhalation exposures in male and female rats that expanded on Borghoff et al. (1996) and Rao and Ginsberg (1997) to include the sex-specific effects of MTBE binding to α_{2u}-globulin, a protein unique to male rats, and to describe the induction of *tert*-butanol metabolism after repeated exposures. Although the primary purpose of the model was to estimate MTBE and *tert*-butanol tissue concentrations after MTBE exposure, the model also was parameterized to include inhalation uptake of *tert*-butanol. The *tert*-butanol portion of the model was calibrated using data from rat exposures to *tert*-butanol and to MTBE. Model compartments included blood, brain, fat, gastrointestinal tissues, kidney, liver, poorly perfused tissues (blood flow <100 mL/min/100 g of tissue: bone, muscle, skin, fat), and rapidly perfused tissues.

Distribution of MTBE and *tert*-butanol was assumed perfusion (i.e., blood-flow) limited. This model used the same assumptions as Borghoff et al. (1996) regarding MTBE metabolism and kinetics and further assumed that *tert*-butanol was metabolized only in the liver through one low-affinity pathway and excreted through urine. The model described binding of MTBE or *tert*-butanol with α_{2u}-globulin in the kidney, due to the high concentration of α_{2u}-globulin in the kidney. As chemicals bind to α_{2u}-globulin, the rate of hydrolysis of the protein decreases and causes accumulation in the kidney; however, there is no evidence that binding of α_{2u}-globulin affects its synthesis, secretion, or circulating concentrations [Borghoff et al. (1990) as cited in Leavens and Borghoff (2009)]. Equations describing this phenomenon were included in the model for male rats only to account for the effects of the binding with α_{2u}-globulin on metabolism of MTBE and *tert*-butanol. Partition coefficient values in the model that differed from those published in previous PBPK models included poorly perfused tissues:blood and kidney:blood values. The kidney:blood value was based on calculated kidney:blood concentrations in female rats only because of the lack of α_{2u}-globulin-associated effects in female rats. The deposition of *tert*-butanol during inhalation in the nasal cavity and upper airways was reflected in the high blood:air partition coefficient for *tert*-butanol, and the ability of *tert*-butanol to induce its own metabolism after chronic exposure also was taken into account. No differences in the induction of metabolism were reported between males and females. The model simulated concentrations of MTBE and *tert*-butanol in the brain, liver, and kidney of male and female rats following inhalation exposure at concentrations of 100, 400, 1,750, or 3,000 ppm MTBE, and compared them to measured concentrations of MTBE and *tert*-butanol from rats exposed at those levels.

Concentrations of MTBE and *tert*-butanol in the brain and liver were similar in male and female rats during exposure and postexposure, but the concentrations of the chemicals in the kidney significantly differed between male rats and female rats. The additional parameter accounting for α_{2u}-globulin protein binding in this PBPK model more accurately reflects the

metabolism of both MTBE and *tert*-butanol in male rat kidneys over time compared with other PBPK models. The model highlights that binding can stimulate increased renal effects in male rats after exposure to MTBE and *tert*-butanol. The assumptions made to reflect *tert*-butanol metabolism induction and deposition in the nasal cavity and upper airways generally were supported by measured data from rats exposed to 250-, 450-, or 1,750-ppm *tert*-butanol as evidenced by the fact that the model was within one standard deviation of the mean concentrations for most data points. The model overpredicted the concentration of *tert*-butanol in the brain, liver, and kidney of male rats, however, after repeated exposures.

Borghoff et al. (2010) modified the PBPK model of Leavens and Borghoff (2009) by adding oral gavage and drinking water exposure components to compare different dose metrics to the toxicity observed across different studies. The Borghoff et al. (2010) model assumed first-order uptake of MTBE absorption from the gut, with 100% of the MTBE dose absorbed for both drinking water and oral gavage exposures. They conducted a series of pharmacokinetic studies comparing the effects of different rat strains and different dosing vehicles on the blood concentration-time profiles of MTBE and *tert*-butanol following MTBE exposure. The effects of exposure to MTBE via drinking water, oral gavage, and inhalation routes over 7 and 91 days on male and female rats were modeled and compared with measured data collected from F344 rats (exposed 28 days) and Wistar Han rats (exposed 14 and 93 days).

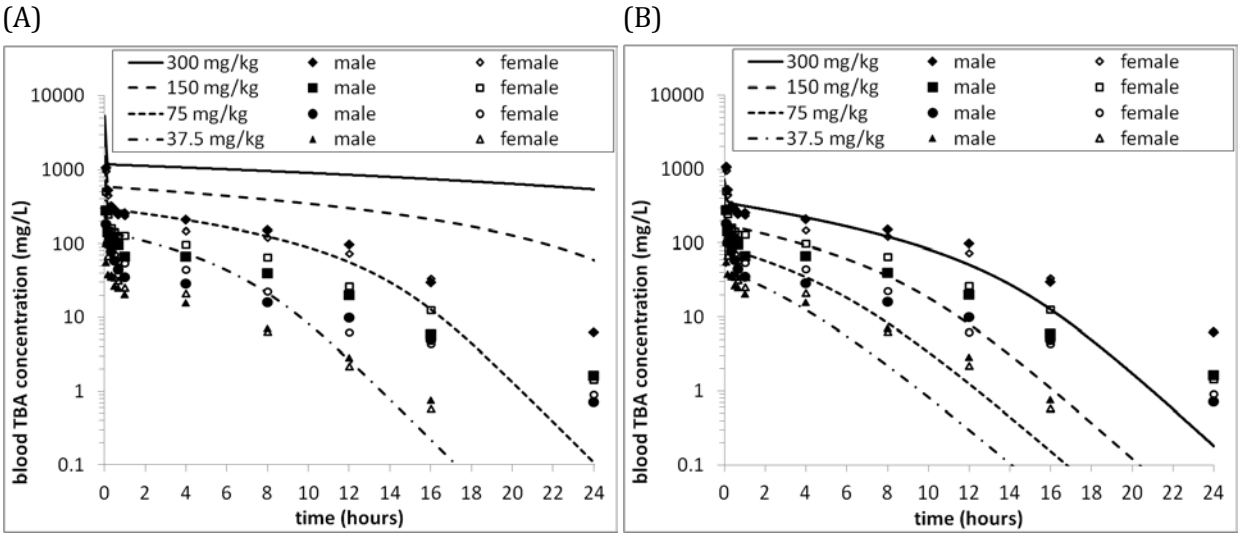
The model predicted the blood concentrations of *tert*-butanol observed after 250 or 1,000 mg/kg-day administration of MTBE in males and females and the blood concentrations of MTBE after 1,000 mg/kg-day. The model did not predict peak concentrations of MTBE, however, after 250 mg/kg-day in males or females using either olive oil or 2% Emulphor as vehicles. When comparing strains, the blood concentrations were similar across strain and sex, except in female Sprague-Dawley rats administered 1,000 mg/kg-day MTBE. Female Sprague-Dawley rats had a significantly (*p*-value not specified) higher blood concentration of both MTBE and *tert*-butanol compared with F344 and Wistar Han females. The study authors considered this an outlier, however, and maintained the metabolic patterns were similar. The model overpredicted the amount of MTBE in the male rat kidney but accurately predicted the level of *tert*-butanol in the male rat kidney at all exposures tested. The model did not accurately predict the kidney concentrations of *tert*-butanol in the female kidney after exposure to MTBE via drinking water, but the study authors attributed the inaccuracies to the study design as opposed to the model formulation. All *tert*-butanol entering the submodel comes from MTBE metabolism in the liver, and the model does not include a separate oral intake of *tert*-butanol.

B.2. PBPK MODEL EVALUATION SUMMARY

B.2.1. Evaluation of Existing *tert*-Butanol Submodels

The Blancato et al. (2007) and Leavens and Borghoff (2009) PBPK models for MTBE were evaluated by comparing predictions from the *tert*-butanol portions of the models with the *tert*-butanol

i.v. data of Poet et al. (1997) (see Figure B-2). Neither model adequately represented the *tert*-butanol blood concentrations. Modifications of model assumptions for alveolar ventilation, explicit pulmonary compartments, and induction of metabolism of *tert*-butanol did not significantly improve model fits to the data. Attempts to reoptimize model parameters in the *tert*-butanol submodels of Blancato et al. (2007) and Leavens and Borghoff (2009) to match blood concentrations from the i.v. dosing study were unsuccessful.



Neither the (A) Blancato et al. (2007) nor the (B) Leavens and Borghoff (2009) model adequately represents the measured *tert*-butanol blood concentrations.

Figure B-2. Comparison of the *tert*-butanol portions of existing MTBE models with *tert*-butanol blood concentrations from i.v. exposure by Poet et al. (1997).

The PBPK submodel for *tert*-butanol in rats was developed in acslX (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, Alabama) by modifying information from the many PBPK models developed in rats and humans for the structurally related substance, MTBE, and its metabolite *tert*-butanol (Borghoff et al., 2010; Leavens and Borghoff, 2009; Blancato et al., 2007; Kim et al., 2007; Rao and Ginsberg, 1997; Borghoff et al., 1996). A brief description comparing the Blancato et al. (2007) and Leavens and Borghoff (2009) models is provided, followed by an evaluation of the MTBE models and the assumptions adopted from MTBE models or modified in the *tert*-butanol model.

The Blancato et al. (2007) model is an update of the earlier Rao and Ginsberg (1997) model, and the Leavens and Borghoff (2009) model is an update of the Borghoff et al. (1996) model. Both the Blancato et al. (2007) and Leavens and Borghoff (2009) models are flow-limited models that predict amounts and concentrations of MTBE and its metabolite *tert*-butanol in blood and six tissue compartments: liver, kidney, fat, brain, and rapidly and slowly perfused tissues. These tissue compartments are linked through blood flow, following an anatomically accurate, typical, physiologically based description (Andersen, 1991). The parent (MTBE) and metabolite

(*tert*-butanol) models are linked by the metabolism of MTBE to *tert*-butanol in the liver. Routes of exposure included in the models are oral and inhalation for MTBE; Leavens and Borghoff (2009) included inhalation exposure to *tert*-butanol. Oral doses are assumed 100% bioavailable and 100% absorbed from the gastrointestinal tract represented with a first-order rate constant. Following inhalation of MTBE or *tert*-butanol, the chemical is assumed to enter the systemic blood supply directly, and the respiratory tract is assumed to be at pseudo-steady state. Metabolism of MTBE by CYP450s to formaldehyde and *tert*-butanol in the liver is described with two Michaelis-Menten equations representing high- and low-affinity enzymes. *tert*-Butanol is either conjugated with glucuronide or sulfate or further metabolized to acetone through MPD and HBA; both processes are described by a single Michaelis-Menten equation in the models. All model assumptions are valid for *tert*-butanol and were applied to the EPA-modified *tert*-butanol PBPK model, except for the separate brain compartment. The brain compartment was lumped with other richly perfused tissues in the EPA-modified *tert*-butanol PBPK model.

In addition to differences in parameter values between the Blancato et al. (2007) and the Leavens and Borghoff (2009) models, the model structure has three differences: (1) the alveolar ventilation was reduced during exposure, (2) the rate of *tert*-butanol metabolism increased over time due to induction of CYP enzymes, and (3) binding of MTBE and *tert*-butanol to α_{2u}-globulin was simulated in the kidney of male rats. The Blancato et al. (2007) model was configured through EPA's PBPK modeling framework, ERDEM (Exposure-Related Dose Estimating Model), which includes explicit pulmonary compartments. The modeling assumptions related to alveolar ventilation, explicit pulmonary compartments, and induction of metabolism of *tert*-butanol are discussed in this model evaluation section.

