EPA/635/R-17/016b
External Review Draft
www.epa.gov/iris

Toxicological Review of Ethyl Tertiary Butyl Ether

(CASRN 637-92-3)

Supplemental Information

June 2017

NOTICE

This document is an **External Review Draft**. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is a preliminary draft for review purposes only. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

CONTENTS

TABLES

FIGURES

ABBREVIATIONS

AIC	Akaike's information criterion
ARCO	ARCO Chemical Company
AUC	area under the curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
CASRN	Chemical Abstracts Service Registry Number
CIIT	Chemical Industry Institute of Toxicology
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
GI	gastrointestinal
HERO	Health and Environmental Research Online
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
HIBA	2-hydroxyisobutyrate
HT	heterogeneous
KO	knockout
JPEC	Japan Petroleum Energy Center
MN	micronucleus, micronucleated
MNNCE	mature normochromatic erythrocyte population
MNPCE	micronucleated polychromatic erythrocyte
MNRETs	micronucleated reticulocytes
MTBE	methyl tertiary butyl ether
MPD	2-methyl-1,2-propane diol
NADPH	nicotinamide adenine dinucleotide phosphate
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
POD	point of departure
RET	reticulocyte
SD	standard deviation
SRBC	sheep red blood cell
TAME	tertiary amyl methyl ether
TBA	*tert*-butyl alcohol, *tert*-butanol
WT	wild type

APPENDIX A. OTHER AGENCY AND INTERNATIONAL ASSESSMENTS

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

Organization	Toxicity value
National Institute for Public Health and the Environment (Bilthoven, The Netherlands)	Oral noncancer tolerable daily intake: 0.25 mg/kg-day Inhalation noncancer tolerable concentration in air: 1.9 mg/m^3

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

B.1. TOXICOKINETICS

B.1.1. Absorption

Absorption in Humans

Most of the available human data on the uptake of ETBE were obtained from volunteers. Nihlén et al. (1998) exposed eight healthy male volunteers (average age: 29 years) to 5, 25, and 50 ppm (20.9, 104, and 210 mg/m^3) ETBE by inhalation for 2 hours. Each volunteer was exposed at each concentration in sequence with 2-week intervals between exposures. The study was performed according to the Declaration of Helsinki after approval by the Regional Ethical Committee of the institution where the study was performed, and written informed consent was obtained from the volunteers. The volunteers performed light physical exercise (50 watts) on a bicycle ergometer during exposure. Exhaled air was collected before exposure, every 30 minutes during exposure, and 6 times after exposure. The concentrations of ETBE and one of its primary metabolites, *tert*-butanol, were determined in exhaled air samples. Blood was drawn before exposure, approximately every 10 minutes during exposure, approximately every 30 minutes from 1 to 4 hours after exposure, and an additional 4 times up to 48 hours after exposure. Urine was collected prior to exposure, at 0 and 2 hours, and at approximately 4, 7, 11, 20, 22, and 46 hours after exposure. ETBE, *tert*-butanol, and acetone (an ETBE metabolite) concentrations were determined in blood and urine. The blood profiles of the parent compound and metabolites were similar at all three exposure levels and reflected exposure concentrations, as judged by linear increases in blood area-under-the-curve (AUC) values for the concentration-time curve calculated (but only reported in graphical form by the authors).

Acetone levels were highly variable and appeared to reflect not only ETBE exposure, but the physical activity of the volunteers. Nihlén et al. (1998) calculated the ETBE doses to the volunteers to be 0.58, 2.9, and 5.8 mmol for the 20.9-, 104-, and 210-mg/m^3 exposure levels, respectively. The concentrations of ETBE in blood rose sharply during the first 30 minutes of exposure and kept rising at a lower rate until the end of exposure, reaching peak concentrations of about 10, 5.4, and 1.1 µM at 210, 104, and 20.9 mg/m^3, respectively. By 6 hours, the concentrations of ETBE had fallen to very low levels (<1 µM) even after the 210-mg/m^3 exposure. Based on blood AUC values for

ETBE, the authors calculated two types of respiratory uptake: net respiratory uptake = (concentration in inhaled air—concentration in exhaled air) multiplied by the pulmonary ventilation; and respiratory uptake = net respiratory uptake + amount exhaled during the exposure. During the 2 hours of exposure, the authors calculated that 32–34% of each dose was retained by the volunteers (respiratory uptake), and the net respiratory uptake was calculated to be 26% of the dose at all three exposure levels. Over 24 hours, the respiratory expiration was calculated as 45−50% of the respiratory uptake, and because the net respiratory uptake and expiration do not consider the amount of ETBE cleared during exposure, the net respiratory excretion was lower, at 30–31% of the net respiratory uptake. These authors determined that the ETBE blood:air partition coefficient in humans was 11.7.

Amberg et al. (2000) exposed six volunteers (three males and three females, average age 28 ± 2 years) to 4.5 ppm (18.8 mg/m^3) and 40.6 ppm (170 mg/m^3) ETBE respectively. The exposures lasted 4 hours, and the two concentrations were administered to the same volunteers 4 weeks apart. These volunteers were healthy nonsmokers and were asked to refrain from alcohol and medication intake from 2 days before until the end of the experiment. The study was performed according to the Declaration of Helsinki after approval by the Regional Ethical Committee of the institution where the study was performed, and written informed consent was obtained from the volunteers. Urine was collected at 6-hour intervals for 72 hours. Blood was drawn immediately after exposure and thereafter every 6 hours for 48 hours. ETBE and its primary metabolite, *tert*-butanol, were determined in blood; the same two substances, plus additional metabolites of *tert*-butanol, were assessed in urine. The authors estimated the retained doses to be 1,090 µmol following 170-mg/m^3 ETBE exposure and 121 µmol following 18.8-mg/m^3 exposure. These estimates were derived using a resting human respiratory rate of 9 L/minute (13 m^3/day) and a retention factor for ETBE of 0.3, which was based on data reported by Nihlén et al. (1998). These estimates of retained dose are lower than those reported during light exercise (Nihlén et al., 1998).

Absorption in Animals

Amberg et al. (2000) exposed F344 NH rats (5/sex/dose group) concurrent with the human volunteers in the same exposure chamber. Blood was taken from the tail vein of each rat at the end of the exposure period, and urine was collected for 72 hours at 6-hour intervals following exposure. Immediately after the 4-hour exposure period, the authors reported that blood levels of ETBE were lower in the rats than in humans, although exact values were not reported. The authors estimated that the rats received doses of 20.5 and 2.3 µmol at the 170- and 18.8-mg/m^3 exposures, respectively, using an alveolar ventilation rate of 0.169 L/minute and a retention factor of 0.3 for rats.

No published oral dosing studies of the absorption of ETBE in humans were identified. The Japan Petroleum Energy Center (JPEC), however, conducted an oral dosing study of the absorption of ETBE in rats after single and repeated dosing for 14 days (JPEC, 2008e, f). Seven-week-old

Crl:CD(SD) male rats (4/dose group) were administered either a single oral dose of 5, 50, or 400 mg/kg [^{14}C]ETBE via gavage or 5 mg/kg-day [^{14}C]ETBE daily for 14 days. In the single-dose study by JPEC (2008f), plasma levels were compared to those observed after a single intravenous dose of 5 mg/kg-day [^{14}C]ETBE. There is no indication that a similar comparison was conducted in the repeated-dose study (JPEC, 2008e). Plasma radioactivity was measured in rats at 1, 2, 4, 6, 8, 10, and 24 hours after the first exposure in the repeated dose study; 8 and 24 hours after the second to 13th exposures; and at 1, 2, 4, 6, 8, 10, 12, 24, 32, 48, 72, 96, 120, 144, and 168 hours after the last exposure in the repeated dose study and after the single dose study.

Plasma radioactivity levels increased following a single dose of [^{14}C]ETBE; this increase was not proportional as the dose increased, especially at the high dose [i.e., the peak plasma radioactivity levels were 2,800, 22,100, and 89,900 ng equivalents of ETBE/mL (ng equivalent ETBE/mL) in the 5-, 50-, and 400-mg/kg dose groups, respectively]. Maximum plasma [^{14}C]ETBE levels (C_{max}) were estimated to be reached at 9.0, 11.5, and 8.0 hours after administration in the 5-, 50-, and 400-mg/kg dose groups, respectively. The [^{14}C]ETBE levels in the plasma were higher following oral exposure than after intravenous exposure (see Table B-2). The estimated elimination plasma half-lives were 17.5, 19.8, and 9.9 hours for the 5-, 50-, and 400-mg/kg dose groups, respectively. With repeated dosing of 5 mg/kg-day [^{14}C]ETBE (JPEC, 2008e), the C_{max} was achieved 6 hours after the first exposure and increased until it reached a steady state around the fifth day of exposure. After the last exposure on Day 14, the C_{max}, of 6,660 ± 407 ng equivalent ETBE/mL was achieved 10 hours after administration of [^{14}C]ETBE, and plasma radioactivity steadily decreased after this point. The elimination plasma half-life from C_{max} to 24 hours was 17.9 hours after the first dose and 14.2 hours after the final dose. The elimination half-life from C_{max} to 168 hours after the final dose following repeated dosing was 24.7 hours. Based on radioactivity levels measured in urine and exhalation, more than 90% of the administered dose was absorbed.

In two parallel studies, the pharmacokinetics of ETBE was studied in mice (Sun and Beskitt, 1995a) and male Fischer 344 rats (Sun and Beskitt, 1995b). Study authors investigated the pharmacokinetics of [^{14}C]ETBE in mice and rats (3/sex/dose) exposed by nose-only inhalation at target concentrations of 500, 750, 1,000, 1,750, 2,500, and 5,000 ppm (2,090, 3,130, 4,180, 7,310, 10,450, and 20,900 mg/m^3) for a single 6-hour period (the true doses differed by less than 10% from the targets). Specific activity of the administered [^{14}C]ETBE and localization of the label were not reported. Note, that in the absence of the specific activity and localization of the label, it is not clear how the "mg ETBE equivalents" were calculated in the Sun and Beskitt (1995a, b) report or for the specific tissues. Of the three animals per sex exposed concurrently, two were used to determine blood and tissue concentrations of radiolabel, and the third was kept in a metabolism cage for up to 118 hours to quantify radiolabel elimination in urine, feces, as volatile in expired air and as exhaled CO_2. Exhaled organic volatiles were trapped in charcoal filters. Exhaled CO_2 was trapped in aqueous 1 M KOH. Samples from the 20,900-mg/m^3 treated animals were collected at 3, 6, 12, 18, 24, 48, 72, 96, and 118 hours after termination of exposure. At the lower exposure concentrations listed above,

samples were collected at fewer time points; generally, at full-day intervals up to 96 hours. Animals were euthanized either immediately after exposure or after being removed from the metabolic cages, and blood and kidneys were collected. Cages were washed and the wash fluid collected. Charcoal traps were eluted with methanol. Urine, cage wash, trapped $^{14}CO_2$, and charcoal filter eluates were measured directly by liquid scintillation spectrometry. Blood and kidney tissue from rats and blood and liver tissue from mice were combusted in a sample oxidizer and analyzed by liquid scintillation spectrometry.

Immediately upon cessation of exposure, radiolabel was quantified in the blood and kidneys of two rats and in the blood and liver of two mice. Results in Table B-1 demonstrate the absorption of radiolabel expressed as mg equivalents of ETBE into blood. Because the ETBE carbon(s) bearing the radiolabel was not identified, further speciation is not possible. The concentration of radiolabel in rat blood is proportionate with exposure concentration to the highest concentration (20,894 mg/m^3), although in mice, such proportionality is absent at concentrations of 10,447 mg/m^3 and above. These data indicate that ETBE is well absorbed following inhalation exposure, but that higher concentrations (e.g., 10,447 mg/m^3 and above) could result in reduced respiration rates or otherwise affect mechanisms of inhalation uptake. Additional support for reduction of absorption is presented in Table B-1, demonstrating the elimination of the radiolabel from rats and mice in these studies (Sun and Beskitt, 1995a, b).

In contrast, Borghoff and Asgharian (1996) evaluated the disposition of ^{14}C radiolabel in F344 rats and CD-1 mice after whole-body and nose-only inhalation exposure to 500, 1,750, or 5,000 ppm [^{14}C]ETBE. Besides recovery of total radioactivity in urine, feces, and expired air, air and urine samples were analyzed for ETBE and *tert*-butanol. Urine samples were also analyzed for *tert*-butanol metabolites HBA and MPD, and $^{14}CO_2$ was measured in exhaled air. Results obtained after both a single 6-hour exposure or after 13 days of pre-exposure to 0, 500, or 5,000 ppm ETBE indicate that total inhalation uptake increases linearly with exposure concentration over this range, although there are dose- and pre-exposure-related shifts in the form and route of elimination. Because the later study used four rats per sex and exposure level, rather than just two, it should be given higher weight.

Table B-1. Radioactivity in blood and kidney of rats and blood and liver of mice, following 6 hours of [^{14}C]ETBE inhalation exposure

Exposure Level (mg/m^3)	F344 Rat[a]		CD-1 Mouse[a]	
	Blood[b]	Kidney[c]	Blood[b]	Liver[c]
2,089	0.037	0.074	0.154	0.208
3,134	0.062	0.094	0.340	0.348
4,179	0.080	0.116	0.336	0.540
7,313	0.124	0.152	0.481	0.724
10,447	0.156	0.185	0.474	0.628
20,894	0.114	0.182	0.408	0.592

[a]Mean values of one male and one female per rat/mouse.
[b]In mg [^{14}C]ETBE equivalents per gram blood.
[c]In mg [^{14}C]ETBE equivalents.

Sources: Sun and Beskitt (1995a) and Sun and Beskitt (1995b).

No studies investigating dermal absorption of ETBE were identified, but because dermal absorption of homologous organic substances is thought to be a function of the octanol:water partition coefficient, ETBE might be assumed to penetrate rat skin relatively well. For humans, Potts RO (1992) have proposed an equation to calculate the dermal permeability coefficient, K_p:

$$\log K_p \text{ (cm/sec)} = -6.3 + 0.71 \times \log K_{ow} - 0.0061 \times (\text{molecular weight}) \quad \text{(B-1)}$$

Using the log K_{ow} [identified as K_{oct} in Potts RO (1992)] values for ETBE (0.95–2.2) (Drogos and Diaz, 2001) and converting cm/second values to cm/hour, the estimated K_p values are 0.0020–0.016 cm/hour for ETBE.

Table B-2. Plasma radioactivity after a single oral or intravenous dose of [^{14}C]ETBE to male Crl:CD(SD) rats

Time (hours)	Radioactive Concentration (ng eq of ETBE/mL)			
	Oral		Intravenous	
Dose administered	5 mg/kg	50 mg/kg	400 mg/kg	5 mg/kg
0.083	-	-	-	918 ± 188[a]
0.25	-	-	-	822 ± 165
0.5	-	-	-	914 ± 156
1	2,150 ± 281	11,100 ± 1,007	47,000 ± 11,900	907 ± 143
2	2,400 ± 151	12,100 ± 883	58,200 ± 7,340	923 ± 158
4	2,620 ± 109	14,800 ± 659	73,300 ± 6,800	929 ± 193
6	2,750 ± 146	18,700 ± 1,550	82,900 ± 12,500	981 ± 216
8	2,760 ± 265	19,900 ± 2,430	89,900 ± 16,300	973 ± 196
10	2,710 ± 303	21,400± 2,830	87,300 ± 15,300	943 ± 203
12	2,660 ± 426	22,000± 3,060	78,500 ± 18,100	862 ± 205
24	1,330 ± 419	10,800 ± 2,820	17,200 ± 6,460	383 ± 184
32	1,170 ± 424	9,310 ± 2,510	13,100 ± 6,580	334 ± 190
48	443 ± 271	3,900 ± 1,480	3,180 ± 1,480	144 ± 93.8
72	204 ± 165	1,660 ± 845	2,000 ± 1,820	65.2 ± 34.0
96	81.3 ± 70.3	792 ± 338	N.D.	31.3 ± 11.4
120	35.9 ± 44.0	385 ± 110	N.D.	16.1 ± 3.8
144	19.6 ± 26.0	179 ± 129	N.D.	11.9 ± 13.8
168	N.D.	85.4 ± 103	N.D.	N.D.

[a]Mean ± standard deviation; *n* = 4.
- = not measured, N.D. = not detected.
Source: JPEC (2008e).

ETBE is moderately absorbed following inhalation exposure in rats and humans, and blood levels of ETBE approached—but did not reach—steady-state concentrations within 2 hours. Nihlén et al. (1998) calculated the net respiratory uptake of ETBE in humans to be 26%. The AUC for the concentration-time curve was linearly related to the ETBE exposure level, suggesting linear kinetics up to 209 mg/m^3. The JPEC studies (JPEC, 2008e, f) demonstrated that ETBE is readily absorbed following oral exposure in rats with >90% of a single dose (5–400 mg/kg-day) or repeated doses (5 mg/kg-day) estimated to be absorbed. In the repeated-dose study, peak plasma [^{14}C]ETBE levels were reached 6 hours after the first dose and 10 hours after the final (14th) dose, and the maximum

plasma concentration reached a steady state on Day 5. No data are available on dermal absorption of ETBE.

B.1.2. Distribution

There are no in vivo data on the tissue distribution of ETBE in humans. Nihlén et al. (1995) measured the partitioning of ETBE and *tert*-butanol in air into human blood from 10 donors (5 males, 5 females), saline, or oil inside of sealed vials. Also, human tissue-to-blood partitioning coefficients were estimated in brain, fat, liver, kidney, lung, and muscle based upon their relative water and fat contents. Kaneko et al. (2000) conducted a similar series of in vitro studies to measure the partitioning of ETBE and *tert*-butanol in air to various rat tissues (5 male Wistar rats), including blood, brain, fat, liver, kidney, lung, muscle, and testes. The blood:air partition coefficients for ETBE were much lower than for *tert*-butanol. Both studies reported efficient uptake of these substances from air into blood, with blood:air partition coefficients of 11.7 and 11.6 for ETBE and 462 and 531 for *tert*-butanol in humans and rats, respectively. Nihlén et al. (1995) also estimated oil:water partition (log K_{ow}) coefficients and obtained values of 0.278 for *tert*-butanol and 22.7 for ETBE. These values have a similar ranking, but are not identical, to those listed in a report by Drogos and Diaz (2001) (namely, 0.35 for *tert*-butanol and 1.48–1.74 for ETBE). Nihlén et al. (1995) used the coefficients of tissue:air and blood:air partition coefficients to calculate human tissue:blood partition coefficients. These values are listed in Table B-3.

Table B-3. Blood:tissue partition coefficients for ETBE and *tert*-butanol

Partition Coefficient	*tert*-Butanol	ETBE
Blood:air	462	11.7
Brain:blood	1.05	2.34
Muscle:blood	1.06	1.78
Fat:blood	0.646	11.6
Lung:blood	1.02	0.835
Kidney:blood	1.06	1.42
Liver:blood	1.05	1.44

Source: Nihlén et al. (1998).

The JPEC (2008e, f) examined the distribution of radioactivity in 7-week-old Crl:CD(SD) male rats (4/dose group) following either a single oral dose of 5 or 400 mg/kg [^{14}C]ETBE via gavage or a repeated dose of 5 mg/kg-day for 7 or 14 days. Tissue samples were collected at 8, 24, 72, and 168 hours after a single dose; 8 and 24 hours after 7 days of repeated dosing; and 8, 24, 72, and 168 hours after 14 days of repeated dosing. Although the highest radioactivity levels were generally

detected in plasma, [^{14}C]ETBE was also detected in all tissues examined (brain, peripheral nerve, eyes, submaxillary gland, thyroid gland, thymus, lungs, kidneys, heart, liver, adrenal glands, spleen, pancreas, bone marrow, mesenteric lymph node, prostate, epididymis, testes, muscle, skin, adipose tissue, stomach, large intestines, and small intestines). Tissue concentrations after a single 400 mg/kg dose of [^{14}C]ETBE were higher than after a single 5 mg/kg dose; however, the percentage distribution of radioactivity in tissues was lower with the higher dose. Tissue radioactivity levels reached a maximum at 8 hours after a single dose of either 5 or 400 mg/kg [^{14}C]ETBE and rapidly decreased by 72 hours. In the repeated dosing study, the radioactivity was the same 8 hours after the seventh administration when compared to 8 hours after the 14th administration. The levels of [^{14}C]ETBE in the tissues declined steadily from 8 hours through 168 hours after the last exposure with the exception of adipose tissue. In adipose tissue, there was a rapid decline between 8 and 24 hours, but the levels remained consistent between the 24- and 168-hour time points. The percentage radioactivity found in red blood cells was estimated to be 20−27% within 72 hours of administration, and little was found to be bound to plasma proteins.

Sun and Beskitt (1995a) and Sun and Beskitt (1995b) studied the distribution of radiolabel derived from [^{14}C]ETBE in rats and mice, respectively. Animals were subjected to a single nose-only inhalation exposure to [^{14}C]ETBE for 6 hours. Immediately upon cessation of exposure, radiolabel was quantified in the blood and kidneys of two rats and in the blood and liver of two mice. Results in Table B-1 (shown earlier) demonstrate the distribution of radiolabel expressed as mg equivalents of ETBE from blood to kidney (rats) and liver (mice) during exposure. The concentration of radiolabel in rat kidney and mouse liver parallels the concentration of radiolabel in blood of the respective species, leading to an expectation of the proportionate distribution of ^{14}C from ETBE to rat kidney and mouse liver up to exposure concentrations of 7,313 mg/m^3 in rats and 10,447 mg/m^3 in mice. Because radiolabel levels do not distinguish between parent ETBE and its metabolites, these results need to be interpreted with some caution, as the distribution of individual chemical species may differ.

Leavens and Borghoff (2009) evaluated the distribution of the structurally similar compound, MTBE, and the common metabolite, *tert*-butanol, after inhalation exposure to those two compounds, specifically in the brain, kidney, and liver of male and female rats and testes of male rats. Concentrations of MTBE and *tert*-butanol were similar in the female rat brain, kidney, and liver, and concentrations in the male rat brain, liver, and testes, were similar for exposure level and across time points, indicating an even distribution of MTBE and *tert*-butanol in those tissues/sexes. While total concentrations of MTBE and *tert*-butanol were higher in male rat kidneys than other tissues, consistent with the mechanism of binding to α_{2u}-globulin for those two compounds (Leavens and Borghoff, 2009), the overall observations are consistent with the conclusion that unbound ETBE and *tert*-butanol distribute rapidly and evenly through the body, although additional accumulation of material bound to α_{2u}-globulin occurs for *tert*-butanol and may occur for ETBE in the male rat kidney.

B.1.3. Metabolism

The metabolism of ETBE has been studied in rats and humans using both in vivo and in vitro methods. A schematic of the proposed metabolism of ETBE is presented in Figure B-1. Based on elucidated structures of urinary metabolites from rats that were exposed to ETBE by inhalation, ETBE is initially metabolized by cytochrome P450 (CYP) enzymes via oxidative deethylation by the addition of a hydroxyl group to the α-carbon of the ethyl ether group (Bernauer et al., 1998). The resulting hemiacetal is unstable and decomposes spontaneously into *tert*-butanol and acetaldehyde. In human liver microsome preparations, this step is catalyzed mainly by CYP2A6, with some contribution from CYP3A4 and CYP2B6 and possible contribution from CYP2E1 (Le Gal et al., 2001; Hong et al., 1999a). Using data from rat hepatic microsome preparations, Turini et al. (1998) suggested that CYP2B1 is the primary enzyme responsible for this step in rats but that CYP2A1 may also have an important role. Acetaldehyde is oxidized to acetic acid by aldehyde dehydrogenase enzymes (some of which are polymorphically expressed) and eventually to carbon dioxide (CO_2). *tert*-Butanol can be sulfated, glucuronidated, and excreted into urine, or it can undergo further oxidation by the CYP enzymes (but not by alcohol dehydrogenases) to form 2-methyl-1,2-propane diol (MPD), and 2-hydroxyisobutyrate (HIBA), acetone, and formaldehyde (Bernauer et al., 1998). It should be noted that these metabolites have been identified in studies using liver preparations from human or rat studies using ETBE, MTBE, or *tert*-butanol (Bernauer et al., 1998; Cederbaum and Cohen, 1980b); however, all the enzymes that perform these metabolic steps have not been fully described. Excretion studies indicate that final metabolism to CO_2 plays only a minor role (see Section B.1.4.).

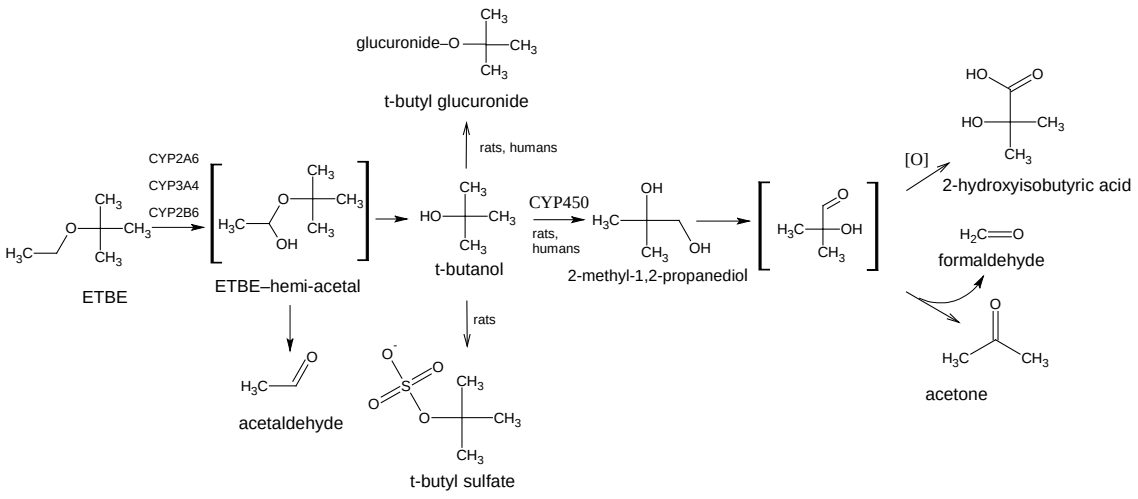


Figure B-1. Proposed metabolism of ETBE.

Source: Adapted from Dekant et al. (2001), NSF International (2003), ATSDR (1996), Bernauer et al. (1998), Amberg et al. (1999), and Cederbaum and Cohen (1980a).

Zhang et al. (1997) used computer models to predict the metabolites of ETBE. The metabolism model correctly predicted cleavage into *tert*-butanol and acetaldehyde and that *tert*-butanol would undergo glucuronidation and sulfation. For the further metabolism of *tert*-butanol, however, the computer model predicted reductive steps leading to metabolites that have not been identified in vivo or in vitro. The software did not predict the formation of MPD or HIBA, which have been found in vivo.

Metabolism in Humans

Metabolism of ETBE in Humans In Vivo

Nihlén et al. (1998) exposed eight healthy male volunteers (average age: 29 years) to 0, 20.9, 104, or 209 mg/m^3 ETBE by inhalation for 2 hours. Profiles of ETBE, *tert*-butanol, and acetone were established for blood throughout exposure and for up to 22 hours thereafter. The blood profiles of parent compounds and metabolites were similar at all three exposure levels and reflected exposure concentrations, as judged by linear increases in concentration-time AUC values calculated by the authors (only reported graphically). Acetone levels were highly variable before, during, and after the exposure period, and the variation could likely be due to variations in endogenous acetone production due to diet or physical activity.

The concentration of ETBE in blood rose sharply during the first 30 minutes of exposure and kept rising at a lower rate until the end of exposure to reach peak concentrations of about 10, 5, and 1 µM at 209, 104, and 20.9 mg/m^3, respectively. By 6 hours, ETBE concentrations had fallen to low levels even after exposure to 209 mg/m^3. The blood concentration of *tert*-butanol continued to rise for the full 2-hour exposure period, with peak values of about 12 and 7 µM at 209 and 104 mg/m^3, respectively. Blood concentrations leveled off for 3–4 hours and then began a slow decline to less than one-half maximum levels by 24 hours (*tert*-butanol levels could not be determined following 20.9 mg/m^3 exposure). Acetone blood levels began to increase after about 1 hour of exposure and continued to increase after the end of exposure (high dose) or leveled off for about 1½ hours after exposure (lower doses and controls). Blood acetone levels fell rapidly during the next half hour but remained slightly above normal for the exposed volunteers until 4 hours after exposure when measurements were terminated.

Amberg et al. (2000) exposed six volunteers (three males and three females; average age: 28 ± 2 years) to 18.8 and 170 mg/m^3 of ETBE. The exposures 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. Blood was drawn immediately, at 4 or 6 hours after exposure, and thereafter every 6 hours for 48 hours. Levels of parent ETBE and its primary metabolite, *tert*-butanol, were determined in blood and urine. In urine, two further metabolites of *tert*-butanol, MPD and HIBA, were also assayed.

At 170 mg/m^3, the mean peak blood concentration of ETBE was 12.1 ± 4.0 µM, although that for *tert*-butanol was 13.9 ± 2.2 µM. The corresponding values at 18.8 mg/m^3 were 1.3 ± 0.7 and

1.8 ± 0.2 μM, respectively. The time courses of metabolite appearance in urine after 170 mg/m^3 and 18.8 mg/m^3 were similar, but relative urinary levels of metabolites after 18.8 mg/m^3 differed from those after 170 mg/m^3. Using parent ETBE as the reference, molar ratios for total urinary excretion (ETBE:*tert*-butanol:MPD:HIBA) were 1:25:107:580 after 170 mg/m^3 and 1:17:45:435 after 18.8 mg/m^3. Individual variations were large, but the authors did not report any gender differences in the metabolism of ETBE based on data from only three subjects of each sex.

In Vitro Metabolism of ETBE Using Human Enzyme Preparations

The metabolism of ETBE has been studied in vitro using microsomal protein derived from human liver and from genetically engineered cells expressing individual human CYP isozymes. Hong et al. (1997b) coexpressed human *CYP2A6* or *CYP2E1* with human CYP reductase in insect SF9 cells. In this heterologous expression system, in the presence of 1 mM ETBE, *tert*-butanol was formed at rates of 13.6 nmol/min-nmol CYP2A6 and 0.8 nmol/min-nmol CYP2E1, indicating a greater capacity for ETBE metabolism by CYP2A6 than by CYP2E1 at high (e.g., 1 mM) concentrations of ETBE.

Hong et al. (1999a) obtained hepatic microsomal protein preparations from 15 human donor liver microsomal samples and used them to evaluate the contributions of several CYP enzymes to ETBE metabolism. The 15 samples displayed very large interindividual variations in metabolic activities towards ETBE ranging from 179 to 3,130 pmol/minute-mg protein. Michaelis constant (K_m) values, estimated in three human liver microsomal samples using MTBE, ranged from 28 to 89 μM, with maximum substrate turnover velocity (V_{max}) values ranging from 215 to 783 pmol/minute-mg protein. The V_{max}/K_m ratios, however, varied only between 7.7 and 8.8. Following an evaluation of the activities of multiple different CYP forms in the 15 donor samples, it was demonstrated that the metabolism of ETBE was highly correlated with certain CYP forms. The highest degree of correlation was found for CYP2A6, which also displayed the highest metabolic capacity for ETBE.

As part of CYP inhibition studies in the same paper, human liver microsomes were coincubated with ETBE in the presence of chemical inhibitors or specific antibodies against either CYP2A6 or CPY2E1. For chemical inhibition, coumarin was added to the liver microsomes prior to initiation of the reaction. For antibody inhibition, monoclonal antibodies against human CPY2A6 or CYP2E1 were preincubated with liver microsomes prior to incubation with the rest of the reaction mixture. Methanol alone caused approximately 20% inhibition of the metabolism of ETBE, and coumarin, a CYP2A6 substrate, caused a significant dose-dependent inhibition of ETBE metabolism which reached a maximal inhibition of 99% at 100-μM coumarin. Antibody against CYP2A6 inhibited metabolism by greater than 75%, but there was no inhibition by the antibody against CYP2E1.

In the same paper, several specific human CYPs were expressed into human β-lymphoblastoid cells which were used to evaluate ETBE metabolism. Based on the ETBE metabolizing activities in the 15 human liver microsomes and the enzyme activity profiles towards

known CYP specific substrates, correlation coefficients (ranging from 0.94 for CYP2A6 to 0 for CYP2D6) were calculated for each CYP enzyme. The correlation ranking for ETBE metabolism by nine human CYP isozymes was as follows: 2A6 > 3A4 ≈ 2B6 ≈ 3A4/5 >> 2C9 > 2E1 ≈ 2C19 >> 1A2 ≈ 2D6. The reported direct enzyme activities towards ETBE by the heterologous expression systems (in pmol *tert*-butanol formed per minute per pmol CYP enzyme) were 1.61 for CYP2A6; 0.34 for CYP2E1; 0.18 for CYP2B6; and 0.13 for CYP1A2. CYPs 1B1, 2C8, 2C9, 2C19, and 2D6 were not investigated. CYP3A4 and 1A1 did not metabolize ETBE. The authors concluded that CYP2A6 is the major enzyme responsible for the oxidative metabolism of ETBE in human livers. Furthermore, they concluded that the results of the correlation analysis and antibody inhibition study strongly suggest that CYP2E1 is not a major enzyme responsible for metabolism of ETBE. Le Gal et al. (2001) used similar human cytochrome preparations as Hong et al. (1999a) (i.e., from human donors) or genetically modified human β-lymphoblastoid cell lines transfected with CYP2A6, CYP2B6, CYP3A4, or CYP2E1 and human CYP reductase to elucidate the metabolism of ETBE, MTBE, and TAME. They identified acetaldehyde and *tert*-butanol as primary metabolites from ETBE.