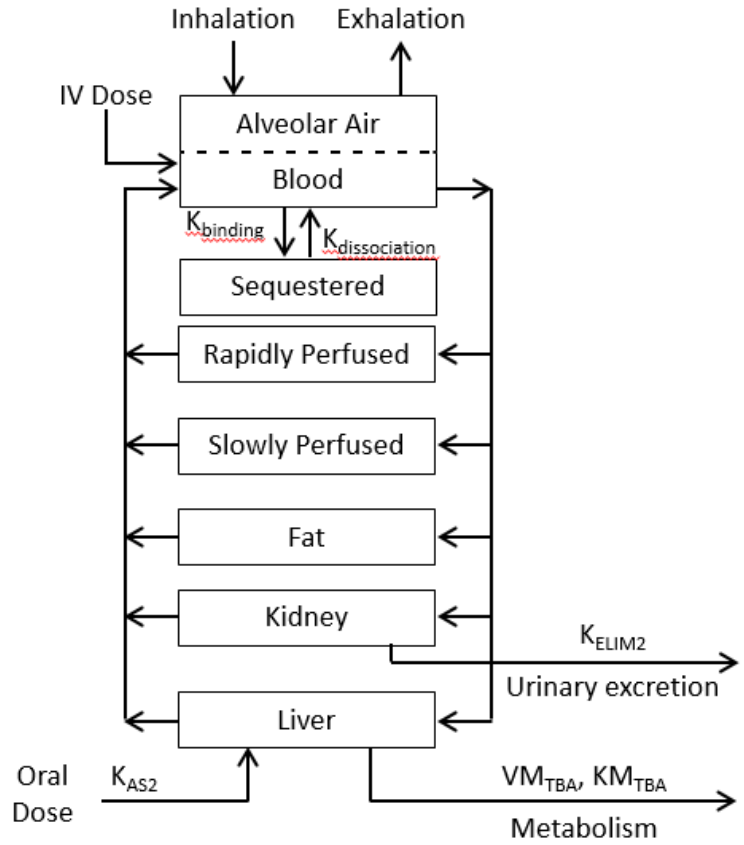
MTBE and *tert*-butanol binding to α_{2u}-globulin in the kidneys of male rats were incorporated in the PBPK model of MTBE by Leavens and Borghoff (2009). Binding to α_{2u}-globulin is one hypothesized mode of action for the observed kidney effects in MTBE-exposed animals. For a detailed description of the role of α_{2u}-globulin and other modes of action in kidney effects, see the kidney mode of action section of the Toxicological Review (Section 1.2.1). In the Leavens and Borghoff (2009) model, binding of MTBE to α_{2u}-globulin was applied to sex differences in kidney concentrations of MTBE and *tert*-butanol, but acceptable estimates of MTBE and *tert*-butanol pharmacokinetics in the blood are predicted in other models that did not consider α_{2u}-globulin binding. Given the uncertainty of *tert*-butanol binding to α_{2u}-globulin, it was not included in the *tert*-butanol PBPK submodel.

B.2.2. Modification of Existing *tert*-Butanol Submodels

To account for the *tert*-butanol blood concentrations after i.v. *tert*-butanol exposure, the model was modified by adding a pathway for reversible sequestration of *tert*-butanol in the blood (see Figure B-3). The PBPK model represented the rate of change in the amount of *tert*-butanol in the sequestered blood compartment (A_{blood2}) with the following equation, where K_{ON} is the binding rate

constant, CV is the free *tert*-butanol concentration in blood, K_{OFF} is the unbinding rate constant, and C_{blood2} is the concentration of *tert*-butanol bound in blood (equal to A_{blood2}/V_{blood}).

$$dA_{blood2}/dt = K_{ON}*CV* - K_{OFF}*C_{blood2}$$



Exposure can be via multiple routes, including inhalation, oral, or i.v. dosing. Metabolism of *tert*-butanol, which occurs in the liver, is described by Michaelis-Menten equations with one pathway for *tert*-butanol. *tert*-Butanol is cleared via exhalation and via urinary excretion. See Table B-1 for definitions of parameter abbreviations.

Figure B-3. Schematic of the PBPK submodel for *tert*-butanol in rats.

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

Body weight and organ volumes as fraction of body weight		
Body weight (kg)	0.25	(Brown et al., 1977)
Body fraction that is blood perfused (F_{perf})	0.8995	(Brown et al., 1977)
Liver	0.034	(Brown et al., 1977)
Kidney	0.007	(Brown et al., 1977)
Fat	0.07	(Brown et al., 1977)

Rapidly perfused	0.04	(Brown et al., 1977)
Slowly perfused	0.7485	[a]
Blood	0.074	(Brown et al., 1977)
Cardiac output and organ blood flows as fraction of cardiac output		
Cardiac output (L/hr)	5.38	(Brown et al., 1977)[b]
Alveolar ventilation (L/hr)	5.38	(Brown et al., 1977)[c]
Liver	0.174	(Brown et al., 1977)[d]
Kidney	0.141	(Brown et al., 1977)
Fat	0.07	(Brown et al., 1977)
Rapidly perfused	0.279	[e]
Slowly perfused	0.336	(Brown et al., 1977)
Partition coefficients for *tert*-butanol		
Blood:air	481	(Borghoff et al., 1996)
Liver:blood	0.83	(Borghoff et al., 1996)
Fat:blood	0.4	(Borghoff et al., 1996)
Rapidly perfused:blood	0.83	(Borghoff et al., 1996)
Slowly perfused:blood	1.0	(Borghoff et al., 1996)
Kidney:blood	0.83	(Borghoff et al., 1996)

[a] F_{perf} – Σ(other compartments).
[b] $15.2*BW^{0.75}$ (BW = body weight).
[c] Alveolar ventilation is set equal to cardiac output.
[d] Sum of liver and gastrointestinal blood flows.
[e] 1 – Σ(all other compartments).

The physiologic parameter values obtained from the literature are shown in Table B-1 (Brown et al., 1977). *tert*-Butanol partition coefficients, determined by the ratios of measured tissue:air and blood:air partition coefficients (Borghoff et al., 1996), also were obtained from literature. The parameters describing rate constants of metabolism and elimination of *tert*-butanol also were obtained from the literature (Blancato et al., 2007) and were kept fixed because they were optimized to *tert*-butanol blood concentrations measured after MTBE exposure, which is also metabolized to *tert*-butanol. The parameters describing *tert*-butanol absorption and *tert*-butanol sequestration in blood were estimated by optimizing the model to the time-course data for blood *tert*-butanol for rats exposed via i.v., inhalation, and oral routes (Leavens and Borghoff, 2009; Poet et al., 1997; ARCO, 1983). The model parameters were estimated with the acslX optimization routine to minimize the log-likelihood function of estimated and measured *tert*-butanol concentrations. The Nedler-Mead algorithm was used with heteroscedasticity and allowed to vary between 0 and 2. The predictions of the model with optimized parameters have a much-improved fit to the *tert*-butanol blood concentrations after *tert*-butanol i.v. exposures, as shown in panel A of Figure B-4. Additionally,

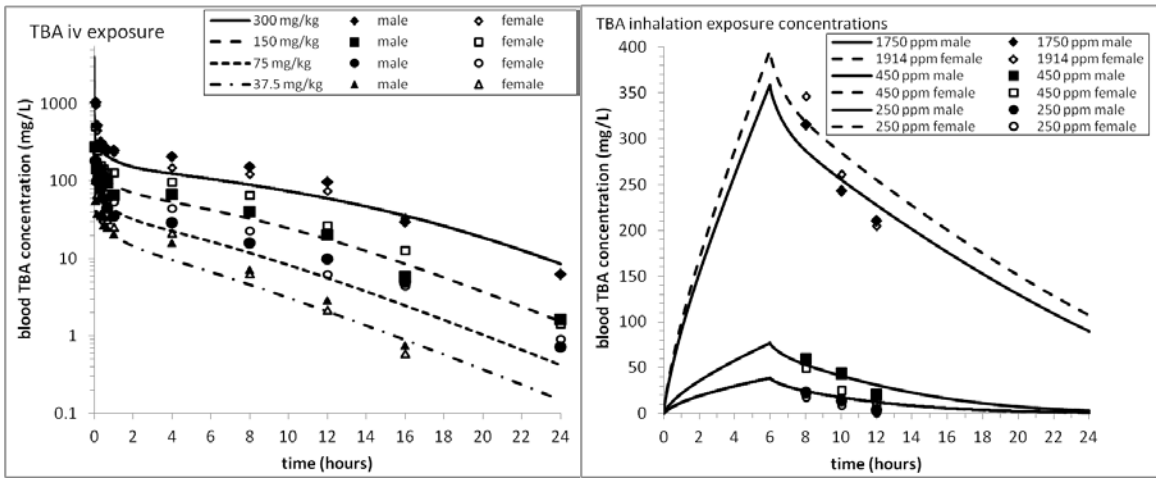
the model adequately estimated the *tert*-butanol blood concentrations after inhalation and oral gavage exposures. The optimized parameter values are shown in Table B-2. The ARCO (1983) study measured *tert*-butanol in plasma only, unlike the Poet et al. (1997) and Leavens and Borghoff (2009) studies, which measured *tert*-butanol in whole blood. Based on the measurements of plasma and whole blood by JPEC (2008), the concentration of *tert*-butanol in plasma is approximately 60% of the concentration in whole blood. The *tert*-butanol plasma concentrations measured by ARCO were increased (divided by 60%) to the expected concentration in whole blood for comparison with the PBPK model.

Induction of *tert*-butanol-metabolizing enzymes was included in the Leavens and Borghoff (2009) model of MTBE based on their study of rats exposed for 8 days to *tert*-butanol via inhalation. The enzyme induction equation and parameters developed in the Leavens and Borghoff (2009) model that were applied to the *tert*-butanol submodel are as follows.

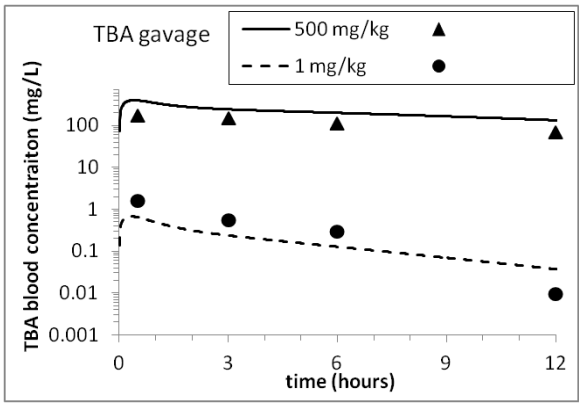
$$V_{max}\ tert\text{-butanol IND} = V_{max}\ tert\text{-butanol} * INDMAX(1-\exp(-KIND*t))$$

V_{max} *tert*-butanol IND is the maximum metabolic rate after accounting for enzyme induction, V_{max} *tert*-butanol is the metabolism rate constant from Table B-2 for both *tert*-butanol pathways, and INDMAX is the maximum percent increase in Vmax *tert*-butanol (124.9). KIND is the rate constant for enzyme induction (0.3977/day). The increased *tert*-butanol metabolism better estimates the measured *tert*-butanol blood concentrations as can be seen in the comparison of the model predictions and experimental measurements shown in Figure B-5. The model better predicted blood concentrations in female rats than male rats. The male rats had lower *tert*-butanol blood concentrations after repeated exposures compared with female rats, and this difference could indicate greater induction of *tert*-butanol metabolism or other physiologic changes such as ventilation or urinary excretion in males. The current data for *tert*-butanol metabolism do not provide sufficient information for resolving this difference between male and female rats.

(A) (B)



(C)



Source: (A) i.v. data from Poet et al. (1997); (B) inhalation data from Leavens and Borghoff (2009); and (C) oral gavage data from ARCO (1983) with the optimized parameter values as shown in Table B-2.

Figure B-4. Comparison of the EPA model predictions with measured *tert*-butanol blood concentrations for i.v., inhalation, and oral gavage exposure to *tert*-butanol.

Table B-2. Rate constants for *tert*-butanol determined by optimization of the model with experimental data

Rate Constant	Value	Source or Reference
Metabolism (VM_{TBA}; mg/kg-hr)[a]	8.0	Blancato et al. (2007)
Metabolism (KM_{TBA}; mg/L)	28.8	Blancato et al. (2007)
Urinary elimination (K_{ELIM2}; 1/hr)	0.5	Blancato et al. (2007)
TBA sequestration rate constant (K_{ON}; L/hr)	0.148	Optimized
TBA unsequestration rate constant (K_{OFF}; L/hr)	0.0134	Optimized
Absorption from gastrointestinal tract (K_{AS2}; 1/hr)	0.5	Optimized

[a] Scaled by $BW^{0.7}$ ($0.25^{0.7}$ = 0.379), BW = body weight.