Metabolism in Animals

Metabolism of ETBE in Animals In Vivo

Bernauer et al. (1998) studied the metabolism and excretion of [^{13}C]ETBE and *tert*-butanol in rats. F344 rats, 2/sex, were exposed via inhalation to 2,000 ppm (8,400 mg/m^3) ETBE; three male F344 rats received 250 mg/kg *tert*-butanol by gavage. Urine was collected for 48 hours. The excretion profile for ETBE metabolites was MPD > HIBA > *tert*-butanol-sulfate > *tert*-butanol-glucuronide. Oral administration of *tert*-butanol produced a similar metabolite profile, with HIBA > *tert*-butanol-sulfate > MPD >> *tert*-butanol-glucuronide ≈ *tert*-butanol. *tert*-Butanol could not be detected in urine following inhalation exposure to ETBE. Traces of acetone were also detected in urine. Amberg et al. (2000) exposed F344 NH rats, 5/sex/dose, to ETBE in the same exposure chamber described earlier for the human volunteers. Urine was collected for 72 hours following exposure. Blood samples were drawn from the tail vein every 6 hours up to 48 hours. Peak blood levels of ETBE and *tert*-butanol were 5.3 ± 1.2 and 21.7 ± 4.9 µM at 170 mg/m^3 and 1.0 ± 0.7 and 5.7 ± 0.8 µM at 18.8 mg/m^3, respectively. Peak levels of *tert*-butanol were higher in rats than in humans. Similar to humans, rats excreted mostly HIBA in urine, followed by MPD and *tert*-butanol. The molar ratios for total urinary excretion of *tert*-butanol:MPD:HIBA were 1:2.3:15 after exposure to 170 mg/m^3 and 1:1.5:11 after exposure to 18.8 mg/m^3. Parent ETBE was not identified in rat urine in this study.

In a review covering mostly their own work on fuel oxygenate metabolism, Dekant et al. (2001) focused on aspects of ETBE metabolism which were considered quantitatively similar in humans and rats, with no sex-dependent differences and no likely accumulation of metabolites or parent compound. They reported that at a high exposure level (8,400 mg/m^3 ETBE), rats predominantly excreted the glucuronide of *tert*-butanol in urine; however at low exposure levels

(16.7 mg/m^3 or 167.1 mg/m^3 ETBE), the relative concentration of *tert*-butanol to the received dose was much smaller. This seems to indicate that at high exposure levels, the normally rapid metabolism of *tert*-butanol to MPD and HIBA became saturated, forcing more of the *tert*-butanol through the glucuronidation pathway. The apparent final metabolite of ETBE was HIBA which can undergo further metabolism to acetone. The latter process appeared to play a minor role in the overall metabolism of ETBE. Dekant et al. (2001) also noted that many metabolites of the fuel oxygenate ethers, such as formaldehyde, acetaldehyde, *tert*-butanol, HIBA, or acetone, occur naturally in normal mammalian physiology, providing a highly variable background that needs to be accounted for in metabolic experiments.

The JPEC (2008e, f) measured metabolite distribution in the plasma and urine of 7-week old Crl:CD(SD) male rats (4/dose group) following either a single oral dose of 5 or 400 mg/kg [^{14}C]ETBE via gavage or a repeated dose of 5 mg/kg-day for 7 or 14 days. Metabolites were measured in the plasma 8 hours after single or repeated dosing. Metabolites were measured in urine collected on Days 1, 7, and 14 after repeated dosing or during a 24-hour period after administration of the single dose. The number of doses did not appear to affect the metabolic pattern. The study authors determined the identities of five metabolites, and the results in plasma and urine are summarized in Table B-4 and Table B-5, respectively. When combined with what is known of the metabolic pathway for ETBE, these data indicate that ETBE is efficiently metabolized to *tert*-butanol, which is then metabolized to *tert*-butanol glucuronide, 2-methyl-1,2-propanediol, and finally to 2-hydroxyisobutyrate.

Although Sun and Beskitt (1995a) did not identify the radiolabel eliminated, their investigations do yield information pertinent to determining whether metabolic saturation might occur under bioassay conditions. In their single-exposure protocol (see Section 0), rats and mice were exposed via inhalation to ETBE. These investigators reported the fraction of absorbed dose that was eliminated in urine and feces, as expired volatiles, and as expired CO_2 from one rat and one mouse. At inhaled concentrations between 4,180 and 7,310 mg/m^3 a shift in the primary route of elimination was observed, as demonstrated by a marked decrease in the fraction of radiolabel eliminated in urine and a marked increase in the fraction of radiolabel eliminated as volatiles in expired air, and (in rats) a doubling of the fraction eliminated as exhaled CO_2. Given the different solubilities, molecular size and other characteristics of ETBE and its multiple metabolites, it is envisioned that this shift in the elimination pattern of radiolabel is indicative of a shift in metabolism at these exposure levels.

Considering the potential shift in metabolic pattern relative to the pattern of toxicity can be informative, especially related to species and dose extrapolation. These data might still be considered preliminary because they are from one animal of each species, have not been replicated by other authors, and the radiolabel has not been speciated as to chemical form. The unfortunate limitation of the application of the PBPK model for human inhalation precludes its combination with rat PBPK models to complete species extrapolation. The inhalation toxicity study by Saito et al.

(2013), however, demonstrated an increased incidence of urothelial hyperplasia at an exposure concentration of 6,270 mg/m^3 and higher, and an increased incidence of hepatocellular adenoma or carcinoma only at an exposure concentration of 20,900 mg/m^3. Additional data are required to determine whether increases in incidence could be related to pharmacokinetic effects (e.g., metabolic saturation).

Table B-4. Unchanged ETBE and its metabolites in plasma 8 hours after a single oral dose or repeated (7 or 14) daily oral dosing of [^{14}C]ETBE to male Crl:CD(SD) rats

Compound	Metabolite	Percentage of Dose			
		1 Dose		7 Doses	14 Doses
		5 mg/kg-day	400 mg/kg-day	5 mg/kg-day	5 mg/kg-day
Unchanged ETBE	ETBE	N.D.	N.D.	N.D.	N.D.
P-1	2-hydroxyisobutyrate	75.4 ± 8.1[a]	35.7 ± 2.5	71.4 ± 4.7	69.8 ± 7.3
P-2	*tert*-butanol glucuronide	N.D.	N.D.	N.D.	N.D.
P-3	Not enough to determine	N.D.	N.D.	N.D.	N.D.
P-4	2-methyl-1,2-propanediol	9.7 ± 2.4	9.328 ± 0.9	9.1 ± 0.8	8.1 ± 1.4
P-5	*tert*-butanol	12.9 ± 3.1	55.0 ± 2.9	18.2 ± 3.8	22.2 ± 6.0

[a]Mean ± standard deviation; *n* = 4.
N.D. = not detected.

Source: JPEC (2008e, f) unpublished reports.

Table B-5. Unchanged ETBE and its metabolites in the urine (measured 0–24 hours) after a single oral dose or repeated (7 or 14) daily oral dosing of [^{14}C]ETBE to male Crl:CD(SD) rats

Compound	Metabolite	Percentage of Dose			
		1 Dose		7 Doses	14 Doses
		5 mg/kg-day	400 mg/kg-day	5 mg/kg-day	5 mg/kg-day
Unchanged ETBE	ETBE	0.7 ± 0.5[a]	N.D.	0.9 ± 0.6	1.4 ± 0.4
P-1	2-hydroxyisobutyrate	53.0 ± 3.4	55.4 ± 4.7	58.9 ± 4.2	56.0 ± 5.2
P-2	*tert*-butanol glucuronide	29.2 ± 3.0	25.9 ± 4.6	22.8 ± 3.2	25.2 ± 5.8
P-3	Not enough to determine	2.5 ± 0.2	1.7 ± 0.4	2.2 ± 0.3	1.7 ± 0.4
P-4	2-methyl-1,2-propanediol	13.1 ± 0.6	13.3 ± 2.5	13.4 ± 1.5	13.9 ± 2.3
P-5	*tert*-butanol	1.5 ± 0.5	3.7 ± 0.6	1.9 ± 0.2	1.8 ± 0.0

[a]Mean ± standard deviation; *n* = 4.
N.D. = not detected.

Source: JPEC (2008e, f) unpublished reports.

Borghoff and Asgharian (1996) evaluated the disposition of a ^{14}C radiolabel in F344 rats and CD-1 mice after whole-body and nose-only inhalation exposure to 500, 1,750, or 5,000 ppm [^{14}C]ETBE. Besides recovery of total radioactivity in urine, feces, and expired air, air and urine samples were analyzed for ETBE and *tert*-butanol. Urine samples were also analyzed for *tert*-butanol metabolites, HBA and MPD. Results obtained after both a single 6-hour exposure or after 13 days of pre-exposure to 0, 500, or 5,000 ppm ETBE indicated dose- and pre-exposure-related shifts in the form and route, likely due to metabolic factors. Elimination shifted from being primarily in the urine after 500 ppm exposure to primarily by exhalation at 5,000 ppm in naïve rats, indicating a saturation of metabolism of ETBE to TBA. This shift was greater in female rats than in males. However, in rats pre-exposed to 5,000 ppm ETBE for 13 days, most of the excretion was in the urine even at 5,000 ppm. Rats pre-exposed to 500 ppm ETBE also showed a shift from exhalation to urinary excretion in comparison to naïve rats, but to a smaller degree than elicited by 5,000 ppm pre-exposure. The changes in elimination after pre-exposure indicated an induction of the metabolism of ETBE to *tert*-butanol. As with rats, the fraction of radiolabel in exhaled volatiles in mice increased with exposure level, while the fraction excreted in urine decreased. The exhalation pattern observed in rats showed levels of ETBE falling ~90% in the first 8 hours postexposure, while levels of TBA exhaled actually rose between 0 and 3 hours postexposure and then fell more slowly between 3 and 16 hours, particularly after 5,000 ppm ETBE exposure. The increase in TBA between 0 and 3 hours postexposure can be explained by the continued metabolism of ETBE during that period. The slower decline after 3 hours can be explained as a

result of the generally slower clearance of TBA, which is saturated by the higher ETBE exposure levels.

Metabolism of ETBE in Animal Tissues In Vitro

Using microsomal protein isolated from the olfactory epithelium from male Sprague-Dawley rats, Hong et al. (1997a) measured ETBE metabolism as the formation of *tert*-butanol (TBA). They found that metabolism occurred only in microsomal protein (not in cytosol) and only in the presence of an NADPH- (nicotinamide adenine dinucleotide phosphate) regenerating system. The metabolic activity was inhibited by 80% after treating the microsomal preparation with carbon monoxide and by 87% in the presence of coumarin (a CYP2A6 inhibitor), which indicates CYP involvement. Using an in vitro concentration of 1 mM ETBE, metabolic activity could not be detected in microsomal protein from the olfactory bulb, lungs or kidneys. Activity toward ETBE was 8.78, 0.95 and 0.24 nmol/minute/mg microsomal protein in olfactory mucosa, respiratory mucosa and liver, respectively. In olfactory mucosa, the authors reported a K_m value of 125 µM for ETBE.

Hong et al. (1999b) used hepatic microsomal protein derived from *Cyp2e1* knockout mice to investigate whether this enzyme plays a major role in ETBE metabolism. They compared the metabolizing activity of liver microsomes (incubated for 30 minutes at 37°C and with 0.1 mM ETBE) between the *Cyp2e1* knockout mice and their parental lineage strains using four or five female mice (7 weeks of age) per group. The ETBE-metabolizing activities were not significantly different between the *Cyp2e1* knockout strain (0.51 ± 0.24 nmol/minute-mg protein) compared to that observed in the *Cyp2e1* wild-type parental strains (0.70 ± 0.12 for C57BL/6N mice, and 0.66 ± 0.14 for 129/Sv mice). Therefore, microsomal protein from mice that did not express any CYP2E1 did not differ from microsomal protein derived from wild-type animals in their ability to metabolize ETBE in vitro, suggesting that CYP2E1 might contribute only little to ETBE metabolism in vivo. Furthermore, these authors evaluated potential sex- and age-dependent differences for the metabolism of 1 mM concentrations of ETBE by hepatic microsomal protein. Although activities in female knockout mice were approximately 60% of those in male knockout mice, the difference did not reach the level of statistical significance. Finally, observed rates of ETBE metabolism (approximately 0.5 to 0.9 nmol/min/mg microsomal protein) did not seem to differ when assayed at 0.1 or 1 mM, indicating that for mouse hepatic microsomal ETBE metabolism, saturation can occur at concentrations no higher than 0.1 mM in vitro, and that K_m values would be expected to be lower than 0.1 mM in vitro.

Turini et al. (1998) investigated the effects of ETBE exposure on P450 content and activities, and characteristics of ETBE metabolism in hepatic microsomal protein from male Sprague-Dawley rats in an attempt to elucidate the role of CYP2E1 in ETBE metabolism. Administration of ETBE at 200 or 400 mg/kg for 4 days did not alter hepatic CYP profiles, but the administration of 2 mL ETBE/kg resulted in significant increases of metabolic activities toward substrates characteristic for CYP2B and CYP2E1 (p-NPH) forms, but not of activities catalyzed by

CYP3A or 1A forms. Studies of ETBE metabolism were based on high performance liquid chromatography (HPLC) detection of the acetaldehyde ETBE metabolite. Induction of CYP2B forms in vivo via the administration of phenobarbital slightly reduced the K_m value and produced a significant, approximate threefold increase in V_{max}; in these preparations, chemical inhibition of CYP2B forms resulted in significant inhibition of ETBE metabolism. Studies with CYP enzymes purified from rats confirmed metabolic competency of several CYP forms, with the activity of purified rat CYP forms 2B1 > 2E1 > 1A1 > 2C11. Chemical inhibition of CYP2E1 did not reduce ETBE metabolic activity; CYP2A forms were not evaluated. In microsomal preparations from rats treated with phenobarbital (a CYP2B inducer), incubation with chemical inhibitors of CYP2B forms produced a significant decrease in ETBE metabolism. Pretreatment of rats with chemicals known as inducers of CYP2E1, CYP3A and CYP1A forms did not result in significant changes in K_m or V_{max} values for ETBE metabolism, measured in vitro. The results of these investigations indicate that, in rats, CYP2E1 is apparently minimally involved in ETBE metabolism, and that under some conditions, CYP2B forms can contribute to ETBE metabolism. The role of CYP2A forms was not studied in this investigation. This study also investigated the kinetic constants for ETBE metabolism in control rat hepatic microsomal protein, indicating a K_m value of 6.3 mM and a V_{max} value of 0.93 nmol/min/mg microsomal protein. When compared to the kinetic constants indicated by the results of Hong et al. (1999b), it can be expected that the rate of ETBE metabolism at in vitro at concentrations below 1 mM would be higher in mouse than in rat microsomal preparations.

The enzymes that metabolize *tert*-butanol to MPD, HIBA, and even acetone, have not been fully characterized; however, *tert*-butanol is not subject to metabolism by alcohol dehydrogenases (Dekant et al., 2001).

B.1.4. Elimination

Elimination in Humans

Nihlén et al. (1998) exposed eight healthy male volunteers (average age, 29 years) to 20.9, 104, and 209 mg/m^3 ETBE by inhalation for 2 hours. ETBE, and two metabolites (*tert*-butanol and acetone) were measured in urine for up to 22 hours after exposure. The blood profiles of the parent compound and metabolites were similar at all three exposure levels and reflected exposure concentrations. The authors estimated the inhaled amount of ETBE in the volunteers to be 0.58, 2.9, and 5.8 mmol for the 20.9-, 104-, and 209-mg/m^3 exposure levels, respectively. Based on blood AUC values for ETBE and metabolites, the authors calculated that respiratory uptake was 32–34% in humans, and net uptake (which excludes ETBE exhaled during exposure) was calculated to be 26% of the dose at all three exposure levels. During the 24 hours following the start of inhalation exposure, respiratory expiration was calculated at 45–50% of the inhaled ETBE (respiratory uptake), and net respiratory expiration was 31% (of the net respiratory uptake), of which *tert*-butanol accounted for only 1.4–3.8%. Urinary excretion of parent ETBE (as percentage of the respiratory uptake of ETBE) accounted for even less: 0.12, 0.061, and 0.056% after the exposures to

20.9, 104, and 209 mg/m^3, respectively. The authors identified four phases of elimination of ETBE from blood, with half-lives of about 2 and 20 minutes and 1.7 and 28 hours. Only one phase for elimination of *tert*-butanol from blood was identified with a half-life of 12 hours compared to 10 hours in another study with volunteers (Johanson et al., 1995). In urine, ETBE displayed two phases of elimination, with half-lives of about 8 minutes and 8.6 hours. The half-life of *tert*-butanol in urine was determined to be 8 hours (Johanson et al., 1995).

ETBE displayed a multiphasic elimination from blood. The first phase likely indicates uptake into highly perfused tissues. The other phases could indicate uptake into less-perfused tissues and fat, and metabolism events. The apparent total body clearance of ETBE (based on the net respiratory uptake) was 0.57 L/hour-kg (average of the three exposure levels). The metabolic clearance was calculated as 0.39 L/hour-kg and the exhalation clearance as 0.35 L/hour-kg. These authors reported that the kinetics of ETBE in humans was linear over the range of concentrations studied (Nihlén et al., 1998).

In the study by Amberg et al. (2000) described earlier (see Section 0), two elimination half-lives were found for ETBE (1.1 ± 0.1 and 6.2 ± 3.3 hours) at the high exposure concentration (170 mg/m^3) although *tert*-butanol displayed only one half-life (9.8 ± 1.4 hours). At the low exposure concentration (18.8 mg/m^3), only the short half-life for ETBE could be measured at 1.1 ± 0.2 hours, although that for *tert*-butanol was 8.2 ± 2.2 hours. The predominant urinary metabolite identified was HIBA, 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). Excretion of unchanged ETBE in urine was minimal. The time courses of urinary elimination after 170 and 18.8 mg/m^3 were similar, but relative urinary levels of HIBA after 18.8 mg/m^3 were higher, although those for MPD were lower, as compared to 170 mg/m^3. HIBA 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 170 and 18.8 mg/m^3, respectively, although *tert*-butanol peaked at 6 hours after both concentrations. The time to peak of the three metabolites reflected the sequence of their formation and interconversion as ETBE is metabolized. Interindividual variations were large, but the authors did not report gender differences in the toxicokinetics of ETBE. Amberg et al. (2000) calculated that 43 ± 12% of the 170 mg/m^3 dose and 50 ± 20% of the 18.8 mg/m^3 dose had been excreted in urine by 72 hours. Respiratory elimination was not monitored.

Elimination in Animals

Amberg et al. (2000) exposed F344 NH rats, 5/sex/dose, concurrent with the human volunteers in the same exposure chamber. Urine was collected for 72 hours following exposure. Similar to humans, rats excreted mostly HIBA in urine, followed by MPD and *tert*-butanol. Parent ETBE was not identified in rat urine. The half-life for *tert*-butanol in rat urine was 4.6 ± 1.4 hours at 170 mg/m^3 but could not be calculated at 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 HIBA. The authors concluded that rats

eliminated ETBE considerably faster than humans. Urinary excretion accounted for 53 ± 15 and 50 ± 30% of the estimated dose at 170- and 18.8-mg/m^3 exposures, respectively, with the remainder of the dose being eliminated via exhalation, as suggested by the authors.

Bernauer et al. (1998) studied the excretion of [^{13}C]ETBE and MTBE in rats. F344 rats, 2/sex, were exposed via inhalation to 8,400 mg/m^3 ETBE or 7,200 mg/m^3 MTBE for 6 hours, or 3 male F344 rats received 250 mg/kg *tert*-butanol by gavage. Urine was collected for 48 hours, and ETBE metabolite prevalence in urine was MPD > HIBA > *tert*-butanol-sulfate > *tert*-butanol-glucuronide. Oral administration of *tert*-butanol produced a similar metabolite profile, with relative amounts of HIBA > *tert*-butanol-sulfate > MPD >> *tert*-butanol-glucuronide ≈ *tert*-butanol.

Although there are several unpublished reports relevant to the elimination of ETBE following inhalation exposure, no additional peer-reviewed publications were identified. Unpublished reports have not gone through the public peer-review process and are of unknown quality. They are included here as additional information only.

Table B-6. Elimination of [^{14}C]ETBE-derived radioactivity from rats and mice within 96 hours following a single 6-hour inhalation exposure

Exposure Level (mg/m^3)	Volatile Organics[a]	Exhaled CO_2[a]	Urine[a]	Feces[a]	Total[b]
F344 Rat[c]					
2,090[d]	37 [28, 32]	1 [1.2, 1.3]	60 [59, 59]	2 [2.8, 1.0]	9.9 [16.1, 13.6]
3,130	36	1	62	2	17.5
4,180	42	1	56	2	22.1
7,310[d]	58 [41, 52]	2 [1.5, 1.7]	38 [53, 41]	3 [0.7, 0.5]	56.9 [45, 31]
10,400	52	2	45	2	56.2
20,900[d, e]	63 (51) [51, 64]	2 (1) [1.6, 2.0]	34 (44) [45, 30]	1 (3) [0.2, 0.2]	97.5 (116) [143, 94]
CD-1 Mouse[f]					
2,090[d]	10 [12.7, 26.8]	1 [1.2, 1.2]	74 [81.3, 67.2]	16 [3.2, 2.3]	6.38 [10.4, 6.8]
3,130	28	2	60	10	7.9
4,180	29	2	64	6	12.8
7,310[d]	42 [23, 36]	2 [2.2, 1.9]	46 [71, 61]	10 [1.1, 0.6]	13.7 [22.4, 17.3]
10,400	42	2	47	10	22.7
20,900[d,e]	44 (37) [40, 47]	5 (2) [2.9, 3.3]	39 (57) [53, 47]	12 (2) [0.6, 0.8]	18.9 (28) [37.1, 25.2]

[a]Percentage of total eliminated radioactivity; mean of one male and one female.
[b]In mg [^{14}C]ETBE equivalents.
[c]Sun and Beskitt (1995b);
[d]values in brackets: [males, females], nose-only exposures, elimination up to 48 hour Borghoff and Asgharian (1996);
[e]values in parentheses: Borghoff (1996); [f]Sun and Beskitt (1995b).

During 96 hours in metabolic cages, rats eliminated approximately 60% of the radioactivity in urine, approximately 38% was recovered as exhaled organic volatiles, and approximately 1% as exhaled CO_2. This pattern was maintained at an exposure concentration of 4,180 mg/m^3; above that, urinary excretion of radioactivity decreased to 34% of the recovered radioactivity, although exhalation of organic volatiles increased to 63%. A shift in the elimination profile of radiolabel was

seen at concentrations of 7,310 mg/m^3 and above, which remained fairly constant to the highest exposure of 20,900 mg/m^3. In this range of concentrations, approximately 39% of the eliminated radiolabel was found in urine, approximately 58% was exhaled as organic volatiles, and 2% was eliminated as exhaled CO_2.

A review of the data demonstrating the percentage of recovered radiolabel via various routes of elimination demonstrate, in the rat and mouse, a pattern indicative of metabolic saturation occurring at inhaled concentrations above 4,180 mg/m^3.

In rats, the time course of elimination indicated that exhalation of organic volatiles was essentially complete by 24 hours, although urinary excretion of ETBE-derived radioactivity displayed a broad peak at 12–48 hours. The bulk of each dose was eliminated within 48 hours after the end of exposure. At 20,900 mg/m^3, $^{14}CO_2$ exhalation and fecal excretion of radioactivity remained rather constant from 12 to 118 hours. In comparing the total radiolabel eliminated to the inhaled concentrations (see Table B-6), a proportionate relationship is observed in rats at all concentrations, but less than proportionate elimination of total radiolabel at the highest concentration in mice. The complete data set led the authors of the report to conclude that saturation of the inhalation absorptive processes might have occurred at concentrations of approximately 7,310 mg/m^3 (see Section 0) The findings of Sun and Beskitt (1995a) in mice at 20,900 mg/m^3 were essentially confirmed by Borghoff (1996) (unpublished report, a pilot study) and Borghoff and Asgharian (1996) (unpublished report, final study) which used the identical species, experimental protocol, materials, and methods but were conducted later at a different laboratory (see Table B-6).

Similarities between rats (Sun and Beskitt, 1995b) and mice (Sun and Beskitt, 1995a) are evident. Both species demonstrate similar elimination pathways and present evidence of saturation of metabolic pathways at concentrations lower than those which demonstrate saturation of absorptive pathways. Metabolic saturation (evidenced as a shift from urine as the predominant elimination pathway and an increase in the fraction of dose eliminated via exhalation) occurred in both species at concentrations approximating 7,310 mg/m^3. Noteworthy differences between the two species were that, in general, mice eliminated a smaller percentage of the dose in the form of volatile organics and a higher amount in urine, at least up to 4,180 mg/m^3 (see Table B-6) and excreted about five times as much [^{14}C]ETBE-derived radioactivity via feces than did rats. The total amounts of eliminated radioactivity (mg equivalents) were considerably higher in rats than in mice; however, the values in the respective columns of Table B-6 are not corrected for body weight. When normalized to body weight, it is apparent that mice absorbed a higher dose than rats; however, the total eliminated radioactivity at 20,900 mg/m^3 showed no further increase over the values at 10,450 mg/m^3, indicating that the absorptive capacities of mice had become saturated; however, this analysis conducted in rats does not indicate a saturation of absorptive capacities over the range of concentrations studied.

Borghoff (1996), in an unpublished report, conducted studies to establish experimental conditions for future bioassays of ETBE, based on the two studies previously conducted by Sun and Beskitt (1995a, b). The experimental protocol and materials were identical to the ones used by Sun and Beskitt (1995a, b); however, in this pilot study, only three male F344 rats and three male CD-1 mice were used per experiment, with the only one exposure level at 20,900 mg/m^3. Also, only blood was collected from the animals, while the whole carcasses were liquefied and assayed for retained radioactivity immediately after exposure and after the end of the animals' stay in metabolic cages. The carbon at "the central position of the *tert*-butyl group" was radiolabeled. Radioactive ETBE was obtained by mixing [^{14}C]ETBE with unlabeled material in the gas phase for a specific activity of 2.74 µCi/mmol. It was found that rats, when assayed immediately after exposure, had absorbed 2.57 ± 0.14 µCi radioactivity, although the balance of radioactivity after 96 hours in metabolic cages from other animals accounted for 3.17 ± 0.08 µCi (mean ± standard deviation [SD], n = 3). The authors could not make any suggestion as to the origin of this discrepancy. Absorbed doses in mice were 0.85 ± 0.08 µCi immediately after exposure and 0.77 ± 0.16 µCi for other mice placed in metabolism cages. Elimination values detected in these rats and mice are shown in parentheses in Table B-6; the percentage values shown in this table were based on the total body burden of the individual animals from which the elimination data were obtained, not on group means.

Mice had eliminated most of the dose within 12 hours after exposure, rats within 24 hours. Organic volatiles collected on charcoal filters were analyzed for ETBE and *tert*-butanol contents. Rats exhaled 22% of the absorbed ETBE within 1 hour after exposure, 12% during the following 2 hours, and only another 3% during the next 3 hours. *tert*-Butanol exhalation accounted for 1% of the total during the first hour, 3% during the following 2 hours, and 4% during the last 3 hours of the experimental period. Mice, on the other hand, exhaled 16% of the unmetabolized ETBE within 1 hour after exposure and 1% during the following 2 hours, with immeasurable amounts thereafter. *tert*-Butanol exhalation made up 6% of total during the first hour, 8% in the next 2 hours, and 4% during the final 3 hours. Elimination of ETBE, *tert*-butanol, HIBA, and MPD in urine were assayed. During 24 hours of collection, rats eliminated about 7 times as much *tert*-butanol as ETBE in urine; in mice, the ratio was >60. HIBA was detected in urine of both species but could not be quantified. MPD was not detected. These results could be interpreted as suggesting that mice metabolize, and hence, eliminate ETBE faster than rats.

A subsequent larger study by Borghoff and Asgharian (1996) (see previous details) essentially confirmed the results of the pilot study (Borghoff, 1996). F344 rats and CD-1 mice were exposed by inhalation to 500, 1,750, or 5,000 ppm [^{14}C]ETBE. Concentrations of ETBE and *tert*-butanol were measured in exhaled breath up to 16 hours postexposure. The exhalation pattern observed in rats showed levels of ETBE falling ~90% in the first 8 hours postexposure, while levels of TBA exhaled actually rose between 0 and 3 hours postexposure and then fell more slowly between 3 and 16 hours, particularly after 5,000 ppm ETBE exposure. The increase in TBA between 0 and 3 hours postexposure can be explained by the continued metabolism of ETBE during that

period. The slower decline after 3 hours can be explained as a result of the generally slower clearance of TBA, which is saturated by the higher ETBE exposure levels. Exhaled breath levels declined much more rapidly in mice than in rats.

Unpublished reports by the JPEC (2008e) determined that following oral exposure of 7-week-old Crl:CD(SD) male rats to [^{14}C]ETBE, the largest amount of radioactivity was recovered in expired air, followed by urinary excretion, with very little excretion occurring via the feces. With increasing dose, increasing proportions of radioactivity were found in expired air. The total radioactivity recovered by 168 hours after a single dose of 5 mg/kg [^{14}C]ETBE was 39.16% in the urine, 0.58% in the feces, and 58.32% in expired air, and, after a single dose of 400 mg/kg, 18.7% in the urine, 0.15% in the feces, and 78.2% in expired air. With repeated dosing, the recovery of radioactivity through excretion increased through day 6 when a steady state was achieved; however, the radioactivity level in the feces increased throughout the 14 days, but the level was too low to affect the total recovery. After 14 days, 36.3% of the administered dose was recovered in the urine, 2.33% was recovered in the feces, and 56.7% was recovered in expired air.

B.1.5. Physiologically Based Pharmacokinetic Models

Two physiologically based pharmacokinetic (PBPK) models have been developed specifically for the administration of ETBE in rats (Borghoff et al., 2016; Salazar et al., 2015). A detailed summary of these and other toxicokinetic models is provided in U.S. EPA (2017).The PBPK model described in Borghoff et al. (2016) and in U.S. EPA (2017) was applied to conduct route-to-route extrapolation based on an equivalent internal dose (the rate of ETBE metabolism in the liver). While the model includes a possible adjustment for induction of *tert*-butanol metabolism, this induction has only been observed in mice exposed directly to *tert*-butanol (McComb and Goldstein, 1979). Further, implementing metabolic induction does not allow for dependence on exposure or dose, nor for any de-induction that might occur during periods without exposure, such as weekends during 5 days/week exposures. Finally, because induction is expected to have an equal impact on oral and inhalation exposures—and only in the case that *tert*-butanol levels or metabolism were used as a dose-metric—induction's potential impact on risk evaluation for ETBE is considered minimal. Therefore, this adjustment was not turned off in the model; instead, the maximum induction level was set to zero.

While model simulations accounted for variations during the day and week (e.g., 6 hours/day, 5 days/week inhalation exposure), simulations reached a condition of "periodicity" by the second week, such that the time-course of internal doses were identical in between the second week and subsequent weeks of exposure with metabolic induction turned off. However, to ensure applicability in the event that metabolic induction is considered (predicted to take 2–3 weeks), simulations were generally run for 7 weeks, with results for the last 1–2 weeks used to estimate average tissue or blood concentrations or metabolic rates.

For simulating exposure to drinking water, the water consumption was modeled as episodic, based on the pattern of drinking observed in rats (Spiteri, 1982). In particular, rats were assumed to ingest water in pulses or "bouts," which were treated as periods of continuous ingestion, interspersed with periods of no ingestion. During the active dark period (12 hours/day), it is assumed that 80% of total daily ingestion occurs in 45-minute bouts alternating with 45 minutes of other activity. During the relatively inactive light period (12 hours/day), it is assumed that the remaining 20% of daily ingestion occurs; the bouts are only assumed to last 30 minutes, with 2.5 hours between. This pattern is thought to be more realistic than assuming continuous 24 hours/day ingestion. The resulting ingestion rate for one exposure is shown in Figure B-2.

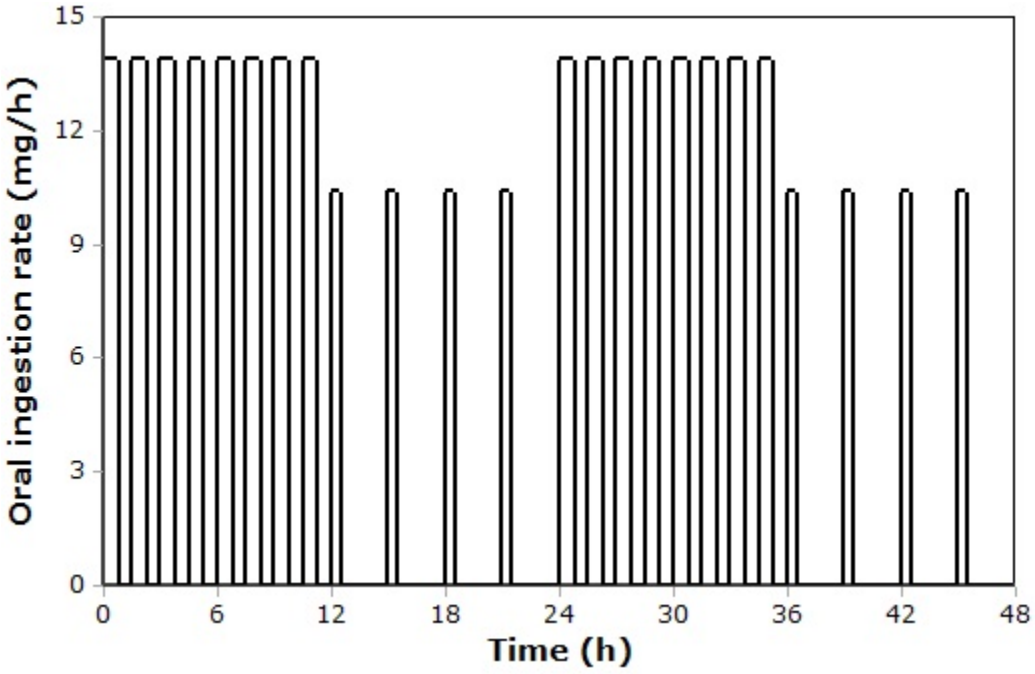
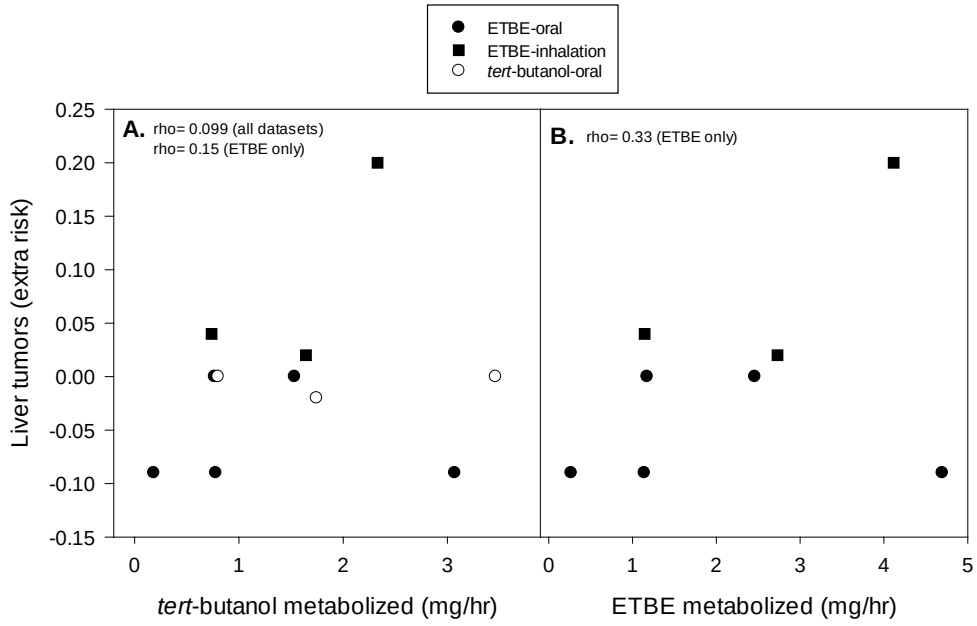