B.2.3. Summary of the PBPK Model for *tert*-Butanol

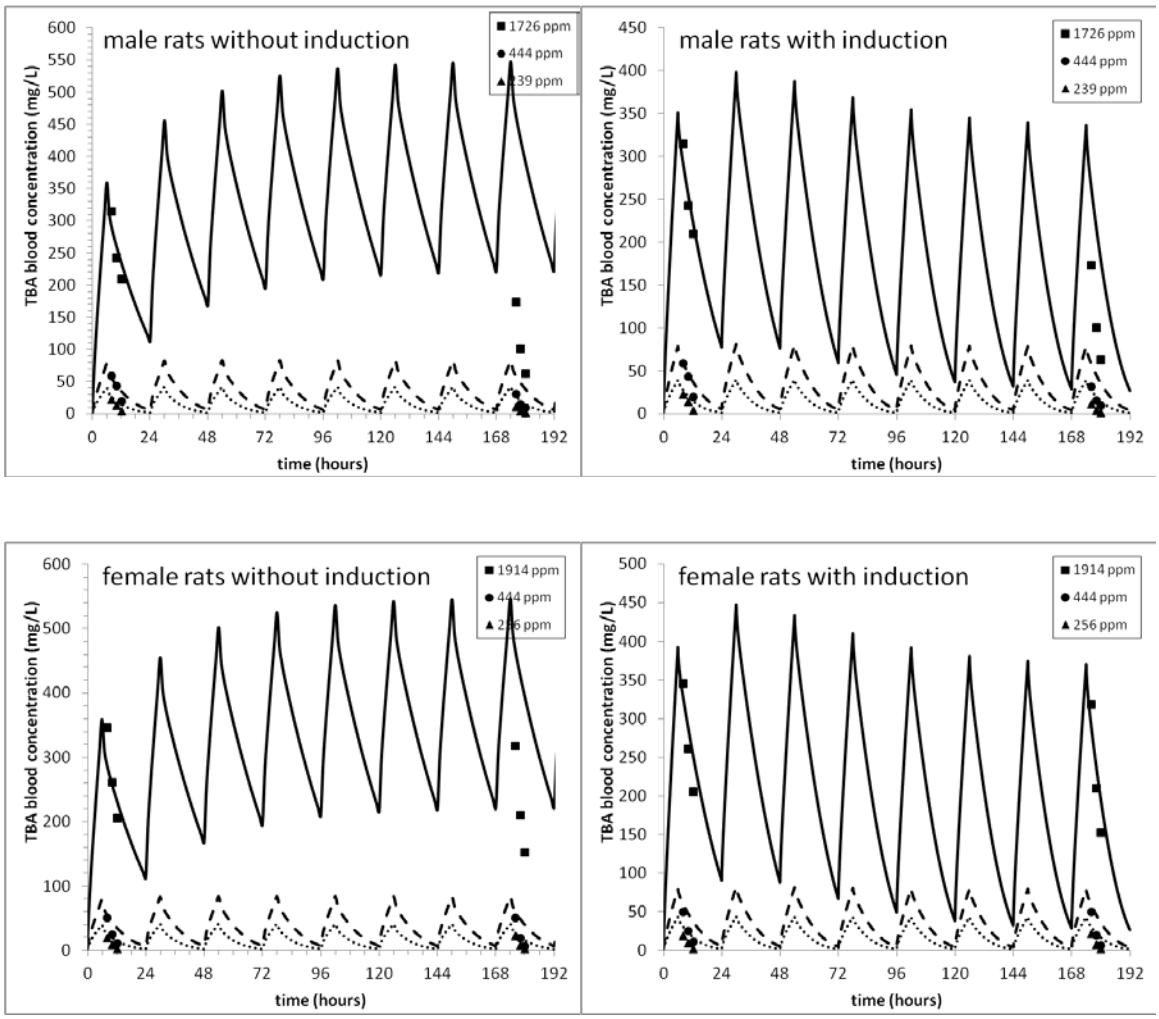
A PBPK model for *tert*-butanol was developed by modifying previous models for MTBE and *tert*-butanol (Leavens and Borghoff, 2009; Blancato et al., 2007). Published *tert*-butanol sub-models do not adequately represent the *tert*-butanol blood concentrations measured in the i.v. study (Poet et al., 1997). The addition of a sequestered blood compartment for *tert*-butanol substantially improved the model fit. The alternative modification—changing to diffusion-limited distribution between blood and tissues—also improved the model fit, but was considered less biologically plausible. Physiological parameters and partition coefficients were obtained from published measurements. The rate constants for *tert*-butanol metabolism and elimination were from a published PBPK model of MTBE with a *tert*-butanol subcompartment (Blancato et al., 2007). Additional model parameters were estimated by calibrating to data sets for i.v., oral, and inhalation exposures and repeated dosing studies for *tert*-butanol. Overall, the model produced acceptable fits to multiple rat time-course datasets of *tert*-butanol blood levels following inhalation or oral gavage exposures.

B.2.4. *tert*-Butanol Model Application

The PBPK model as described above was applied to toxicity studies to predict *tert*-butanol blood concentrations (the preferred internal dose metric in the absence of evidence linking any specific metabolite of *tert*-butanol to any toxic effect). For simulation studies where *tert*-butanol was administered in drinking water, the consumption was modeled as episodic, based on the pattern of drinking observed in rats (Spiteri, 1982).

B.2.5. 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, 201#, HEROID##).



Male rats were exposed to 239, 444, or 1726 ppm and female rats were exposed to 256, 444, or 1914 ppm *tert*-butanol for up to 8 consecutive days (Borghoff et al., 2001). *tert*-Butanol blood concentrations are better predicted by the model after 8 days of exposure with enzyme induction (right panels) compared to without enzyme induction (left panels).

Figure B-5. Comparison of the EPA model predictions with measured amounts of *tert*-butanol in blood after repeated inhalation exposure to *tert*-butanol.

B.3. OTHER PERTINENT TOXICITY INFORMATION

B.3.1. Other Toxicological Effects

B.3.1.1. *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 ethanol causes, in terms of 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 such as withdrawal increased with dose and duration of exposure. Study quality and utility concerns (e.g., inappropriate exposure durations, lack of data reporting, small number of animals per treatment group) associated with these studies (Grant and Samson, 1981; Snell, 1980; Thurman et al., 1980; McComb and Goldstein, 1979a, b; Wood and Laverty, 1979), however, preclude an understanding of potential neurotoxicity following *tert*-butanol exposure, and therefore, central nervous system studies are not discussed further.

Exposure-response arrays of these effects on liver and urinary bladder are provided in Figure B-6 and Figure B-7 for oral and inhalation studies, respectively.

Kidney effects

Absolute and relative kidney weight numerical data are presented in Table B-3.

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-4). 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-6), 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 to females.

B.3.1.2. *Mechanistic Evidence*

No mechanistic evidence is available for these effects.

B.3.1.3. *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-3. 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-4. Changes in liver weight in animals following exposure to *tert*-butanol

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

Reference and study design	Results					
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	Dose (mg/kg-d)	Absolute weight	Relative weight	Dose (mg/kg-d)	Absolute weight	Relative weight
	0	0	0	0	0	0
	90	+2	+7	180	−14*	−8
	200	+8	+11	330	−3	−1
	420	+1	+14*	650	−6	+9*
	Only animals sacrificed at 15 months were evaluated for organ weights. Organ weights were not measured in the 2-year mouse study					
NTP (1997) F344/N rat; 10/sex/treatment 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/m3)	Absolute weight	Relative weight	Absolute weight	Relative weight	
	0	0	0	0	0	
	406	−8	−8	0	+3	
	824	−2	−1	0	0	
	1,643	+1	−1	+3	+2	
	3,273	+10	+7	+9	+9*	
	6,368	+5	+5	+4	+8*	
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment 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/m3)	Absolute weight	Relative weight	Absolute weight	Relative weight	
	0	0	0	0	0	
	406	−1	0	+1	−4	
	824	+4	+9	+1	+5	
	1,643	+7	+5	+5	+1	
	3,273	−8	-2	+2	+9*	
	6,368	+5	+7	+8	+21*	

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

Table B-5. 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.

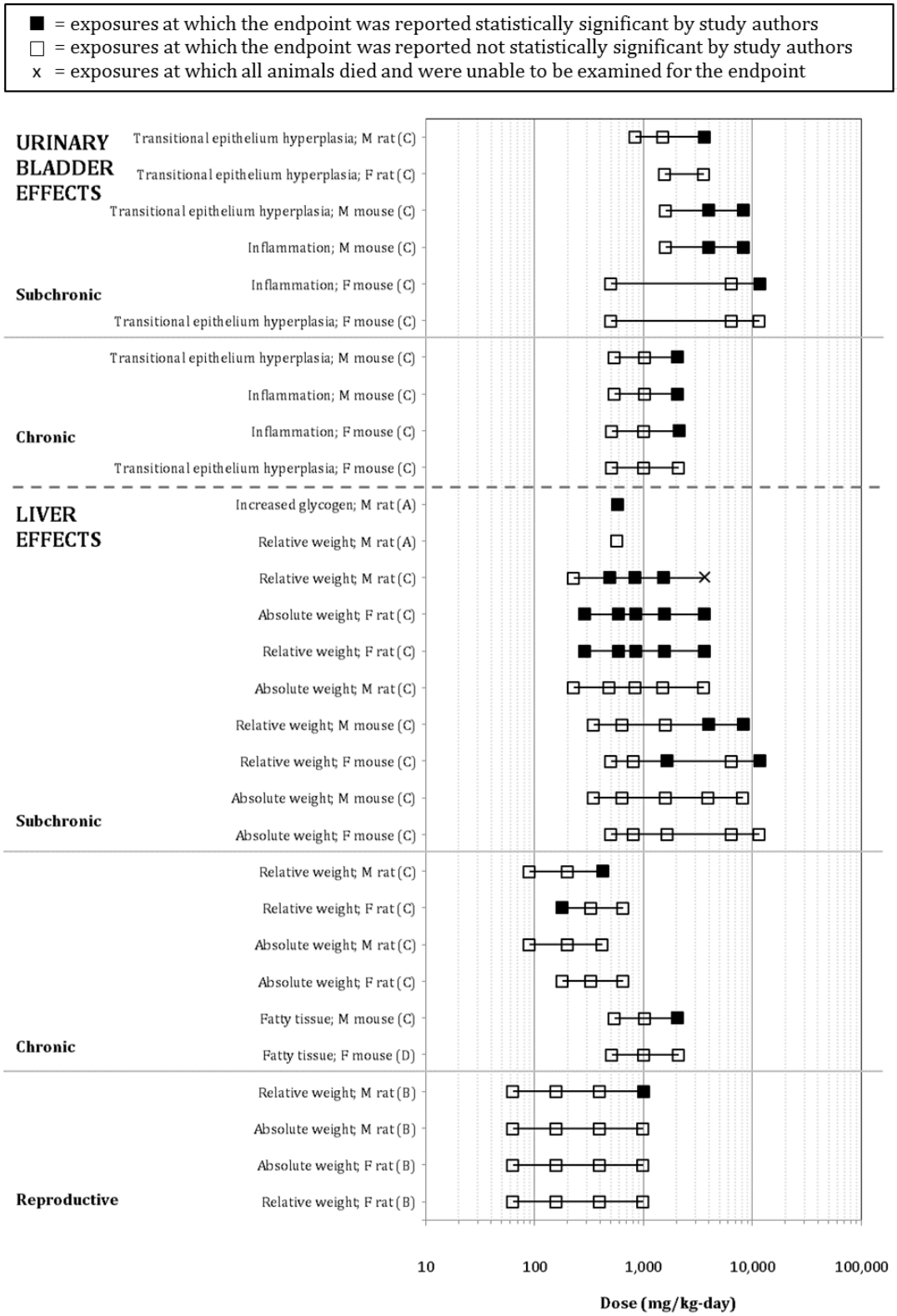
[a]The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.