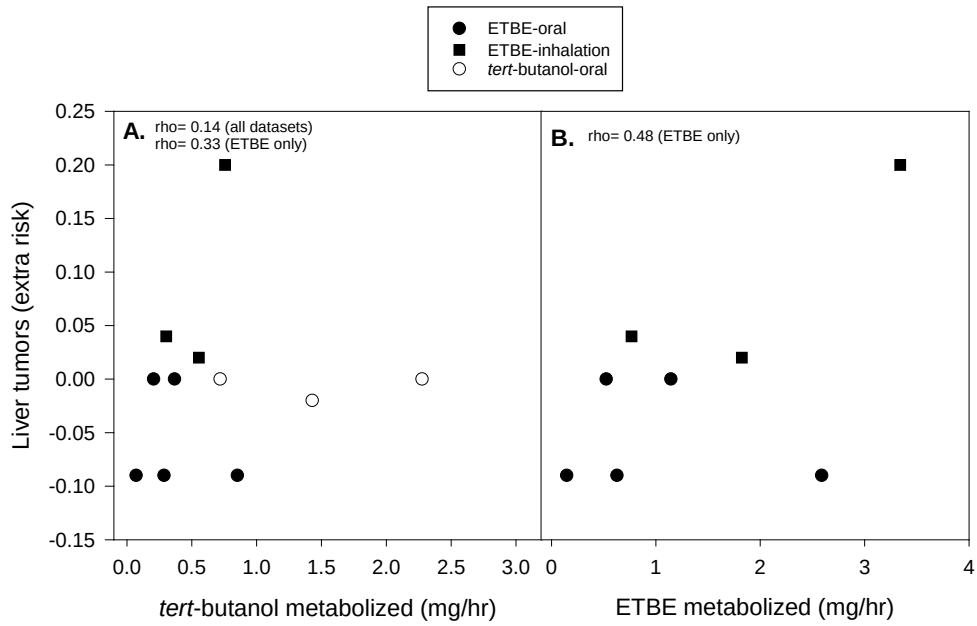
Figure B-2. Example oral ingestion pattern for rats exposed via drinking water.

PBPK modeling was also used to evaluate a variety of internal dose metrics (daily average TBA blood concentration, daily amount of TBA metabolized in liver, daily average of ETBE blood concentration, and daily amount of ETBE metabolized in liver) to assess the correlation with different endpoints following exposure to ETBE or TBA (Salazar et al., 2015). Administering ETBE either orally or via inhalation achieved similar or higher levels of TBA blood concentrations or TBA metabolic rates as those induced by direct TBA administration. Altogether, the PBPK model-based analysis by Salazar et al. (2015) [which applied a model structurally similar to (Borghoff et al., 2016)] indicates a consistent dose-response relationship between kidney weight, urothelial hyperplasia, and chronic progressive nephropathy (CPN) and TBA blood concentration (as the dose

metric for both ETBE and TBA). Kidney and liver tumors, however, were not consistently correlated with any dose metric. These data are consistent with TBA mediating the noncancer kidney effects following ETBE administration, but additional factors besides internal dose are necessary to explain the induction of liver and kidney tumors.



Salazar et al. (2015)



Borghoff et al. (2016)

Figure B-3. Comparisons of liver tumors in male rats following 2-year oral or inhalation exposure to ETBE or *tert*-butanol with internal dose metrics calculated from the PBPK model. Results applying the model of Salazar et al. (2015) (top) and Borghoff et al. (2016) (bottom)

Dose metrics expressed are metabolism rate of *tert*-butanol (A) and metabolism rate of ETBE (B). Liver tumor incidences following ETBE oral or inhalation exposure did not present a consistent dose-response relationship using either the ETBE or *tert*-butanol metabolism rate dose metric, and the correlation coefficients were not statistically significant. These data indicate that internal dose is inadequate to explain differences in tumor response across these studies.

B.1.6. PBPK Model Code

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

B.2. OTHER PERTINENT TOXICITY INFORMATION

B.2.1. Other Toxicological Effects

Synthesis of Other Effects

The database for effects other than kidney, liver, reproductive, and cancer contain only 11 rodent studies. These effects included decreased body weight, increased adrenal weights, altered spleen weights, and increased mortality. All selected studies used inhalation, oral gavage, or drinking water exposures for ≥90 days. Shorter duration, multiple-exposure studies that examined immunological endpoints were also included. No studies were removed for methodological concerns.

Kidney Effects

Numerical absolute kidney weight data are presented in Table B-7.

Table B-7. Evidence pertaining to kidney weight effects in animals exposed to ETBE

Reference and Study Design	Results (percent change compared to control)			
Fujii et al. (2010); JPEC (2008d) rat, Sprague-Dawley oral—gavage P0, male (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 16 wk beginning 10 wk prior to mating P0, female (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 17 wk beginning 10 wk prior to mating to lactation day 21	**P0, Male**		**P0, Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	100	5%	100	−2%
	300	8%	300	0%
	1,000	18%*	1,000	7%*
Gaoua (2004b) rat, Sprague-Dawley oral—gavage P0, male (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wks beginning 10 wk before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wk beginning 10 wk before mating until PND 21 F1, males and females (25/group/sex): via P0 dams in utero daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups	**P0, Male**		**P0, Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	250	11%*	250	−1%
	500	15%*	500	2%
	1,000	21%*	1,000	5%
	F1, Male		**F1, Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	250	10%	250	4%
	500	22%*	500	3%
	1,000	58%*	1,000	11%*
Hagiwara et al. (2011); JPEC (2008c) rat, Fischer 344 oral—gavage male (12/group): 0, 1,000 mg/kg-day daily for 23 wk	**Male**			
	Dose (mg/kg-day)	Absolute weight		
	0	-		
	1,000	19%*		

Reference and Study Design	Results (percent change compared to control)			
Miyata et al. (2013); JPEC (2008b) rat, CRL:CD(SD) oral—gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-day; female (15/group): 0, 5, 25, 100, 400 mg/kg-day daily for 26 wk	**Male**		**Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	5	1%	5	1%
	25	6%	25	0%
	100	5%	100	7%
	400	25%*	400	10%*
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3); dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	627	10%	627	1%
	2,090	11%	2,090	−1%
	6,270	18%*	6,270	4%
	20,900	16%*	20,900	7%
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[a]; female (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk followed by a 28 day recovery period; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	20,900	19%	20,900	8%
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation—vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	2,090	7%	2,090	4%
	7,320	10%*	7,320	12%*
	20,900	19%*	20,900	21%*

Reference and Study Design	Results (percent change compared to control)			
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation—vapor male (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	2,090	9%	2,090	0%
	7,320	10%	7,320	6%
	20,900	5%	20,900	4%

[a]4.18 mg/m^3 = 1 ppm.
* result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
- for controls, no response relevant; for other doses, no quantitative response reported.
(n) number evaluated from group.

Body Weight

As presented in Table B-7, body weights were significantly reduced compared with vehicle controls following 2-year oral and inhalation exposures to ETBE (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Reductions were also reported in studies of exposure durations shorter than 2 years (Banton et al., 2011; Hagiwara et al., 2011; Fujii et al., 2010; JPEC, 2008a, b; Gaoua, 2004b; Medinsky et al., 1999); however, these effects were frequently not statistically significant. Food consumption did not correlate well with body weight (Saito et al., 2013; Suzuki et al., 2012; JPEC, 2010a, b). Water consumption was reduced in the 2-year oral exposure study (JPEC, 2010a). Palatability and reduced water consumption due to ETBE exposure might contribute to the reduced body weight, particularly for oral exposures. Ptyalism, which is frequently observed with unpalatable chemicals following gavage, was observed in rats gavaged for 18 weeks (Gaoua, 2004b). Body weight changes are poor indicators of systemic toxicity but are important when evaluating relative organ weight changes. Body weight was most severely affected in 2-year studies, and 2-year kidney and liver weights are not appropriate for analysis as stated in Sections 1.2.1 and 1.2.2. Thus, the body weight effects data are inadequate to draw conclusions as a human hazard of ETBE exposure.

Adrenal Weight

Adrenal weights were increased in 13-week and 23-week studies (see Table B-8). For instance, a 13-week inhalation study found that absolute adrenal weights were increased in male and female rats (Medinsky et al., 1999). In another study, absolute and relative adrenal weights were increased in male rats (Hagiwara et al., 2011). None of the observed organ weight changes

corresponded with functional or histopathological changes; thus, adrenal effect data are inadequate to draw conclusions as a human hazard of ETBE exposure.

Immune System

Functional immune assays represent clear evidence of immunotoxicity and generally outweigh immune organ weight and cell population effects when establishing hazard (WHO, 2012) (see Table B-10). The single published functional assay available reported that the number of IgM^+ sheep red blood cell (SRBC)-specific antibody forming cells was not significantly affected after a 28-day oral exposure to ETBE (Banton et al., 2011). Relative spleen weights were inconsistently affected in male and female rats following oral and inhalation >13-week exposures to ETBE (see Table B-10). The only dose-responsive changes in spleen weights were increased relative weights in male rats and decreased absolute weights in female rats following 2-year inhalation exposure (Saito et al., 2013; JPEC, 2010b) and increased relative weights in female rats following 2-year oral exposure (Suzuki et al., 2012; JPEC, 2010a). Spleen weights are heavily influenced by the proportion of red blood cells which do not impact immune function of the organ (Elmore, 2006). Thus, spleen weight changes must be correlated with histopathological and functional changes for evidence of immunotoxicity (Elmore, 2006), none of which are observed for ETBE. $CD3^+$, $CD4^+$, and $CD8^+$ T cells were modestly reduced in male mice after 6 or 13 weeks of ETBE exposure via inhalation but are not correlated with any change in T cell function as indicated by the SRBC assay (Li et al., 2011). No other indicators of histopathological or functional changes were reported with a single chemical exposure. The ETBE database contains no evidence of altered immune function that correlate with modest T cell population reductions and altered splenic organ weights, thus the immune effect data are inadequate to draw conclusions as a human hazard of ETBE exposure.

Mortality

Mortality was significantly increased in male and female rats following a 2-year ETBE inhalation exposure (Saito et al., 2013; JPEC, 2010b) but not significantly affected following a 2-year drinking water exposure (Suzuki et al., 2012; JPEC, 2010a). Increased mortality in male rats correlated with increased CPN severity in the kidney. Increased mortality in females was attributed to pituitary tumors by the study authors; however, pituitary tumors were not dose responsively increased by ETBE exposure. Survival was also reduced in a lifetime gavage study at the highest exposure in males and females after 72 weeks (data not shown), and after 104 weeks, survival was reduced 54% in males at the highest dose (Maltoni et al., 1999). After 104 weeks, however, survival in the controls was approximately 25% in males and 28% in females which is much lower than anticipated for a 2-year study (Maltoni et al., 1999). The survival data in this study was likely confounded by chronic respiratory infections which could have contributed to the reduced survival (Malarkey and Bucher, 2011). These data do not suggest that mortality was increased in these

studies due to excessively high exposure concentrations of ETBE; thus, the mortality data are inadequate to draw conclusions as a human hazard of ETBE exposure.

Mechanistic Evidence

No relevant mechanistic data are available for these endpoints.

Summary of Other Toxicity Data

EPA concluded that the evidence does not support body weight changes, adrenal and immunological effects, and mortality as potential human hazards of ETBE exposure based on confounding factors, lack of progression, and study quality concerns.

Table B-8. Evidence pertaining to body weight effects in animals exposed to ETBE

Reference and Study Design	Results (percent change compared to control)
Banton et al. (2011) rat, Sprague-Dawley oral—gavage female (10/group): 0, 250, 500, 1,000 mg/kg-day daily for 28 consecutive days	**Female** Dose (mg/kg-day) / Body weight 0 / - 250 / 3% 500 / 5% 1,000 / −1%
Fujii et al. (2010); JPEC (2008d) rat, Sprague-Dawley oral—gavage P0, male (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 16 wk beginning 10 wk prior to mating; P0, female (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 17 wk beginning 10 wk prior to mating to lactation day 21	**P0, Male** / **P0, Female** Dose (mg/kg-day) / Body weight / Dose (mg/kg-day) / Body weight 0 / - / 0 / - 100 / −4% / 100 / 1% 300 / −4% / 300 / 1% 1,000 / −7% / 1,000 / 5%
Gaoua (2004b) rat, Sprague-Dawley oral—gavage P0, male (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wk beginning 10 wk before mating until PND 21 F1, male (25/group): 0, 250, 500, 1,000 mg/kg-day dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups F1, female (24−25/group): 0, 250, 500, 1,000 mg/kg-day P0 dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups	**P0, Male** / **P0, Female** Dose (mg/kg-day) / Final body weight / Dose (mg/kg-day) / Final body weight 0 / - / 0 / - 250 / −1% / 250 / −7% 500 / −3% / 500 / −2% 1,000 / −5%* / 1,000 / 0% **F1, Male** / **F1, Female** Dose (mg/kg-day) / Final body weight / Dose (mg/kg-day) / Final body weight 0 / - / 0 / - 250 / 0% / 250 / −2% 500 / 3% / 500 / −3% 1,000 / 1% / 1,000 / 2%

Reference and Study Design	Results (percent change compared to control)
Hagiwara et al. (2011); JPEC (2008c) rat, Fischer 344 oral—gavage male (12/group): 0, 1,000 mg/kg-day daily for 23 wk	**Male** Dose (mg/kg-day) / Final body weight 0 / - 1,000 / −5%*
Miyata et al. (2013);JPEC (2008b) rat, CRL:CD(SD) oral—gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-day; female (15/group): 0, 5, 25, 100, 400 mg/kg-day daily for 26 wk	**Male** / **Female** Dose (mg/kg-day) / Body weight / Dose (mg/kg-day) / Body weight 0 / - / 0 / - 5 / −6% / 5 / −5% 25 / 0% / 25 / −2% 100 / −5% / 100 / −2% 400 / 2% / 400 / −3%
Maltoni et al. (1999) rat, Sprague-Dawley oral—gavage male (60/group): 0, 250, 1,000 mg/kg-day; female (60/group): 0, 250, 1,000 mg/kg-day; 4 d/wk for 104 wk; observed until natural death	Male No significant difference at any dose Female No significant difference at any dose
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral—water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-day);[a] female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a] daily for 104 wk	**Male** / **Female** Dose (mg/kg-day) / Terminal body weight / Dose (mg/kg-day) / Terminal body weight 0 / - / 0 / - 28 / −4% / 46 / −10%* 121 / −7%* / 171 / −11%* 542 / −9%* / 560 / −17%*
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3) dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male** / **Female** Dose (mg/m^3) / Body weight / Dose (mg/m^3) / Body weight 0 / - / 0 / - 627 / 0% / 627 / −6% 2,090 / 1% / 2,090 / −7% 6,270 / −1% / 6,270 / −7% 20,900 / −7% / 20,900 / −11%

Reference and Study Design	Results (percent change compared to control)			
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b]; female (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk followed by a 28 day recovery period; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Body weight	Dose (mg/m^3)	Body weight
	0	-	0	-
	20,900	3%	20,900	4%
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation—vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Body weight	Dose (mg/m^3)	Body weight
	0	-	0	-
	2,090	2%	2,090	−3%
	7,320	4%	7,320	3%
	20,900	2%	20,900	6%*
Medinsky et al. (1999); Bond et al. (1996b) mice, CD-1 inhalation—vapor male (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (40/group): 0, 500, 1,750, 50,00 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Body weight	Dose (mg/m^3)	Body weight
	0	-	0	-
	2,090	0%	2,090	−2%
	7,320	−1%	7,320	−1%
	20,900	−3%	20,900	2%
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation—vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration, and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Body weight	Dose (mg/m^3)	Body weight
	0	-	0	-
	2,090	−7%*	2,090	−6%*
	6,270	−7%*	6,270	−10%*
	20,900	−26%*	20,900	−23%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
Percentage change compared to controls calculated as 100 × ((treated value—control value) ÷ control value).

Table B-9. Evidence pertaining to adrenal effects in animals exposed to ETBE

Reference and Study Design	Results (percentage change compared to control)			
Adrenal Weight				
Hagiwara et al. (2011); JPEC (2008c) rat, Fischer 344 oral—gavage male (12/group): 0, 1,000 mg/kg-day daily for 23 wk	**Male** Dose (mg/kg-day) 0 1,000	 Absolute weight - 16%*	 Relative weight - 19%*	
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation—vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male** Dose (mg/m^3) 0 2,090 7,320 20,900	 Absolute weight - 11% 9% 34%*	**Female** Dose (mg/m^3) 0 2,090 7,320 20,900	 Absolute weight - 7% 7% 18%*
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation—vapor male (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a]; female (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male** Dose (mg/m^3) 0 2,090 7,320 20,900	 Absolute weight - 0% 50% 0%	**Female** Dose (mg/m^3) 0 2,090 7,320 20,900	 Absolute weight - −8% 8% −8%

[a]4.18 mg/m^3 = 1 ppm.
*: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
(n): number evaluated from group.

Table B-10. Evidence pertaining to immune effects in animals exposed to ETBE

Reference and Study Design	Results (percent change compared to control)
Functional Immune Effects	
Banton et al. (2011) rat, Sprague-Dawley oral—gavage female (10/group): 0, 250, 500, 1,000 mg/kg-day daily for 28 consecutive days immunized i.v. 4 days prior to sacrifice with sheep red blood cells	**Female** Dose (mg/kg-day) / IgM antibody forming cells/10^6 spleen cells / IgM antibody forming cells/spleen 0 / - / - 250 / −21% / −20% 500 / 42% / 36% 1,000 / 8% / 8%
Immune Cell Populations	
Li et al. (2011) mice, 129/SV inhalation—vapor male (6/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hr/d for 5 d/wk over 6 wk; generation method not reported; analytical concentration and method were reported	**Male** Dose (mg/m^3) / Number of CD3+ T cells / Number of CD4+ T cells / Number of CD8+ T cells 0 / - / - / - 2,090 / −18%* / −16% / −13% 7,320 / −16% / −11% / −14% 20,900 / −21%* / −17%* / −25%
Li et al. (2011) mice, C57BL/6 inhalation—vapor male (6/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hr/d for 5 d/wk over 6 wk; generation method not reported; analytical concentration and method were reported	**Male** Dose (mg/m^3) / Number of CD3+ T cells / Number of CD4+ T cells / Number of CD8+ T cells 0 / - / - / - 2,090 / −14% / −15% / −12% 7,320 / −13% / −11% / −13%* 20,900 / −24%* / −23%* / −23%*
Li et al. (2011) mice, C57BL/6 inhalation—vapor male (5/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[a] whole body, 6 hr/d for 5 d/wk over 13 wk; generation method not reported; analytical concentration, and method were reported	**Male** Dose (mg/m^3) / Number of CD3+ T cells / Number of CD4+ T-cells / Number of CD8+ T cells 0 / - / - / - 2,090 / −9% / −11% / −8% 7,320 / −17%* / −28%* / −12% 20,900 / −24%* / −37%* / −20%

Reference and Study Design	Results (percent change compared to control)
Spleen Weight	
Banton et al. (2011) rat, Sprague-Dawley oral—gavage female (10/group): 0, 250, 500, 1,000 mg/kg-day daily for 28 consecutive days	**Female** Dose (mg/kg-day) / Absolute weight / Relative weight 0 / - / - 250 / −3% / 0% 500 / −15% / −18% 1,000 / −9% / 0%
Fujii et al. (2010); JPEC (2008d) rat, Sprague-Dawley oral—gavage P0, male (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 16 wk beginning 10 wk prior to mating P0, female (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	**P0, Male** Dose (mg/kg-day) / Absolute weight / Relative weight 0 / - / - 100 / −4% / −1% 300 / −2% / 2% 1,000 / 0% / 8% **P0, Female** Dose (mg/kg-day) / Absolute weight / Relative weight 0 / - / - 100 / 0% / −2% 300 / −2% / −3% 1,000 / −1% / −5%
Hagiwara et al. (2011); JPEC (2008c) rat, Fischer 344 oral—gavage male (12/group): 0, 1,000 mg/kg-day daily for 23 wk	**Male** Dose (mg/kg-day) / Absolute weight / Relative weight 0 / - / - 1,000 / −5% / 0%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral—water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a] daily for 104 wk	**Male** Dose (mg/kg-day) / Absolute weight / Relative weight 0 / - / - 628 / −3% / −35% 121 / 19% / 3%* 542 / 39% / −45% **Female** Dose (mg/kg-day) / Absolute weight / Relative weight 0 / - / - 46 / −35% / 2% 171 / −1% / 28% 560 / −50%* / 55%*

Reference and Study Design	Results (percent change compared to control)					
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3) dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**			**Female**		
	Dose (mg/m^3)	Absolute weight	Relative weight	Dose (mg/m^3)	Absolute weight	Relative weight
	0	-	-	0	-	-
	627	0%	0%	627	−9%	−3%
	2,090	7%	5%	2,090	−2%	5%
	6,270	−1%	1%	6,270	−5%	1%
	20,900	−9%	−2%	20,900	1%	12%
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b]; female (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk followed by a 28-day recovery period; generation method, analytical concentration and method were reported	**Male**			**Female**		
	Dose (mg/m^3)	Absolute weight	Relative weight	Dose (mg/m^3)	Absolute weight	Relative weight
	0	-	-	0	-	-
	20,900	10%	6%	20,900	6%	0%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation—vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration and method were reported	**Male**			**Female**		
	Dose (mg/m^3)	Absolute weight	Relative weight	Dose (mg/m^3)	Absolute weight	Relative weight
	0	-	-	0	-	-
	2,090	4%	15%	2,090	5%	30%
	6,270	32%	43%*	6,270	−39%	−31%
	20,900	17%	66%*	20,900	−43%*	−25%

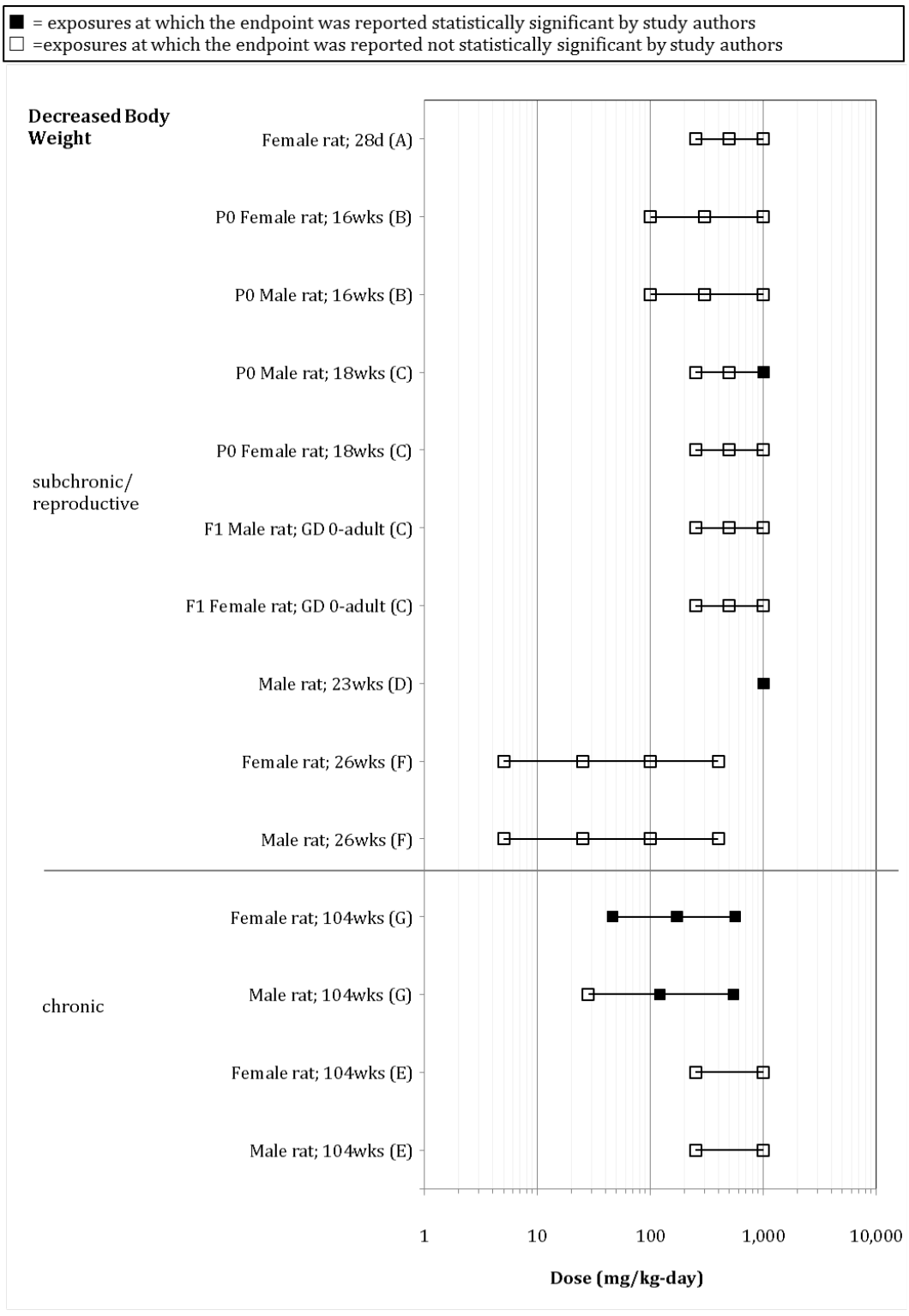
Reference and Study Design	Results (percent change compared to control)			
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation—vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	2,090	6%	2,090	−3%
	7,320	3%	7,320	3%
	20,900	5%	20,900	0%
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation—vapor male (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (40/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	2,090	−5%	2,090	−11%
	7,320	0%	7,320	−2%
	20,900	−15%	20,900	−11%

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
(n): number evaluated from group.

Table B-11. Evidence pertaining to mortality in animals exposed to ETBE

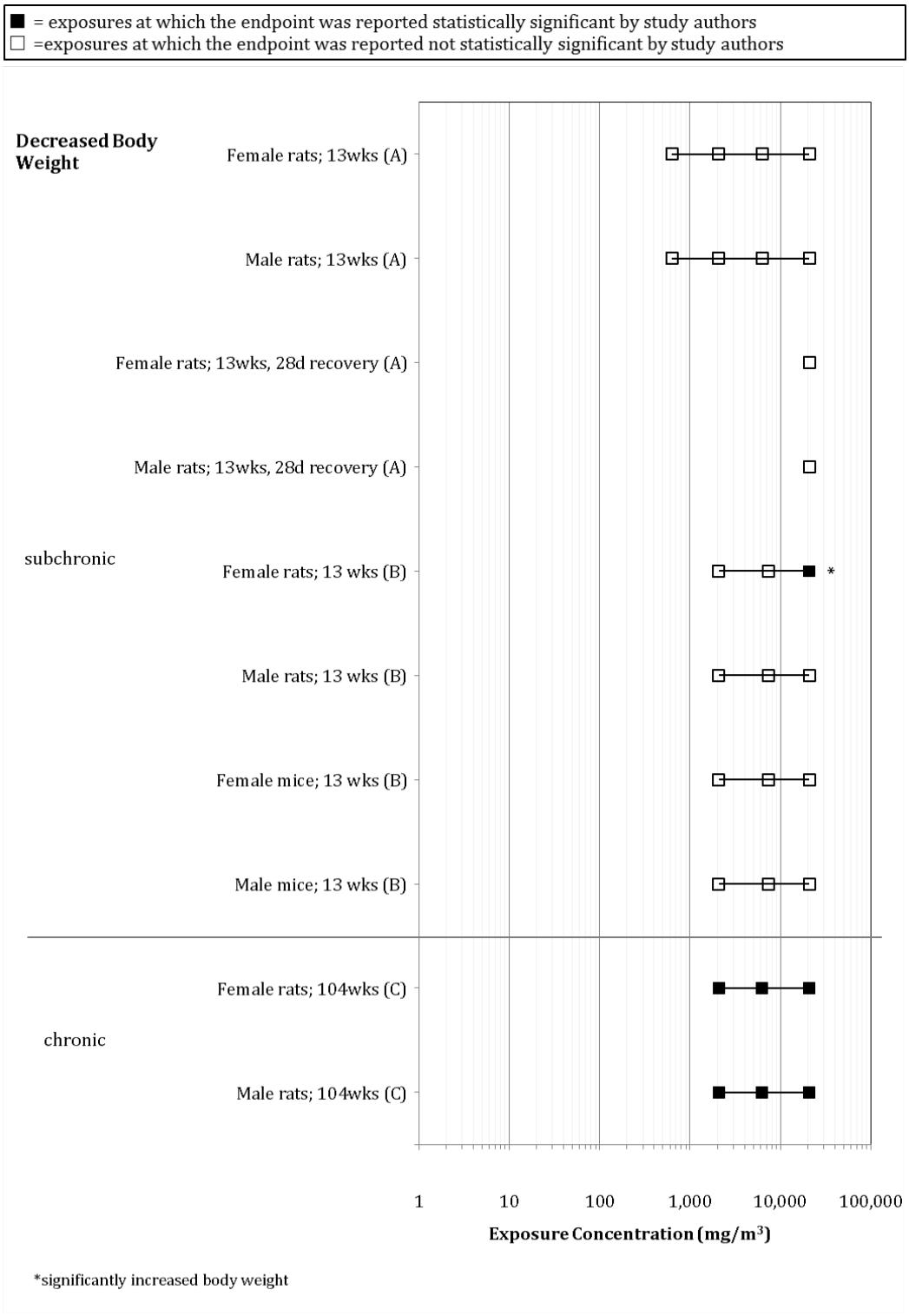
Reference and Study Design	Results (percentage change compared to control)			
Maltoni et al. (1999) rat, Sprague-Dawley oral—gavage male (60/group): 0, 250, 1,000 mg/kg-day; female (60/group): 0, 250, 1,000 mg/kg-day 4 d/wk for 104 wk; observed until natural death	**Male**		**Female**	
	Dose (mg/m^3)	Survival at 104 wk	Dose (mg/m^3)	Survival at 104 wk
	0	-	0	-
	250	−8%	250	−8%
	1,000	−54%	1,000	18%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral—water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a] daily for 104 wk	**Male**		**Female**	
	Dose (mg/kg-day)	Percentage survival	Dose (mg/kg-day)	Percentage survival
	0	-	0	-
	628	−3%	46	3%
	121	−11%	171	6%
	542	−11%	560	6%
Saito et al. (2013);JPEC (2010b) rat, Fischer 344 inhalation—vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Survival at 104 wk	Dose (mg/m^3)	Survival at 104 wk
	0	-	0	-
	2,090	−14%	2,090	3%
	6,270	−9%	6,270	−21%*
	20,900	−32%*	20,900	−21%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
(n): number evaluated from group.



Sources: (A) Banton et al., 2011 (B) Fujii et al., 2010; JPEC, 2008e (C) Gaoua, 2004b (D) Hagiwara et al., 2011 (E) Maltoni et al., 1999 (F) Miyata et al., 2013; JPEC, 2008c (G) Suzuki et al., 2012; JPEC, 2010a

Figure B-4. Exposure-response array of body weight effects following oral exposure to ETBE.



Sources: (A) JPEC, 2008b (B) Medinsky et al., 1999; Bond et al., 1996 (C) Saito et al., 2013; JPEC, 2010b

Figure B-5. Exposure-response array of body weight effects following inhalation exposure to ETBE.

B.2.2. Genotoxicity Studies

Bacterial Systems

Mutagenic potential of ETBE has been tested by Zeiger et al. (1992) using different *Salmonella typhimurium* strains for 311 chemicals, including ETBE, both in the absence and presence of metabolic activation (S9). Preincubation protocol was followed and precaution was exercised to account for the volatility of the compound. Five doses ranging from 100 to 10,000 µg/plate were tested using different Salmonella strains including TA97, TA 98, TA100, TA1535. The results showed that the ETBE did not cause mutations in any of the Salmonella strains tested. It should be noted that TA102, a sensitive strain for oxidative metabolite, was not used in this study. The available genotoxicity data for *tert*-butanol are discussed below, and the summary of the data is provided in Table B-12.

In Vitro Mammalian Studies

Limited available studies (two) in in vitro mammalian systems were unpublished reports. Vergnes and Kubena (1995b) evaluated the mutagenicity of ETBE using the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward mutation assay in Chinese hamster ovary K1-BH4 cells. Duplicate cultures were treated with five concentrations of ETBE (>98% purity; containing 13 ppm AO22, an antioxidant stabilizer) ranging from 100 to 5,000 µg/mL, both in the presence and absence of S9 activation. No statistically significant or concentration-related increase in the HGPRT mutation frequencies were observed at any of the ETBE concentrations tested, either in the absence or in the presence of metabolic (S9) activation.

The same authors [(Vergnes and Kubena, 1995b) unpublished report] studied the clastogenic potential of ETBE in vitro using chromosome aberration assay in Chinese hamster ovary cells. The cells were exposed from 100 to 5,000 µg/mL of ETBE in culture medium, both in the presence and absence of S9 metabolic activation system. No statistically significant or concentration-related increase in the frequency of chromosomal aberrations, in the presence or absence of the S9 metabolic activation system, was observed. Neither the effect of the antioxidant stabilizer used in ETBE nor control for volatility of the compound was described for both studies although capped glass bottles were used in the experiments.