Table B-6. 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

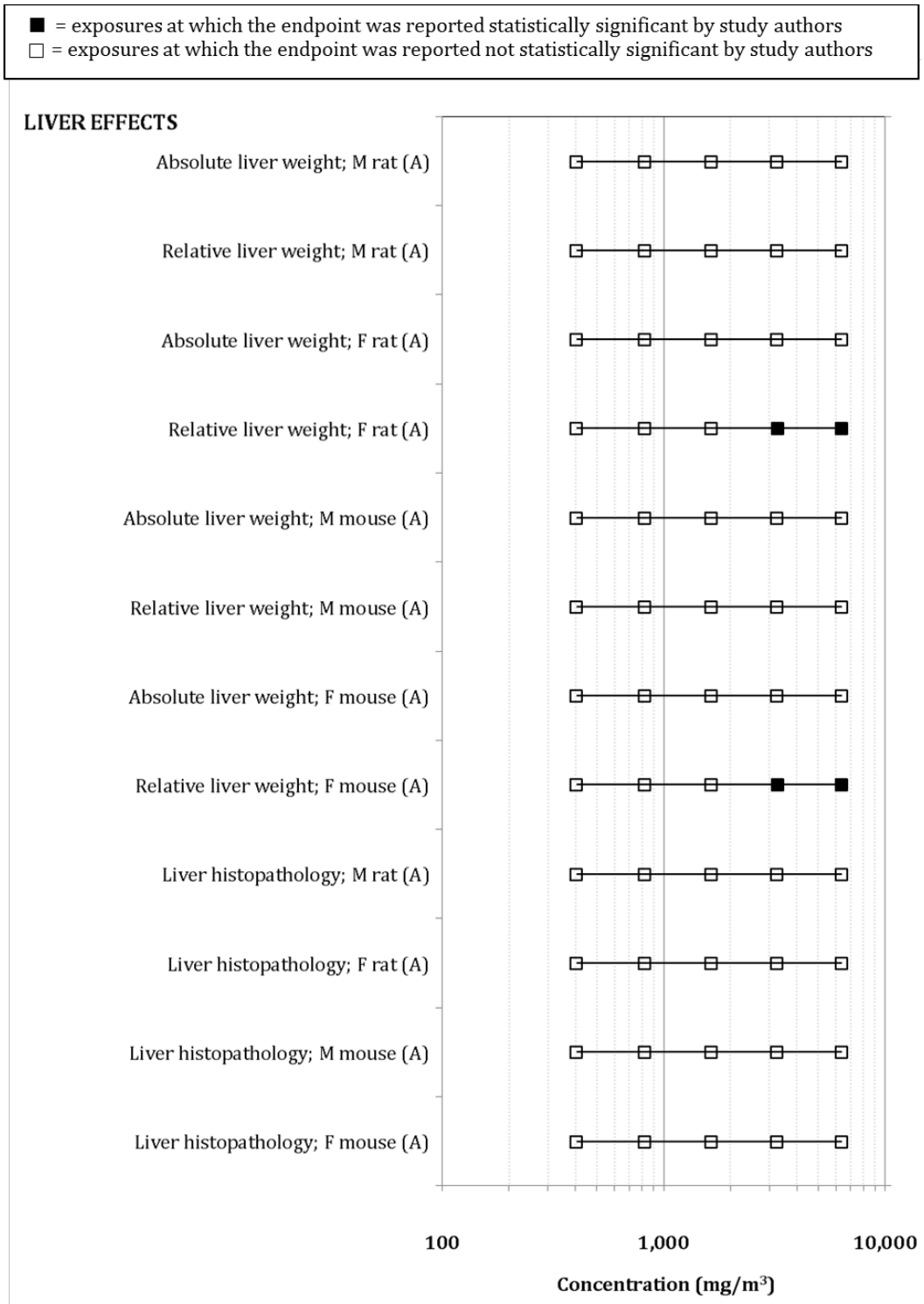
Reference and study design	Results
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, 2,070[a] mg/kg-d F: 0, 510, 1,020, 2,110 mg/kg-d 2 years	Incidence (severity): **Males** Dose (mg/kg-d) / Transitional epithelial hyperplasia / Inflammation 0 / 1/59 (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-6. Exposure-response array of other effects following oral exposure to *tert*-butanol.



Source: (A) NTP (1997)

Figure B-7. Exposure-response array of other effects following inhalation exposure to *tert*-butanol.

B.3.2. 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 formation, 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-7.

B.3.2.1. *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 strain *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 in 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. In one experiment where *tert*-butanol was dissolved in water, a significant, dose-related increase in the number of revertants occurred, reaching almost twice the control value at a concentration of 2,250 µg/plate. Of note is that DMSO is known to be a free radical scavenger, and its presence at high concentrations might mask a mutagenic response caused by oxidative damage.

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.3.2.2. *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 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 in 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 in 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 in a dose-dependent manner 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.3.2.3. *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 formation 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, 1995).

Male Kumming 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-7. 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, 3,333, 10,000 µg/plate	-	-	Preincubation procedure was followed. This study was part of the NTP 1995 testing results.	Zeiger et al. (1987);NTP (1995)
Reverse Mutation Assay *Salmonella typhimurium* (TA102)	1,000–4,000 µg/plate	ND	+	Only tested with S9 activation	Williams-Hill et al. (1999)
Reverse Mutation Assay *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537)	5, 15, 50, 100, 150, 200, 500, 1,000, 1,500, 2,500, 5,000 µg/plate	-	-	Experiments conducted in two different laboratories, two vehicles – distilled water and DMSO were used, different concentrations were used in experiments from different laboratories	Mcgregor et al. (2005)
Reverse mutation *Neurospora crassa*, ad-3A locus (allele 38701)	1.75mol/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, 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, 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 formation, B6C3F1 mouse peripheral blood cells	3,000, 5,000, 10,000, 20,000, 40,000 ppm	-		13-week, subchronic, drinking water study	NTP (1995)
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.3.3. Summary

tert-Butanol has been tested for its genotoxic potential using a variety of genotoxicity assays. Bacterial assays that detect reverse mutations have been thought to predict carcinogenicity with accuracy up to 80%. *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 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 and micronucleus formation. 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 formation 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 genotoxicty 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; 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; 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 from 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	1,643	3,274	6,369
		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	1,643	3,274	6,369

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.

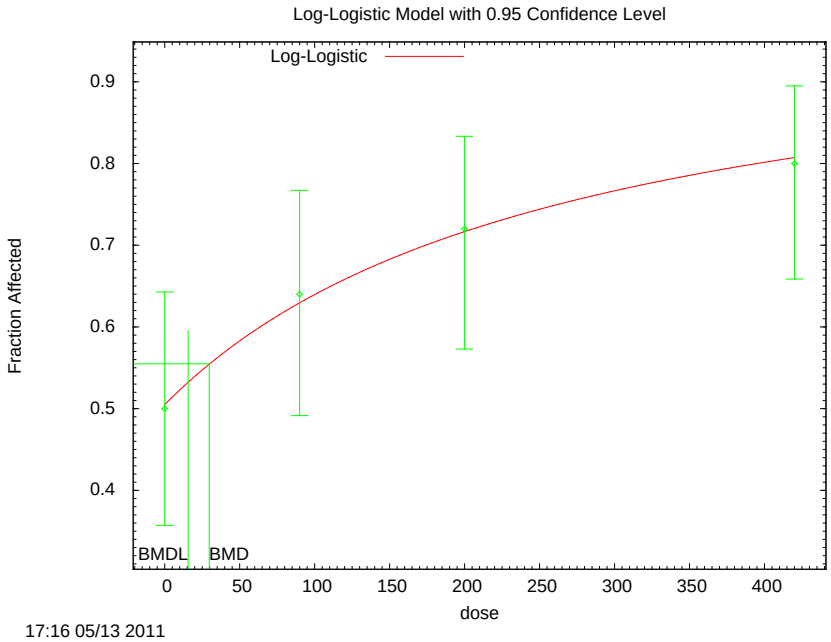


Figure C-1. Plot of incidence by dose, with fitted curve for LogLogistic model for kidney transitional epithelial hyperplasia in male F344 rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d

```
 ================================================================
        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.

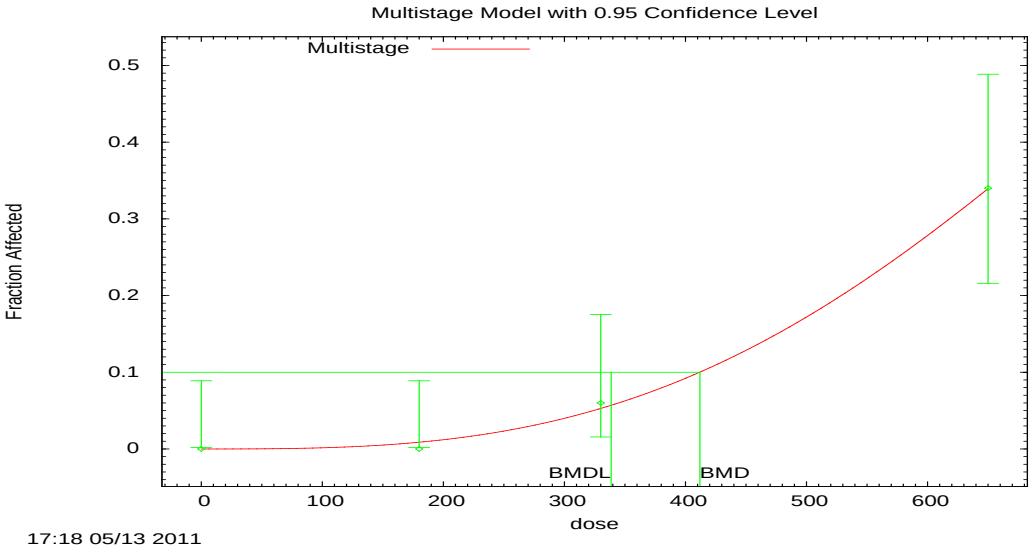


Figure C-2. Plot of incidence by dose, with fitted curve for Multistage 3° model for kidney transitional epithelial hyperplasia in female F344 rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d

```
================================================================
        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.

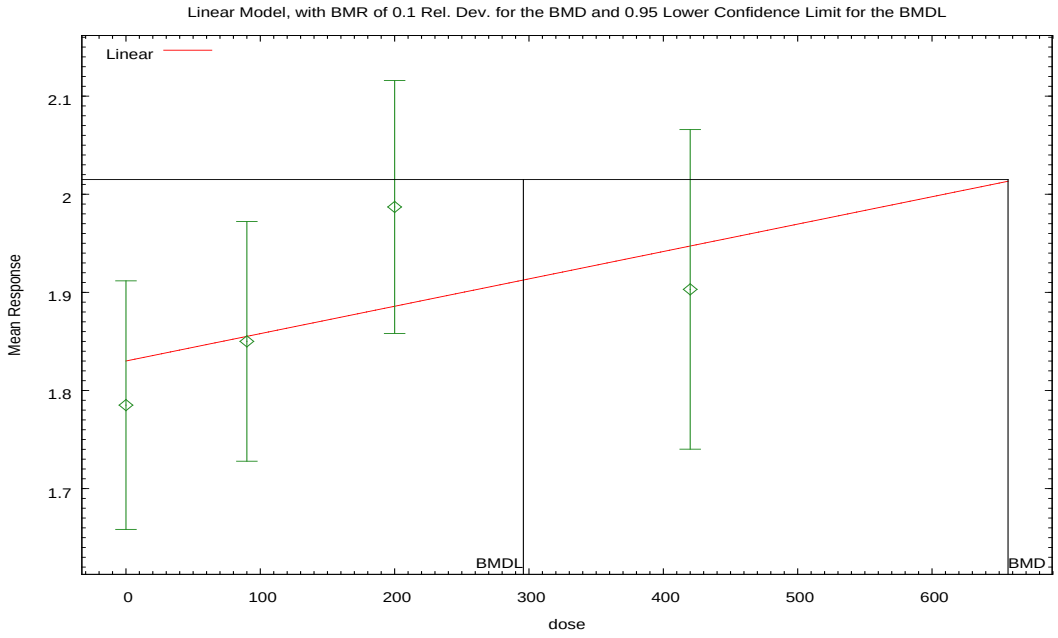


Figure C-3. Plot of mean response by dose, with fitted curve for Linear model with constant variance for absolute kidney weight in male F344 rats exposed to *tert*-butanol in drinking water for 15 months (NTP, 1995); BMR = 10% rel. dev. from control mean; dose shown in mg/kg-d

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.