In Vivo Animal Studies

In vivo studies were conducted by same authors that tested ETBE for in vitro genotoxicity. Vergnes and Kubena (1995a), unpublished report, performed an in vivo bone marrow micronucleus (MN) test in mice in response to ETBE exposure. Male and female CD-1 mice (5 animals/sex/group) were exposed to ETBE by inhalation at target concentrations of 0, 400, 2,000, and 5,000 ppm (0, 1,671, 8,357, and 20,894 mg/m^3) for 6 hours/day, for 5 days. Following treatment, polychromatic erythrocytes (PCE) from bone marrow were analyzed for micronucleus formation. The results showed that no statistically significant increases in the mean percentages of

micronucleated polychromatic erythrocytes (MNPCE) were observed in mice (male or female) when exposed to ETBE.

In addition to Vergnes and Kubena (1995a), four animal studies were conducted by the JPEC in rats using different routes of exposure (oral, inhalation, intraperitoneal or drinking water) to detect micronucleus as a result of exposure to ETBE [JPEC (2007c); JPEC (2007a); JPEC (2007d); JPEC (2007b) published as Noguchi et al. (2013)].

The first two studies (oral and intraperitoneal injection) were part of an acute (2-day) exposure. In the first study, both male and female F344 rats (5 animals/sex/dose group) were administered ETBE (99.3% pure) via gavage at doses of 0, 500, 1,000, or 2,000 mg/kg-day separated by 24 hours in olive oil [(JPEC, 2007a), unpublished report]. Animals were sacrificed, and bone marrow smears were collected and stained 24 hours after the final administration. Following treatment, polychromatic erythrocytes from bone marrow were analyzed for MN formation. The results were expressed as the ratio of polychromatic erythrocytes/total erythrocytes. There were no treatment-related effects on the number of MNPCE or the ratio of PCE/total erythrocytes. ETBE was determined to be negative for micronuclei induction in rat bone marrow cells after acute oral exposure.

In the second study (intraperitoneal injection), male and female F344 rats (5 animals/sex/dose group) were administered two ETBE intraperitoneal injections separated by 24 hours at doses of 0, 250, 500, 1,000, or 2,000 mg/kg-day in olive oil (Noguchi et al., 2013; JPEC, 2007b). Animals were sacrificed, and bone marrow smears were collected and stained 24 hours after the final injection. All animals in the 2,000 mg/kg-day group died on the first day of treatment. There were no treatment-related effects on either the number of MNPCEs or the ratio of polychromatic erythrocytes/total erythrocytes. In addition, no dose-dependent tendencies for increase in MNPCE/PCE or alterations in the ratios of PCE/total erythrocytes were noted in either sex of the treated groups. ETBE was determined to be negative for micronuclei induction in rats after acute intraperitoneal exposure.

The next two studies (drinking water and inhalation) were part of 13-week toxicity studies in rats where ETBE effects on the micronuclei in PCE were examined at the end of the study. In the first 13-week study, male and female F344 rats (10 animals/sex/dose group) were administered drinking water containing 0, 1,600, 4,000, or 10,000 ppm ETBE for 13 weeks (Noguchi et al., 2013; JPEC, 2007d). The concentrations were stated to be equivalent to 0, 101, 259, and 626 mg/kg-day in males and 0, 120, 267, and 629 mg/kg-day in females. Following treatment, polychromatic erythrocytes from bone marrow were analyzed for MN formation. The results were expressed as the ratio of PCE/total erythrocytes. There were no treatment-related effects on the number of MNPCEs or the ratio of PCE/total erythrocytes.

In the second 13-week study (inhalation), male and female F344 rats (10 animals/sex/dose group) were exposed to ETBE (99.2–99.3% pure) through whole-body inhalation exposure at 0, 500, 1,500, or 5,000 ppm (0, 2,089, 6,268, or 20,894 mg/m^3) for 6 hours/day, 5 days/week

(Noguchi et al., 2013; JPEC, 2007b). Normochromatic and polychromatic erythrocytes and micronuclei were counted as in the previous study. There were no treatment-related effects on the number of MNPCE or the ratio of PCE/total erythrocytes. ETBE was determined to be negative for micronuclei induction in rat bone marrow cells after a 13-week inhalation exposure.

Furthermore, NTP (1996a, 1996b) performed an in vivo bone marrow micronucleus test both in B6C3F1 mice and Fischer rats. The animals were exposed through intraperitoneal injection 3 times in a period of 72 hours (n = 5). Doses for the mice were 0, 1,300, 1,700, 2,100 and 2,500 mg/kg, and the doses for rats were 0, 625, 1,250, 2,500 mg/kg. No increase in micronucleated PCEs were observed in either mice or rats. Two of five mice died in the 1,700 mg/kg dose group, while 3 of 5 and 4 of 5 animals died in the 2,100 and 2,500 mg/kg dose groups, respectively, and the surviving animals in the two highest dose groups were not scored. In the rat study, 2 of 5 animals died in the highest dose group.

Weng et al. (2011) conducted several studies evaluating the differential genotoxicity of ETBE in various tissues or systems (i.e., erythrocytes, leukocytes, liver, and sperm) in C57BL/6 wild-type and *Aldh2* knockout mice after subchronic inhalation exposure. All studies used the same exposures (i.e., to 0, 500, 1,750 and 5,000 ppm ETBE for 6 hours/day, 5 days/week for 13 weeks). Deoxyribonucleic acid (DNA) strand breaks were observed in leukocytes of male (all concentrations) and female (high dose only) *Aldh2* knockout mice and with the high dose in wild type male mice (Weng et al., 2011).

Weng et al. (2012) studied the differential genotoxic effects of subchronic exposure to ETBE in the liver of C57BL/6 wild-type and *Aldh2* knockout mice. DNA strand breaks in the hepatocytes of male and female with different *Aldh2* genotypes were determined using alkaline comet assay. In addition, 8-hydroxyguanine DNA-glycosylase (hOGG1)-modified oxidative base modification, and 8-hydroxydeoxyguanosine were determined as endpoints for genetic damage. There was significant increase in damage in all three exposure groups in the knockout male mice, although the increase was only found in 5,000 ppm exposure group for the knockout female mice. In the wild-type, significant DNA damage was seen only in males in the 5,000 ppm group, but not in females. This indicates the sensitivity of sex differences both in knockout and wild-type mice.

In another study by the same authors (Weng et al., 2013), the authors performed in vivo micronucleus tests (on what appear to be the same set of animals), in addition to the DNA strand breaks, 8-hydroxyguanine DNA-glycosylase (hOGG1)-modified oxidative base modification, and 8-hydroxydeoxyguanosine. The mice (wild-type and knockout, males and females) were exposed to 0, 500, 1,750 and 5,000 ppm ETBE for 6 hours/day, 5 days/week for 13 weeks. Peripheral blood samples were obtained and processed to detect micronucleated reticulocytes (MN-RETs) and micronuclei in the mature normochromatic erythrocyte population (MN-NCE). The results indicate that ETBE significantly affected frequencies of MN-RETs in male and female mice. In knockout male mice, the frequencies of MN-RETs of the 1,750- and 5,000-ppm exposure groups were significantly increased when compared with the control group. In the wild-type male mice, however, only the

5,000 ppm group had a higher frequency of MN-RETs than that of the control group. In female mice, there was no difference in the frequencies of MN-RETs between exposure groups and the control group in wild-type mice. In the same exposure group (5,000 ppm), the knock-out mice had a higher frequency of MN-RETs compared to the wild-type. These results inform the influence of *Aldh2* and sex difference on genotoxicity as a result of exposure to ETBE.

In yet another study by the same authors (Weng et al., 2014), DNA strand breaks and 8-hydroxyguanine DNA-glycosylase (hOGG1)-modified oxidative base modification were measured in sperm collected from the left caudia epididymis. In addition to the 13-week protocol used in the other studies, Weng et al. (2014) also included an additional 9-week study where the male mice (wild-type, knockout, and heterogeneous [HT]) were exposed to 0, 50, 200 and 500 ppm ETBE for 6 hours/day, 5 days/week for 9 weeks. In the 13-week study, there were significant increases in damage in all three exposure groups in the knockout male mice, but only in the two highest dose groups in the wild-type males. In the 9-week study, there was no change in the wild-type mice, but both the heterogeneous and the knockout mice had significant increases in the two highest doses.

Table B-12. Summary of genotoxicity (both in vitro and in vivo) studies of ETBE

Species	Test System	Dose/Conc.	Results[a]		Comments	Reference
Bacterial systems						
			–S9	+S9		
Salmonella typhimurium (TA97, TA98, TA100, TA1535)	Mutation Assay	100, 333, 1,000, 3,333, 10,000 µg/plate	-	-	Preincubation procedure was followed. Experiment was conducted in capped tubes to control for volatility	Zeiger et al. (1992)
In vitro systems						
Chinese Hamster Ovary cells (*hgprt* locus)	Gene Mutation Assay	100, 300, 1,000, 3,000, 5,000 µg/mL	-	-	Experiments conducted both with and without metabolic activation	Vergnes and Kubena (1995b) (unpublished report)
Chinese Hamster Ovary cells	Chromosomal Aberration Assay	100, 300, 1,000, 3,000, 5,000 µg/mL	-	-	Experiments conducted both with and without metabolic activation	Vergnes (1995) (unpublished report)
In vivo animal studies						
CD-1 mice (male and female)	Bone Marrow Micronucleus test	0, 400, 2,000, 5,000 ppm (0, 1,670, 8,360, 20,900 mg/m^3)[b]	-		Whole body Inhalation, 6 hr/d, 5 d, 5 animals/sex/group	Vergnes and Kubena (1995a) (unpublished report)

Species	Test System	Dose/Conc.	Results[a]	Comments	Reference
B6C3F1 mice (male)	Bone Marrow Micronucleus test	0, 1,300, 1,700, 2,100, 2,500 mg/kg	-	Intraperitoneal injection 3×, 72 hr. Five animals/group, 3 animals in dose 1,700 mg/kg dose. Surviving animals were not scored at doses of 2,100 and 2,500 mg/kg	NTP (1996a)
Fischer 344 rats (male)	Bone Marrow Micronucleus test	0, 625, 1,250, 2,500 mg/kg	-	Intraperitoneal injection 3×, 72 hr. Five animals/group, 3 animals in 2,500 mg/kg dose group.	NTP (1996b)
Fischer 344 rats (male and female)	Bone Marrow Micronucleus test	0, 500, 1,000, 2,000 mg/kg-day	-	Oral gavage, 24 hr apart, 2 d, 5 animals/sex/group	JPEC (2007b) (unpublished report)
Fischer 344 rats (male and female)	Bone Marrow Micronucleus test	0, 250, 500, 1,000, 2,000 mg/kg-day	-	Intraperitoneal injection, 24 hr apart, 2 d, 5 animals/sex/group	Noguchi et al. (2013); JPEC (2007b),unpublished report
Fischer 344 rats (male and female)	Bone Marrow Micronucleus test	0, 1,600, 4,000, 10,000 ppm (0, 101, 259, 626 mg/kg-day in males; 0, 120, 267, 629 mg/kg-day in females)[c]	-	Drinking water, 13 wk, 10 animals/sex/group	Noguchi et al. (2013); JPEC (2007c), unpublished report
Fischer 344 rats (male and female)	Bone Marrow Micronucleus test	0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]	-	Whole body inhalation, 6 hr/d, 5 d/wk, 13 wk. 10 animals/sex/group	Noguchi et al. (2013); JPEC (2007c), unpublished report
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	DNA strand breaks (alkaline comet assay), leukocytes	0, 500, 1,750 and 5,000 ppm	Male—WT/KO: +[d]/+ Female WT/KO: −/+[d]	Whole body inhalation, 6 hr/d, 5 d/wk, 13 wk	Weng et al. (2011)

Species	Test System	Dose/Conc.	Results[a]		Comments	Reference
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	DNA strand breaks (alkaline comet assay)	0, 500, 1,750 and 5,000 ppm	Male—WT/KO	+[d]/+	Whole body inhalation, 6 hr/d, 5 d/wk, 13 wk	Weng et al. (2012)
			Female WT/KO	−/+[d]		
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	Micronucleus assay, erythrocytes	0, 500, 1,750 and 5,000 ppm	Male* WT/KO	+[d]/+	Whole body inhalation, 6 hr/d, 5 d/wk, 13 wk	Weng et al. (2013)
			Female* WT/KO	−/+		
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	DNA strand breaks (alkaline comet assay); sperm	0, 50, 200 and 500 ppm	WT/HT/KO	−/+/+	Whole body inhalation, 6 hr/d, 5 d/wk, 9 wk	Weng et al. (2014)
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	DNA strand breaks (alkaline comet assay); sperm	0, 500, 1,750 and 5,000 ppm	WT/KO	+/+	Whole body inhalation, 6 hr/d, 5 d/wk, 13 wk	Weng et al. (2014)

[a]+ = positive; − = negative; (+), equivocal.
[b]4.18 mg/m^3 = 1 ppm.
[c]Conversions performed by study authors.
[d]Positive in highest dose tested.
*When the data of ETBE-induced MN-RETs (micronucleated reticulocytes) were normalized with corresponding control, the effect disappeared.

Summary

Limited studies have been conducted to understand the genotoxic potential of ETBE. Most studies indicate that ETBE does not induce genotoxicity in the systems tested. More recently, Weng and coauthors seem to illustrate the influence of *Aldh2* on the genotoxic effects of ETBE. With respect to overall existing database, it should be noted that the array of genotoxic tests conducted are limited. The inadequacy of the database is two dimensional: (a) the coverage of the studies across the genotoxicity tests needed for proper interpretation of the weight of evidence of the data; (b) the quality of the available data. With respect to the array of types of genotoxicity tests available, ETBE has only been tested in one bacterial assay. Limited (two) studies are available with respect to in vitro studies. Existing in vivo studies have all been tested only for the micronucleus assay, DNA strand breaks, or both. Key studies in terms of chromosomal aberrations and DNA

adducts are missing. It should also be noted that the few existing studies are unpublished reports lacking peer review. Given the above limitations; significant deficiencies; and sparse database both in terms of quality and quantity; it is implicit that the database is inadequate or insufficient to draw any conclusions on the effect of ETBE with respect to genotoxicity.

B.3. SUPPLEMENTAL ORGAN WEIGHT DATA

B.3.1. Relative Kidney Weight Data

Table B-13. Evidence pertaining to relative kidney weight effects in animals exposed to ETBE

Reference and Study Design	Results (percent change compared to control)			
Fujii et al. (2010); JPEC (2008d) rat, Sprague-Dawley oral—gavage P0, male (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 16 wk beginning 10 wk prior to mating P0, female (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 17 weeks beginning 10 weeks prior to mating to lactation day 21	**P0, Male**		**P0, Female**	
	Dose (mg/kg-day)	Relative weight	Dose (mg/kg-day)	Relative weight
	0	-	0	-
	100	8%*	100	−3%
	300	12%*	300	−1%
	1,000	26%*	1,000	2%
Gaoua (2004b) rat, Sprague-Dawley oral—gavage P0, male (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wk beginning 10 wk before mating until PND 21 F1, males and females (25/group/sex): via P0 dams in utero daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups	**P0, Male**		**P0, Female**	
	Dose (mg/kg-day)	Relative weight	Dose (mg/kg-day)	Relative weight
	0	-	0	-
	250	11%*	250	9%
	500	18%*	500	5%
	1,000	28%*	1,000	3%
	F1, Male		**F1, Female**	
	Dose (mg/kg-day)	Relative weight	Dose (mg/kg-day)	Relative weight
	0	-	0	-
	250	10%*	250	6%
	500	19%*	500	6%
	1,000	58%*	1,000	10%*

Reference and Study Design	Results (percent change compared to control)			
Hagiwara et al. (2011); JPEC (2008c) rat, Fischer 344 oral—gavage male (12/group): 0, 1,000 mg/kg-day daily for 23 wk	**Male** Dose (mg/kg-day) 0 1,000	 Relative weight - 25%*		
Miyata et al. (2013);JPEC (2008b) rat, CRL:CD(SD) oral—gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-day; female (15/group): 0, 5, 25, 100, 400 mg/kg-day daily for 26 wk	**Male** Dose (mg/kg-day) 0 5 25 100 400	 Relative weight - 8% 6% 12%* 21%*	**Female** Dose (mg/kg-day) 0 5 25 100 400	 Relative weight - 7% 4% 11%* 15%*
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral—water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a] daily for 104 wk	**Male** Dose (mg/kg-day) 0 28 121 542	 Relative weight - 0% 12%* 31%*	**Female** Dose (mg/kg-day) 0 46 171 560	 Relative weight - 13%* 22%* 37%*
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3) dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male** Dose (mg/m^3) 0 627 2,090 6,270 20,900	 Relative weight - 10% 9% 20%* 24%*	**Female** Dose (mg/m^3) 0 627 2,090 6,270 20,900	 Relative weight - 8% 7% 12%* 20%*

Reference and Study Design	Results (percent change compared to control)			
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b]; female (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk followed by a 28 day recovery period; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Relative weight	Dose (mg/m^3)	Relative weight
	0	-	0	-
	20,900	15%*	20,900	5%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation—vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Relative weight	Dose (mg/m^3)	Relative weight
	0	-	0	-
	2,090	19%*	2,090	11%*
	6,270	26%*	6,270	16%*
	20,900	66%*	20,900	51%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
Percentage change compared to controls calculated as 100 × ((treated value—control value) ÷ control value).