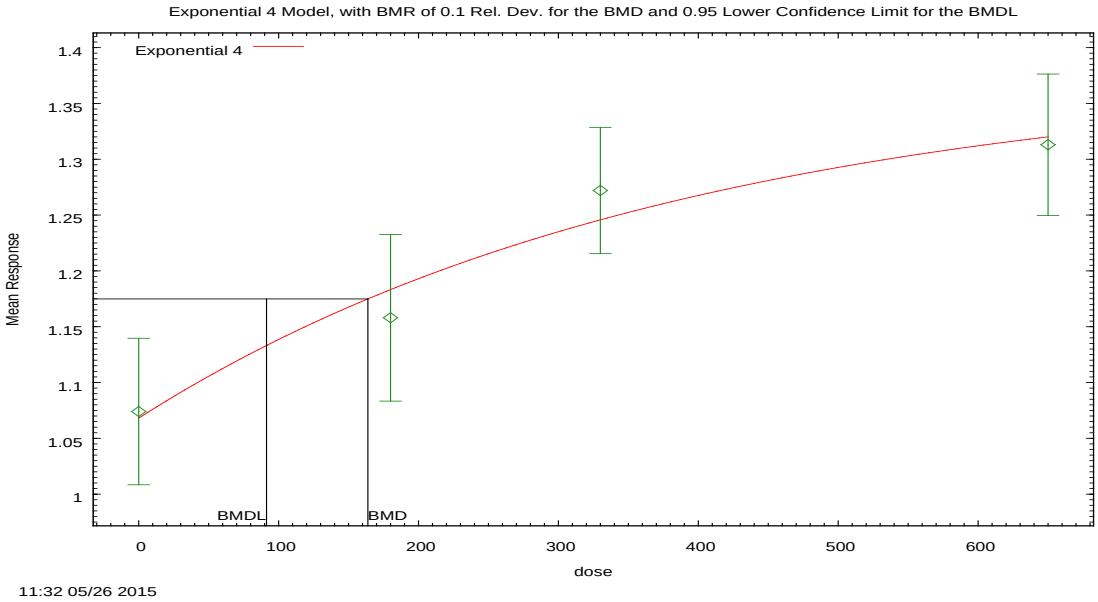


Figure C-4. Plot of mean response by dose, with fitted curve for Exponential (M4) model with constant variance for absolute kidney weight in female F344 rats exposed to *tert*-butanol in drinking water for 15 months (NTP, 1995); BMR = 10% rel. dev. from control mean; dose shown in mg/kg-d

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.

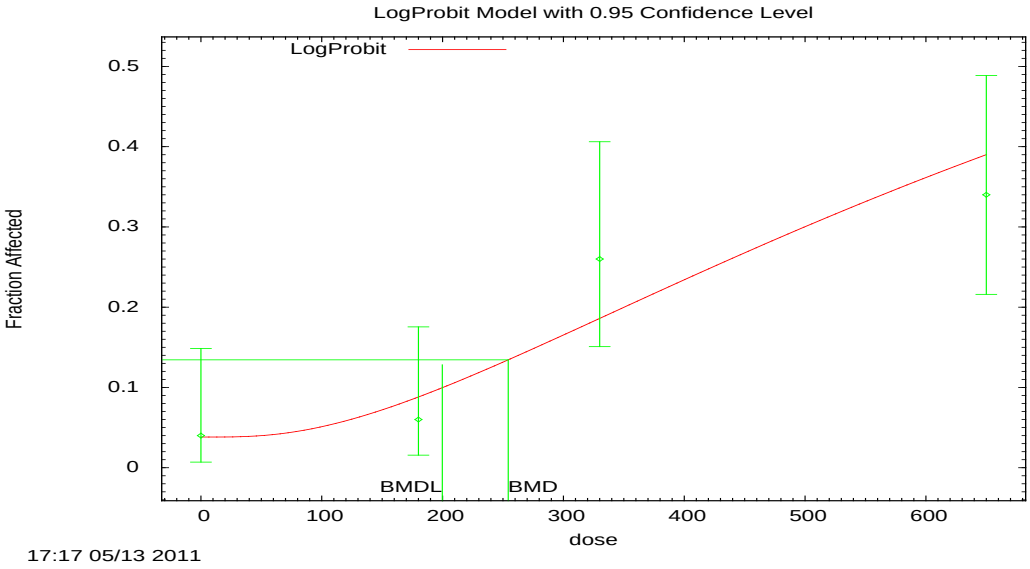


Figure C-5. Plot of incidence by dose, with fitted curve for Logprobit model for kidney inflammation in female rats exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d

```
        ================================================================
                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.

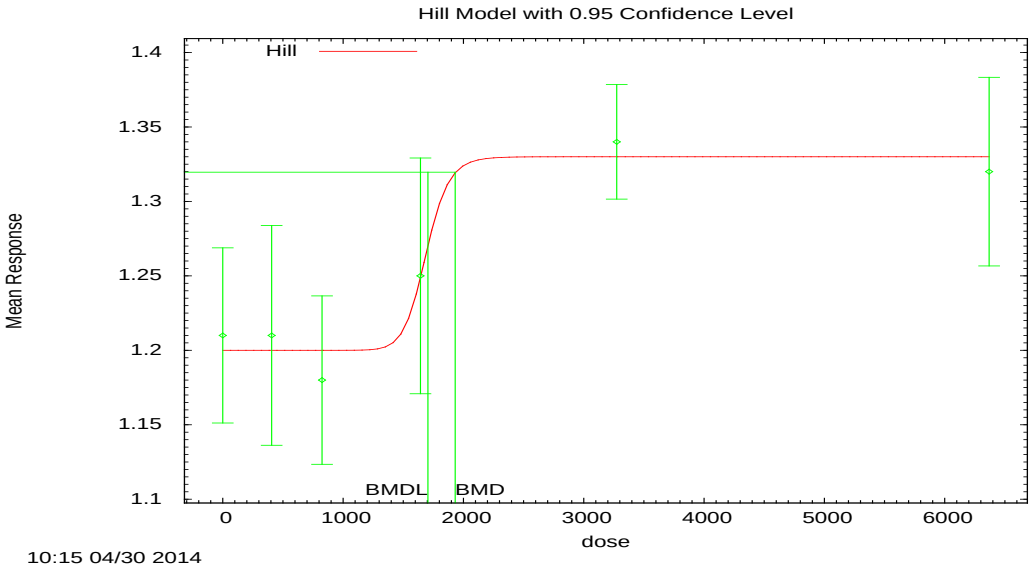


Figure C-6. Plot of mean response by concentration, with fitted curve for Hill model for absolute kidney weight in male F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m^3

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.

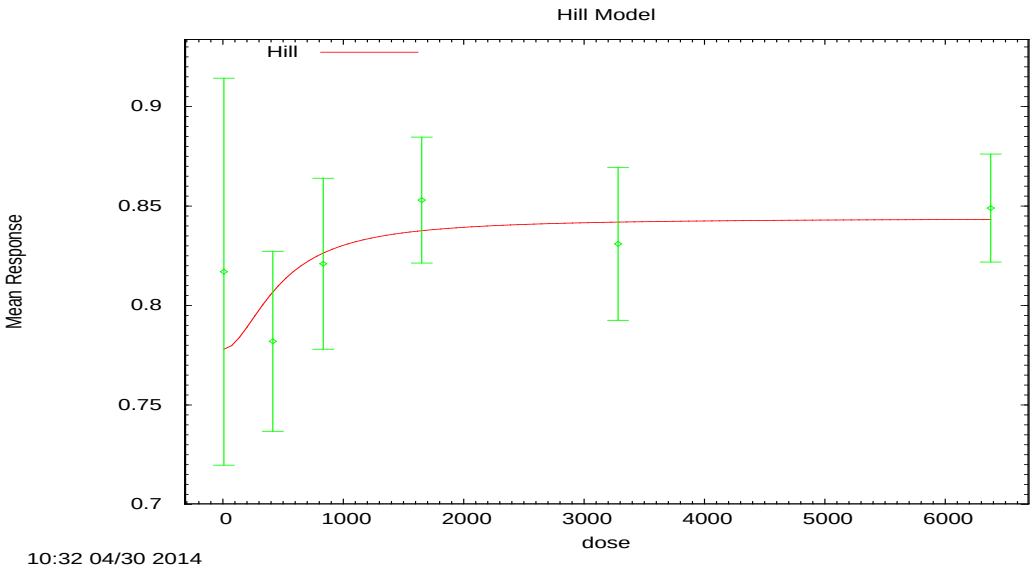


Figure C-7. Plot of mean response by concentration, with fitted curve for Hill model for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m³

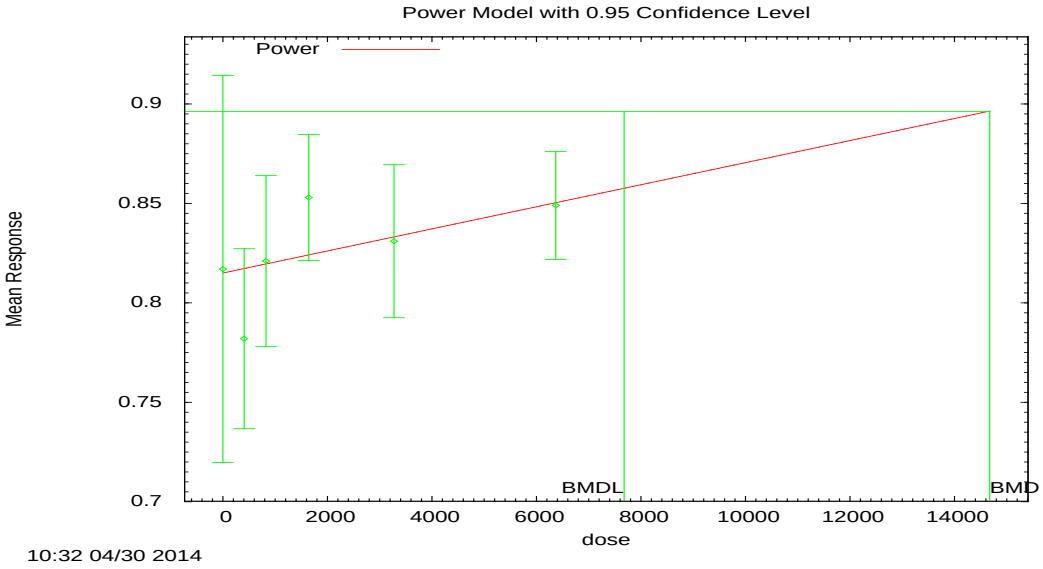


Figure C-8. Plot of mean response by concentration, with fitted curve for Power model for absolute kidney weight in female F344 rats exposed to *tert*-butanol via inhalation for 6 hr/d, 5d/wk for 13 weeks (NTP, 1997); BMR = 10% relative deviation from the mean; concentration shown in mg/m³

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	Dose (PBPK, mg/L)	0	4.6945	12.6177	40.7135
		Incidence / Total	8 / 50	13 / 50	19 / 50	13 / 50
Renal tubule adenoma or carcinoma NTP (1995)	Rat (F344) / Male	Dose (PBPK, mg/hr)	0	0.7992	1.7462	3.4712
		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	Dose (PBPK, mg/L)	0	4.6945	12.6177	40.7135
		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	Dose (PBPK, mg/hr)	0	0.7992	1.7462	3.4712
		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.1.2.3. *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.

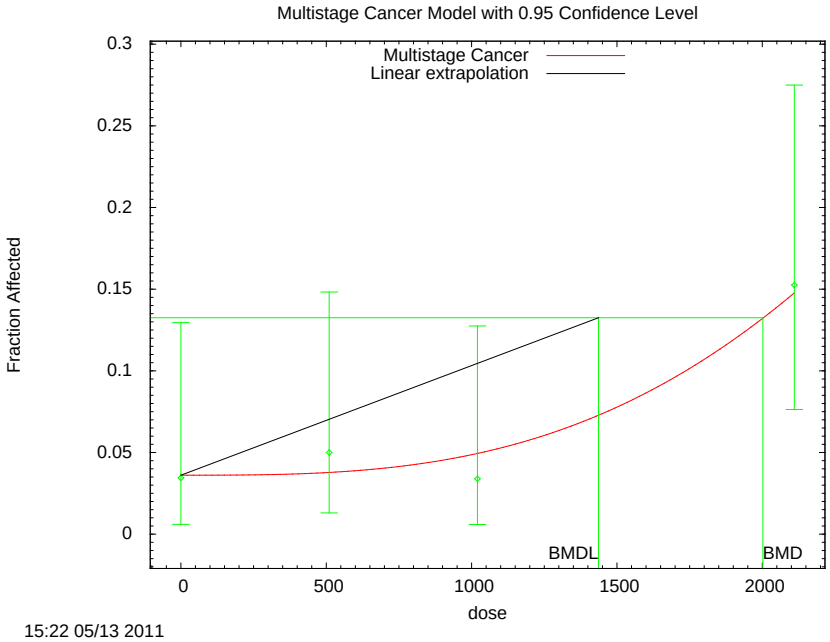


Figure C-9. Plot of incidence by dose, with fitted curve for Multistage 3° model for thyroid follicular cell adenomas in female B6C3F1 mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 10% extra risk; dose shown in mg/kg-d

```
        ================================================================
                 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.

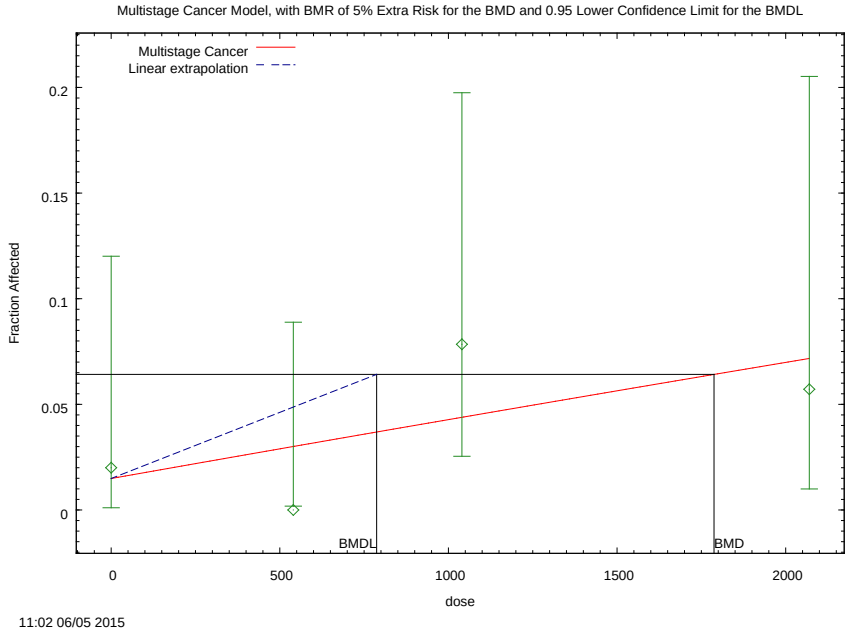


Figure C-10. Plot of incidence by dose, with fitted curve for Multistage 1° model for thyroid follicular cell adenomas or carcinomas in male B6C3F1 mice exposed to *tert*-butanol in drinking water for 2 years (NTP, 1995); BMR = 5% extra risk; dose shown in mg/kg-d

```
 ======================================================================
         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.

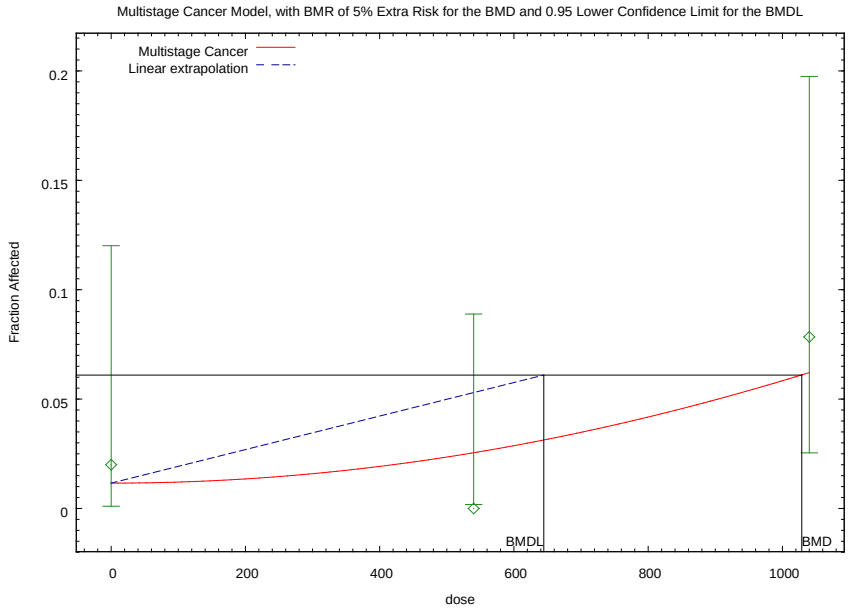


Figure C-11. Plot of incidence by dose, with fitted curve for Multistage 2° model for thyroid follicular cell adenomas or carcinomas in male B6C3F1 mice exposed to *tert*-butanol in drinking water for 2 years, high dose omitted (NTP, 1995); BMR = 5% extra risk; dose shown in mg/kg-d

```
=================================================================
         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.

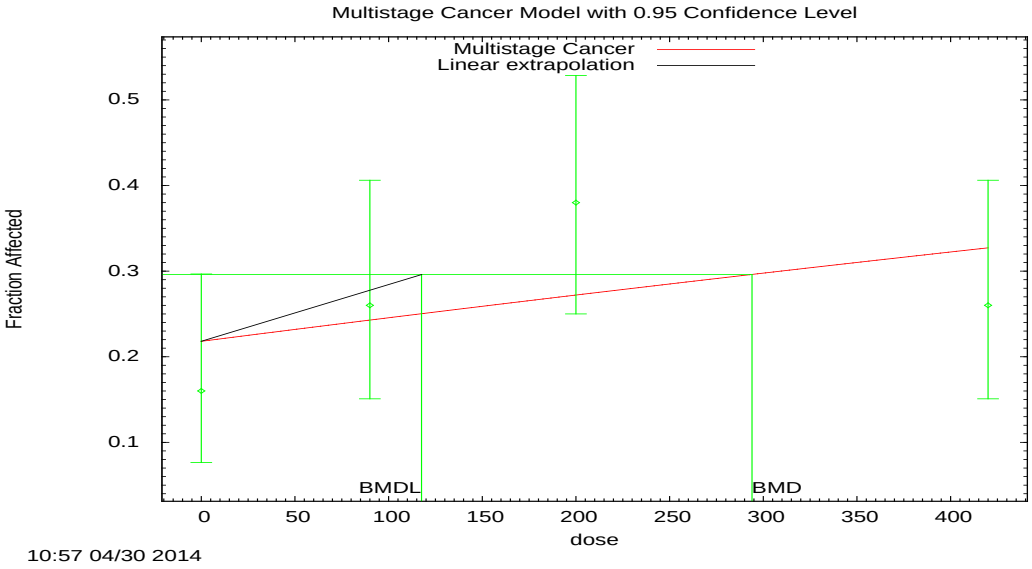


Figure C-12. Plot of incidence by dose, with fitted curve for Multistage 2° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and including all dose groups (NTP, 1995); BMR = 10% extra risk; dose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

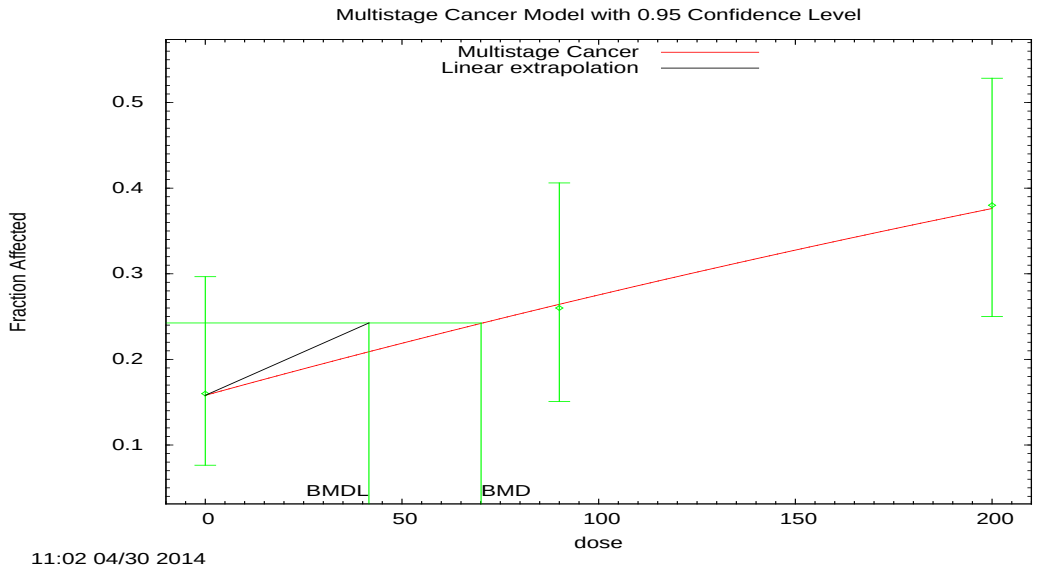


Figure C-13. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk.; dose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

Model[a]	Goodness of fit			BMD_{10Pct} (mg/L)	$BMDL_{10Pct}$ (mg/L)	Basis for model selection
	p-value	Scaled residuals	AIC			
Three Two One	**0.0518**	**-1.373, 0.155, 1.889, and -0.668**	**234.83**	**51.8**	**13.9**	**Multistage 1° was selected as the most parsimonious model of adequate fit.**

[a] Selected model in bold.

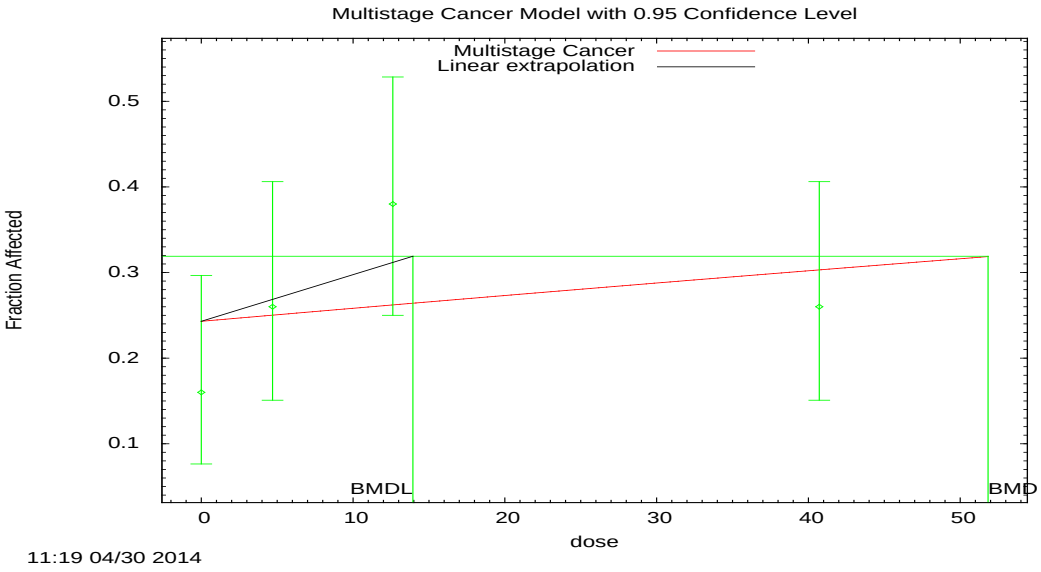


Figure C-14. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with PBPK (*tert*-butanol, mg/L) dose units and including all dose groups (NTP, 1995); BMR = 10% extra risk.; dose shown in mg/L.

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 = 51.8357
BMDL at the 95% confidence level = 13.9404
BMDU at the 95% confidence level = error
Taken together, (13.9404, error) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = error

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0.243327	0.253053
Beta(1)	0.00203259	0.00150893

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	p-value
Full model	-112.492	4			
Fitted model	-115.417	2	5.84883	2	0.0537
Reduced model	-115.644	1	6.30404	3	0.09772

AIC: = 234.834

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0.2433	12.166	8	50	-1.373
4.6945	0.2505	12.526	13	50	0.155
12.6177	0.2625	13.124	19	50	1.889
40.7135	0.3034	15.171	13	50	-0.668

Chi^2 = 5.92 d.f = 2 P-value = 0.0518

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 PBPK (*tert*-butanol, mg/L) dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMD_{10Pct} (mg/L)	$BMDL_{10Pct}$ (mg/L)	Basis for model selection
	p-value	Scaled residuals	AIC			
Two One	**0.891**	**-0.054, 0.113, and -0.057**	**171.70**	**4.33**	**2.54**	Multistage 1° was selected as the most parsimonious model of adequate fit.

[a] Selected model in bold.

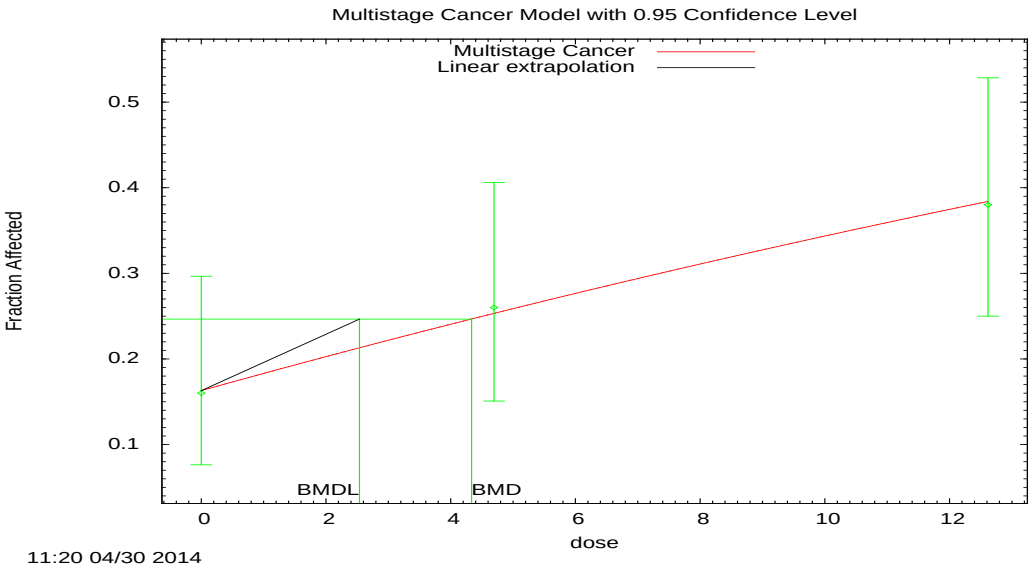


Figure C-15. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with PBPK (*tert*-butanol, mg/L) dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk; dose shown in mg/L.

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 = 4.33496
BMDL at the 95% confidence level = 2.53714
BMDU at the 95% confidence level = 12.8097
Taken together, (2.53714, 12.8097) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.0394144

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0.162798	0.164724
Beta(1)	0.0243048	0.0238858

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	p-value
Full model	-83.8395	3			
Fitted model	-83.8489	2	0.0187339	1	0.8911
Reduced model	-86.9873	1	6.29546	2	0.04295

AIC: = 171.698

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0.1628	8.14	8	50	-0.054
4.6945	0.2531	12.654	13	50	0.113
12.6177	0.3839	19.195	19	50	-0.057

Chi^2 = 0.02 d.f = 1 P-value = 0.891

Table C-18. 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 PBPK (metabolized, mg/hr) dose units and including all dose groups (NTP, 1995); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMD_{10Pct} (mg/hr)	$BMDL_{10Pct}$ (mg/hr)	Basis for model selection
	p-value	Scaled residuals	AIC			
Three **Two** **One**	**0.0885**	**-0.920, 0.301, 1.677, and -1.049**	**233.76**	**2.28**	**0.954**	Multistage 1° was selected as the most parsimonious model of adequate fit.

[a] Selected model in bold.

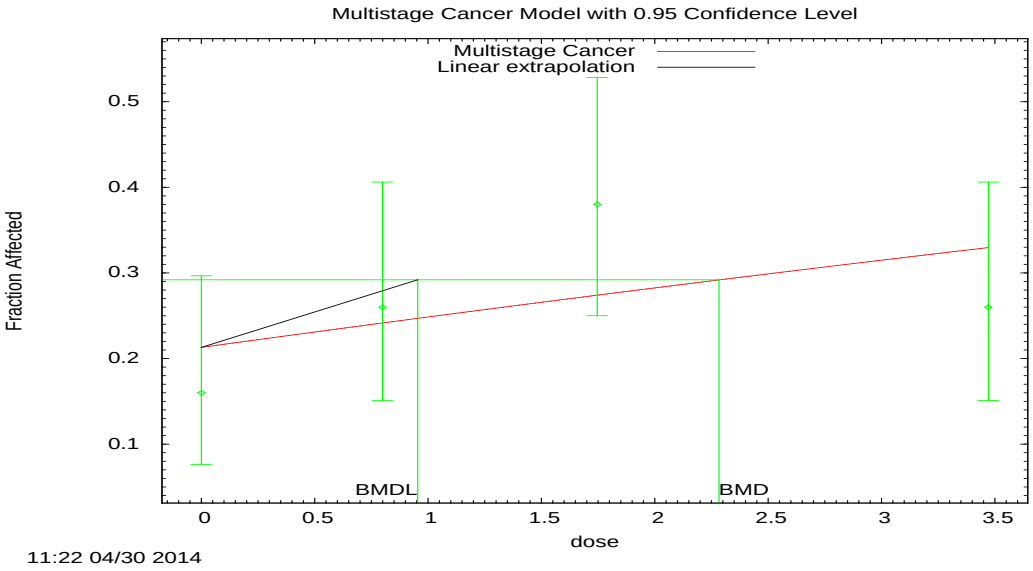


Figure C-16. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to tert-butanol in drinking water for 2 years modeled with PBPK (metabolized, mg/hr) dose units and including all dose groups (NTP, 1995); BMR = 10% extra risk; dose shown in mg/hr.

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 = 2.28299
BMDL at the 95% confidence level = 0.95436
BMDU at the 95% confidence level = error
Taken together, (0.95436, error) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = error

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0.21328	0.229822
Beta(1)	0.0461502	0.0349139

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	p-value
Full model	-112.492	4			
Fitted model	-114.879	2	4.77309	2	0.09195
Reduced model	-115.644	1	6.30404	3	0.09772

AIC: = 233.758

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0.2133	10.664	8	50	-0.92
0.7992	0.2418	12.088	13	50	0.301
1.7462	0.2742	13.71	19	50	1.677
3.4712	0.3297	16.487	13	50	-1.049

Chi^2 = 4.85 d.f = 2 P-value = 0.0885

Table C-19. 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 PBPK (metabolized, mg/hr) dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMD_{10Pct} (mg/hr)	$BMDL_{10Pct}$ (mg/hr)	Basis for model selection
	p-value	Scaled residuals	AIC			
Two	N/A[b]	-0.000, -0.000, and -0.000	173.68	0.673	0.365	Multistage 1° was selected on the basis of lowest AIC.
One	**0.906**	**0.037, -0.096, and 0.057**	**171.69**	**0.614**	**0.364**	

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

Data from NTP1995

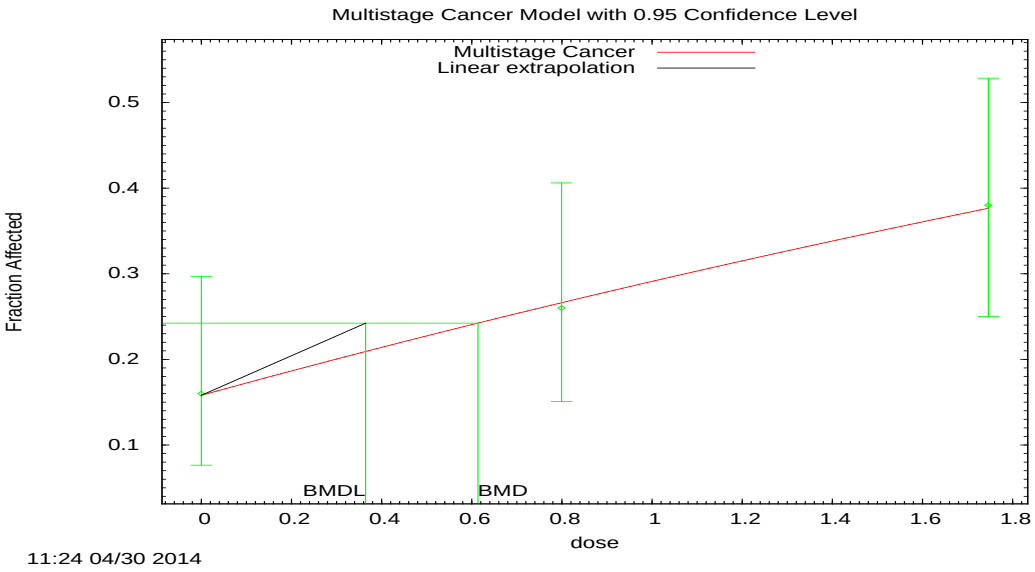


Figure C-17. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to tert-butanol in drinking water for 2 years modeled with PBPK (metabolized, mg/hr) dose units and excluding high-dose group (NTP, 1995); BMR = 10% extra risk; dose shown in mg/hr.

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 = 0.613798
BMDL at the 95% confidence level = 0.364494
BMDU at the 95% confidence level = 1.77845
Taken together, (0.364494, 1.77845) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.274353

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0.158068	0.156284
Beta(1)	0.171653	0.174305

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	p-value
Full model	-83.8395	3			
Fitted model	-83.8465	2	0.0138544	1	0.9063
Reduced model	-86.9873	1	6.29546	2	0.04295

AIC: = 171.693

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0.1581	7.903	8	50	0.037
0.7992	0.266	13.3	13	50	-0.096
1.7462	0.3761	18.806	19	50	0.057

Chi^2 = 0.01 d.f = 1 P-value = 0.9064

Table C-20. 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-21. 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.

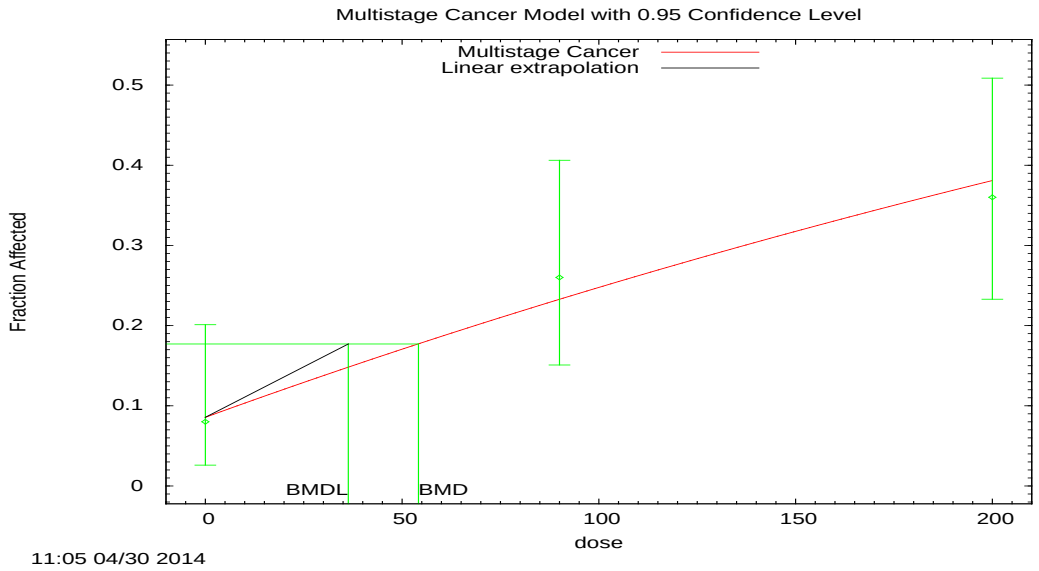


Figure C-18. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to tert-butanol in drinking water for 2 years modeled with administered dose units and excluding high-dose group; re-analyzed data (Hard et al., 2011; NTP, 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

Table C-22. 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 PBPK (*tert*-butanol, mg/L) 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/L)	$BMDL_{10Pct}$ (mg/L)	Basis for model selection
	p-value	Scaled residuals	AIC			
Three Two One	0.0048	-2.089, 0.864, 2.165, and -0.929	220.82	31.4	11.7	No model fit the data.

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

Table C-23. 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 PBPK (*tert*-butanol, mg/L) dose units and excluding high-dose group; reanalyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMD_{10Pct} (mg/L)	$BMDL_{10Pct}$ (mg/L)	Basis for model selection
	p-value	Scaled residuals	AIC			
Two One	**0.364**	**-0.285, 0.750, and -0.424**	**155.33**	**3.35**	**2.21**	Multistage 1° was selected as the most parsimonious model of adequate fit.

[a] Selected model in bold.

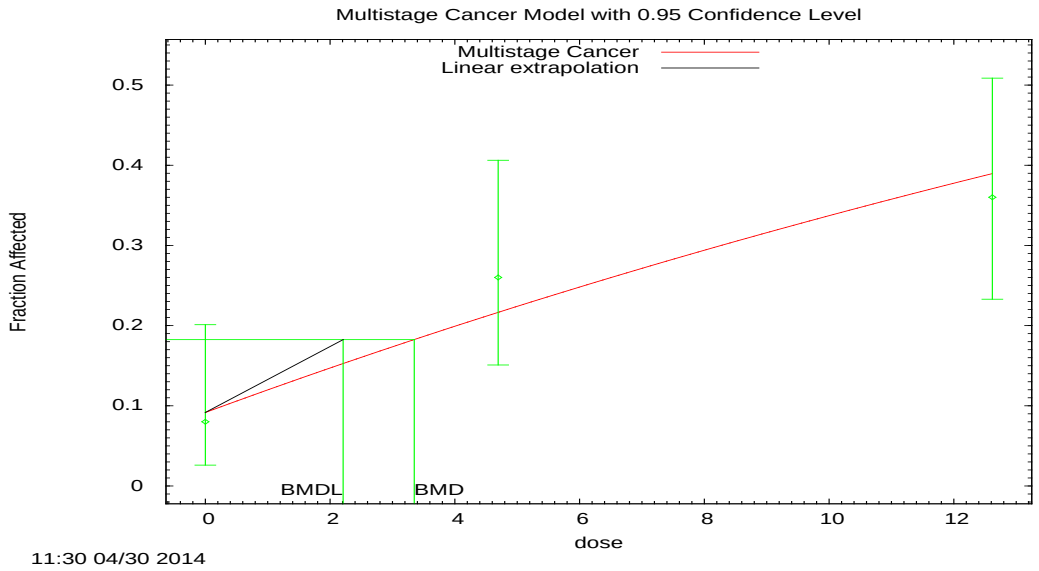


Figure C-19. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to tert-butanol in drinking water for 2 years modeled with PBPK (*tert*-butanol, mg/L) dose units and excluding high-dose group; reanalyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk; dose shown in mg/L.

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 = 3.34903
BMDL at the 95% confidence level = 2.20865
BMDU at the 95% confidence level = 6.49702
Taken together, (2.20865, 6.49702) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.0452765

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0.0916116	0.110649
Beta(1)	0.03146	0.0276674

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	p-value
Full model	-75.2622	3			

Fitted model	-75.664	2	0.803466	1	0.3701
Reduced model	-81.4909	1	12.4574	2	0.001972

AIC: = 155.328

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0.0916	4.581	4	50	-0.285
4.6945	0.2163	10.817	13	50	0.75
12.6177	0.3892	19.462	18	50	-0.424

Chi^2 = 0.82 d.f = 1 P-value = 0.3643

Table C-24. 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 PBPK (metabolized, mg/hr) 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/hr)	$BMDL_{10Pct}$ (mg/hr)	Basis for model selection
	p-value	Scaled residuals	AIC			
Three Two One	0.0142	-1.367, 1.119, 1.783, and -1.484	218.26	1.44	0.770	No model fit the data.

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

Table C-25. 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 PBPK (metabolized, mg/hr) dose units and excluding high-dose group; reanalyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMD_{10Pct} (mg/hr)	$BMDL_{10Pct}$ (mg/hr)	Basis for model selection
	p-value	Scaled residuals	AIC			
Two One	**0.593**	**-0.130, 0.435, and -0.281**	**154.81**	**0.474**	**0.319**	**Multistage 1° was selected as the most parsimonious model of adequate fit.**

[a] Selected model in bold.

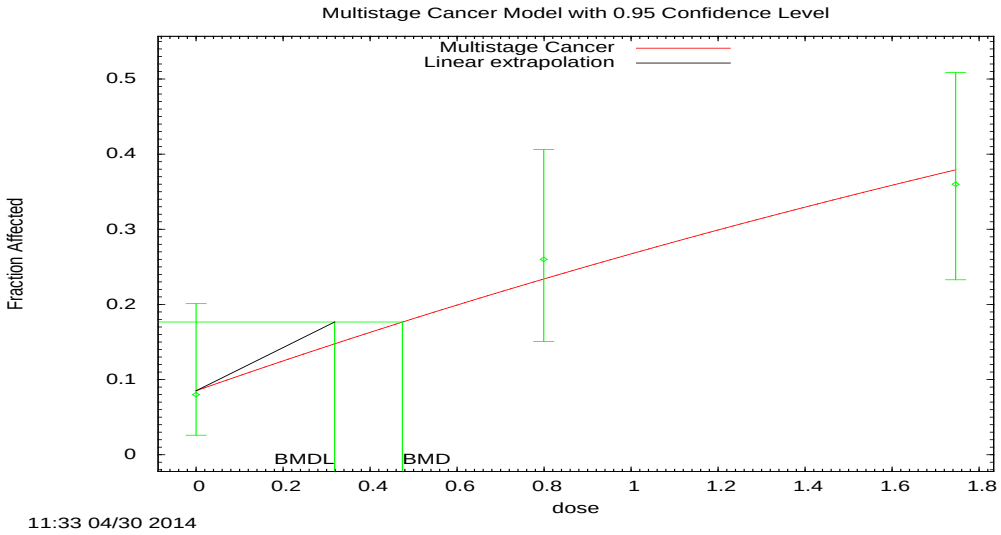


Figure C-20. Plot of incidence by dose, with fitted curve for Multistage 1° model for renal tubule adenoma or carcinoma in male F344 rats exposed to *tert*-butanol in drinking water for 2 years modeled with PBPK (metabolized, mg/hr) dose units and excluding high-dose group; reanalyzed data (Hard et al., 2011; NTP, 1995); BMR = 10% extra risk.; dose shown in mg/hr.

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 = 0.474241
BMDL at the 95% confidence level = 0.318504
BMDU at the 95% confidence level = 0.882859
Taken together, (0.318504, 0.882859) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.313968

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0.0851364	0.0969736
Beta(1)	0.222167	0.206161

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	p-value
Full model	-75.2622	3			
Fitted model	-75.4029	2	0.281435	1	0.5958
Reduced model	-81.4909	1	12.4574	2	0.001972

AIC: = 154.806

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0.0851	4.257	4	50	-0.13
0.7992	0.234	11.699	13	50	0.435
1.7462	0.3793	18.966	18	50	-0.281

Chi^2 = 0.29 d.f = 1 P-value = 0.5933

C.1.2.4. *Inhalation Unit Risk for Cancer*

An inhalation unit risk was not derived because the relative contribution of the α_{2u}-globulin and other, unknown, processes to renal tumor formation cannot be determined (U.S. EPA, 1991), and therefore the male rat renal tumors are not considered suitable for quantitative analysis. However, if renal tumors are considered suitable for analysis then route-to-route extrapolation could be performed.

Dose Response Analysis – Adjustments and Extrapolation Methods

A PBPK model for *tert*-butanol in rats has been developed, as described in Appendix B. Using this model, route-to-route extrapolation of the oral BMDL to derive an inhalation POD was performed as follows. First, the internal dose in the rat at the oral BMDL (assuming continuous exposure) was estimated using the PBPK model, to derive an "internal dose BMDL." Then, the inhalation air concentration (again assuming continuous exposure) that led to the same internal dose in the rat was estimated using the PBPK model, resulting in a route-to-route extrapolated BMCL.

A critical decision in the route-to-route extrapolation is the selection of the internal dose metric to use that established "equivalent" oral and inhalation exposures. For *tert*-butanol-induced kidney effects, the two options are the concentration of *tert*-butanol in blood and rate of *tert*-butanol metabolism. Note that using the kidney concentration of *tert*-butanol will lead to the same route-to-route extrapolation relationship as *tert*-butanol in blood, since the distribution from blood to kidney is independent of route. There are no data that suggest metabolites of *tert*-butanol mediate its renal toxicity. In the absence of evidence that would suggest otherwise, it is assumed that *tert*-butanol itself is the active toxicological agent. Therefore, the concentration of *tert*-butanol in blood was selected as the dose metric to derive the BMCL.

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

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

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended if the MOA of carcinogenicity has not been established (U.S. EPA, 2005). In the case of *tert*-butanol, the mode of carcinogenic action for renal tubule tumors is not fully understood (see Section 1.2.1). Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with *tert*-butanol exposure.

Inhalation Unit Risk Derivation

The results from route-to-route extrapolation of the male rat renal tubule tumor data are summarized in Table C-26. The lifetime inhalation unit risk for humans is defined as the slope of the line from the lower 95% bound on the exposure at the POD to the control response (inhalation unit risk = $0.1/BMCL_{10}$). This slope, a 95% upper confidence limit represents a plausible upper bound on the true risk. Using linear extrapolation from the $BMCL_{10}$, a human equivalent inhalation unit risk was derived, as listed in Table C-26.

Two inhalation unit risks were derived from the NTP (1995) bioassay: one based on the original reported incidences and one based on the Hard et al. (2011) reanalysis. The two estimates differ by less than 20%, but the Hard et al. (2011) reanalysis is considered preferable, as it is based on a PWG analysis. Therefore, the recommended inhalation unit risk for providing a sense of the magnitude of potential carcinogenic risk associated with lifetime inhalation exposure to *tert*-butanol is **1×10^{-3} per mg/m³**, or **2×10^{-3} per µg/m³**, based on the renal tubule tumor response in male F344 rats.

Table C-26. Summary of the inhalation unit risk derivation

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

[a] Average blood concentration of *tert*-butanol under continuous oral exposure at the BMDL.
[b] Continuous inhalation human equivalent concentration that leads to the same average blood concentration of *tert*-butanol as continuous oral exposure at the BMDL.
[c] Human equivalent inhalation unit risk = $0.1/BMCL_{HEC}$.

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