B.3.2. Absolute Liver Weight Data

Table B-14. Evidence pertaining to absolute liver weight effects in animals exposed to ETBE

Reference and Study Design	Results (percentage change compared to control)			
Fujii et al. (2010); JPEC (2008d) rat, Sprague-Dawley oral—gavage P0, male (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 16 wk beginning 10 wk prior to mating P0, female (24/group): 0, 100, 300, 1,000 mg/kg-day daily for 17 wk beginning 10 wk prior to mating to lactation day 21	**P0, Male**		**P0, Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	100	−3%	100	−1%
	300	−1%	300	3%
	1,000	13%*	1,000	14%*

Reference and Study Design	Results (percentage change compared to control)			
Gaoua (2004b) rat, Sprague-Dawley oral—gavage P0, male (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wk beginning 10 wk before mating until after weaning of the pups P0, female (25/group): 0, 250, 500, 1,000 mg/kg-day daily for a total of 18 wk beginning 10 wk before mating until PND 21 F1, male (25/group): 0, 250, 500, 1,000 mg/kg-day P0 dams dosed daily through gestation and lactation, then F1 doses beginning PND 22 until weaning of the F2 pups F1, female (24–25/group): 0, 250, 500, 1,000 mg/kg-day P0 dams dosed daily through gestation and lactation, then F1 dosed beginning PND 22 until weaning of the F2 pups	**P0, Male**		**P0, Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	250	2%	250	–1%
	500	2%	500	4%
	1,000	17%*	1,000	6%
	F1, Male		**F1, Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	250	0%	250	1%
	500	14%*	500	3%
	1,000	27%*	1,000	10%*
Hagiwara et al. (2011); JPEC (2008c) rat, Fischer 344 oral—gavage male (12/group): 0, 1,000 mg/kg-day daily for 23 wk	**Male**			
	Dose (mg/kg-day)	Absolute weight		
	0	-		
	1,000	21%*		
Miyata et al. (2013); JPEC (2008b) rat, CRL:CD(SD) oral—gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-day; female (15/group): 0, 5, 25, 100, 400 mg/kg-day daily for 26 wk	**Male**		**Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	5	–2%	5	–4%
	25	7%	25	–1%
	100	4%	100	2%
	400	19%	400	9%
Suzuki et al. (2012); JPEC (2010a) rat, Fischer 344 oral—water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-day)[a]; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-day)[a] daily for 104 wk	**Male**		**Female**	
	Dose (mg/kg-day)	Absolute weight	Dose (mg/kg-day)	Absolute weight
	0	-	0	-
	28	–11%*	46	–5%
	121	–4%	171	–2%
	542	2%	560	–10%

Reference and Study Design	Results (percentage change compared to control)			
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3)[b]; female (NR): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m^3) dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	627	5%	627	−3%
	2,090	6%	2,090	−8%
	6,270	4%	6,270	−2%
	20,900	2%	20,900	5%
JPEC (2008a) rat, CRL:CD(SD) inhalation—vapor male (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b]; female (6/group): 0, 5,000 ppm (0, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk followed by a 28 day recovery period; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	20,900	13%	20,900	11%
Saito et al. (2013); JPEC (2010b) rat, Fischer 344 inhalation—vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b]; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m^3)[b] dynamic whole body inhalation; 6 hr/d, 5 d/wk for 104 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	2,090	1%	2,090	−3%
	6,270	11%*	6,270	−8%
	20,900	10%	20,900	1%
Medinsky et al. (1999); Bond et al. (1996b) rat, Fischer 344 inhalation—vapor male (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (48/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	2,090	6%	2,090	2%
	7,320	14%*	7,320	9%
	20,900	32%*	20,900	26%*

Reference and Study Design	Results (percentage change compared to control)			
Medinsky et al. (1999); Bond et al. (1996a) mice, CD-1 inhalation—vapor male (40/group): 0, 500, 1,750, 5,000 ppm (0, 2,090, 7,320, 20,900 mg/m^3)[b]; female (40/group): 0, 500, 1,750, 5,000 ppm(0, 2,090, 7,320, 20,900 mg/m^3)[b] dynamic whole body chamber; 6 hr/d, 5 d/wk for 13 wk; generation method, analytical concentration and method were reported	**Male**		**Female**	
	Dose (mg/m^3)	Absolute weight	Dose (mg/m^3)	Absolute weight
	0	-	0	-
	2,090	4%	2,090	2%
	7,320	13%*	7,320	19%*
	20,900	18%*	20,900	33%*

[a]Conversion performed by study authors.
[b]4.18 mg/m^3 = 1 ppm.
NR: not reported; *: result is statistically significant ($p < 0.05$) based on analysis of data by study authors.
-: for controls, no response relevant; for other doses, no quantitative response reported.
Percent change compared to controls calculated as 100 × ((treated value—control value) ÷ control value).

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

C.1. BENCHMARK DOSE MODELING SUMMARY

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant toxicological endpoints. The endpoints were modeled using EPA's Benchmark Dose Software (BMDS, version 2.2). Sections 0 and 0 (noncancer) and Section C.1.2 (cancer) 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, 2012). In some cases, it might be appropriate to use alternative methods based on statistical judgment; exceptions are noted as necessary in the summary of the modeling results.

C.1.1. Noncancer Endpoints

Evaluation of Model Fit

For each dichotomous endpoint, BMDS dichotomous models[1] were fitted to the data using the maximum likelihood method. 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 were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the benchmark response (BMR).

For each continuous endpoint, BMDS continuous models[2] were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. 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 fitted 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

[1]Unless otherwise specified, all available BMDS dichotomous models besides the alternative and nested dichotomous models were fitted. The following parameter restrictions were applied: For the log-logistic model, restrict slope ≥ 1; for the gamma and Weibull models, restrict power ≥ 1.

[2]Unless otherwise specified, all available BMDS continuous models were fitted. The following parameter restrictions were applied: For the polynomial models, restrict the coefficients b1 and higher to be nonnegative or nonpositive if the direction of the adverse effect is upward or downward, respectively; for the Hill, power, and exponential models, restrict power ≥ 1.

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 were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

Model Selection

For each endpoint, the BMDL estimate (95% lower confidence limit on the benchmark dose (BMD), as estimated by the profile likelihood method and 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 at most threefold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

Table C-1. Noncancer endpoints selected for dose-response modeling for ETBE

Endpoint, Study	Sex, Strain, Species	Doses and Effect Data				
ORAL						
Urothelial hyperplasia of the renal pelvis Suzuki et al. (2012); JPEC (2010a)	Male F344 rats	Dose (mg/kg-day)	0	28	121	542
		Incidence/Total	0/50	0/50	10/50	25/50
Increased absolute kidney weight Miyata et al. (2013); JPEC (2008b)	Male Sprague-Dawley rats	Dose (mg/kg-day)	0	5	25	100	400
		No. of animals	15	15	14	15	13
		Mean ± SD	3.27 ± 0.34	3.29 ± 0.3	3.47 ± 0.32	3.42 ± 0.48	4.09 ± 0.86
Increased absolute kidney weight Miyata et al. (2013); JPEC (2008b)	Female Sprague-Dawley rats	Dose (mg/kg-day)	0	5	25	100	400
		No. of animals	15	15	15	15	15
		Mean ± SD	1.88 ± 0.2	1.89 ± 0.16	1.88 ± 0.15	2.02 ± 0.21	2.07 ± 0.23
Increased absolute kidney weight Gaoua (2004b)	P0 Male Sprague-Dawley rats	Dose (mg/kg-day)	0	250	500	1,000
		No. of animals	25	25	25	25
		Mean ± SD	3.58 ± 0.413	3.96 ± 0.446	4.12 ± 0.624	4.34 ± 0.434
Increased absolute kidney weight Gaoua (2004b)	P0 Female Sprague-Dawley rats	Dose (mg/kg-day)	0	250	500	1,000
		No. of animals	25	24	22	25
		Mean ± SD	2.24 ± 0.185	2.22 ± 0.16	2.29 ± 0.207	2.35 ± 0.224
Increased absolute kidney weight Gaoua (2004b)	F1 Male Sprague-Dawley rats	Dose (mg/kg-day)	0	250	500	1,000
		No. of animals	24	25	24	25
		Mean ± SD	3.38 ± 0.341	3.73 ± 0.449	4.13 ± 0.64	5.34 ± 5.39

1

Table C-1. Noncancer endpoints selected for dose response modeling for ETBE (continued)

Endpoint, Study	Sex, Strain, Species	Doses and Effect Data				
Increased absolute kidney weight Gaoua (2004b)	F1 Female Sprague-Dawley rats	Dose (mg/kg-day)	0	250	500	1,000
		No. of animals	25	24	25	23
		Mean ± SD	2.24 ± 0.178	2.34 ± 0.242	2.3 ± 0.226	2.49 ± 0.284
Increased absolute kidney weight Fujii et al. (2010); JPEC (2008d)	Male Sprague-Dawley rats	Dose (mg/kg-day)	0	100	300	1,000
		No. of animals	24	24	24	24
		Mean ± SD	3.46 ± 0.57	3.62 ± 0.45	3.72 ± 0.35	4.07 ± 0.53
Increased relative kidney weight Fujii et al. (2010); JPEC (2008d)	Male Sprague-Dawley rats	Dose (mg/kg-day)	0	100	300	1,000
		No. of animals	24	24	24	24
		Mean ± SD	0.546 ± 0.059	0.592 ± 0.06	0.609 ± 0.042	0.689 ± 0.049
Increased absolute kidney weight Fujii et al. (2010); JPEC (2008d)	Female Sprague-Dawley rats	Dose (mg/kg-day)	0	100	300	1,000
		No. of animals	21	22	23	19
		Mean ± SD	2.17 ± 0.18	2.13 ± 0.14	2.17 ± 0.17	2.33 ± 0.24
Increased relative kidney weight Fujii et al. (2010); JPEC (2008d)	Female Sprague-Dawley rats	Dose (mg/kg-day)	0	100	300	1,000
		No. of animals	24	24	24	24
		Mean ± SD	0.674 ± 0.053	0.656 ± 0.048	0.668 ± 0.057	0.687 ± 0.045
INHALATION						
Urothelial hyperplasia of the renal pelvis Saito et al. (2013); JPEC (2010b)	Male F344 rats	Exposure concentration (mg/m^3)	0	2,090	6,270	20,900
		Incidence/Total	2/50	5/50	16/49	41/50

Table C-1. Noncancer endpoints selected for dose response modeling for ETBE (continued)

Endpoint, Study	Sex, Strain, Species	Doses and Effect Data					
Increased absolute kidney weight JPEC (2008a)	Male Sprague-Dawley rats	Exposure concentration (ppm)	0	150	500	1,500	5,000
		No. of animals	10	10	10	10	10
		Mean ± SD	3.15 ± 0.243	3.45 ± 0.385	3.49 ± 0.314	3.72 ± 0.365	3.64 ± 0.353
Increased absolute kidney weight JPEC (2008a)	Female Sprague-Dawley rats	Exposure concentration (ppm)	0	150	500	1,500	5,000
		No. of animals	10	10	10	10	10
		Mean ± SD	1.84 ± 0.129	1.85 ± 0.18	1.83 ± 0.118	1.92 ± 0.173	1.97 ± 0.16
Increased absolute kidney weight Medinsky et al. (1999); Bond et al. (1996b)	Male F344 rats	Exposure concentration (ppm)	0	500	1,750	5,000	
		No. of animals	11	11	11	11	
		Mean ± SD	1.73 ± 0.155	1.85 ± 0.137	1.903 ± 0.1	2.067 ± 0.124	
Increased absolute kidney weight Medinsky et al. (1999); Bond et al. (1996b)	Female F344 rats	Exposure concentration (ppm)	0	500	1,750	5,000	
		No. of animals	10	11	11	11	
		Mean ± SD	1.077 ± 0.069	1.125 ± 0.048	1.208 ± 0.076	1.306 ± 0.055	

Modeling Results

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

Oral Exposure Endpoints

Table C-2. Summary of BMD modeling results for urothelial hyperplasia of the renal pelvis in male F344 rats exposed to ETBE in drinking water for 104 weeks (IPEC, 2010a) modeled with doses as mg/kg-day (calculated by study authors); BMR = 10% extra risk

Model[a]	Goodness of Fit		BMD_{10Pct} (mg/kg-day)	$BMDL_{10Pct}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Gamma	0.196	127.93	88.1	60.9	Of the models that provided an adequate fit and a valid BMDL estimate, the Quantal-Linear model was selected based on lowest AIC.
Logistic	1.00×10^{-3}	139.54	217	177	
LogLogistic	0.264	127.28	85.3	49.5	
Probit	0.0015	138.30	197	162	
LogProbit	0.374	126.14	85.8	51.3	
Weibull	0.202	128.00	85.7	60.7	
Multistage 3°[b] Multistage 2°[c]	0.395	126.07	79.3	60.5	
Quantal-Linear[c]	**0.395**	**126.07**	**79.3**	**60.5**	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 28, 121, and 542 mg/kg-day were 0.000, −1.377, 1.024, and −0.187, respectively.
[b]For the Multistage 3° model, the beta coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Multistage 2° model.
[c]The Multistage 2° model and Quantal-Linear models appear equivalent; however, differences exist in digits not displayed in the table.

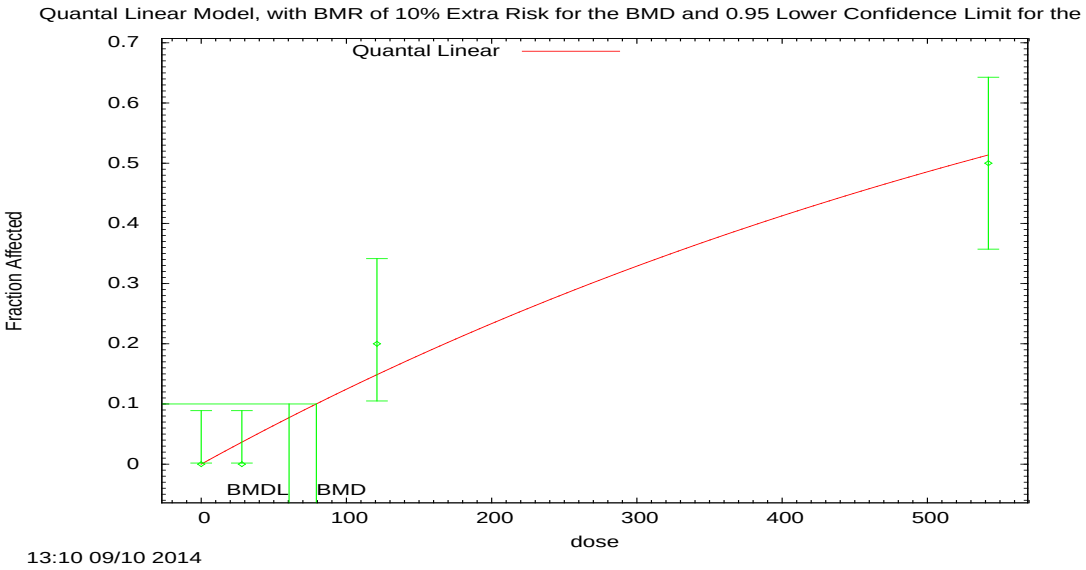


Figure C-1. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Quantal Linear Model using Weibull Model (Version: 2.16; Date: 2/28/2013)
The form of the probability function is: P[response] = background + (1-background) × [1-exp(− slope × dose)]

Benchmark Dose Computation.
BMR = 10% Extra risk
BMD = 79.3147
BMDL at the 95% confidence level = 60.5163

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0	0.0192308
Slope	0.00132839	0.00124304
Power	n/a	1

Analysis of Deviance Table

Model	Log (likelihood)	# Param's	Deviance	Test d.f.	*p*-value
Full model	−59.6775	4			
Fitted model	−62.0369	1	4.71891	3	0.1936
Reduced model	−92.7453	1	66.1356	3	<.0001

AIC = 126.074

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0	0	0	50	0
28	0.0365	1.826	0	50	−1.377
121	0.1485	7.424	10	50	1.024
542	0.5132	25.662	25	50	−0.187

χ^2 = 2.98; d.f = 3; *p*-value = 0.3948

Table C-3. Summary of BMD modeling results for increased absolute kidney weight in male S-D rats exposed to ETBE by daily gavage for 26 weeks (Miyata et al., 2013; JPEC, 2008d); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.752	−47.963	186	126	The linear model was selected based on lowest AIC.
Exponential (M4) Exponential (M5)[c]	0.603	−46.156	157	67.7	
Hill	0.605	−46.161	156	63.6	
Power[d] Polynomial 2°[e] Linear[f]	**0.774**	**−48.055**	**176**	**115**	
Polynomial 3°[g]	0.774	−48.055	176	115	

[a]Modeled variance case presented (BMDS Test 2 *p*-value = <0.0001), selected model in bold; scaled residuals for selected model for doses 0, 5, 25, 100, and 400 mg/kg-day were −0.421, −0.288, 1.29, −0.669, and 0.15, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.
[f]The Linear and Polynomial 3° models appear equivalent; however, differences exist in digits not displayed in the table.
[g]The Linear model, Polynomial 2° and 3° models and the Power models appear equivalent; however, differences exist in digits not displayed in the table.

1

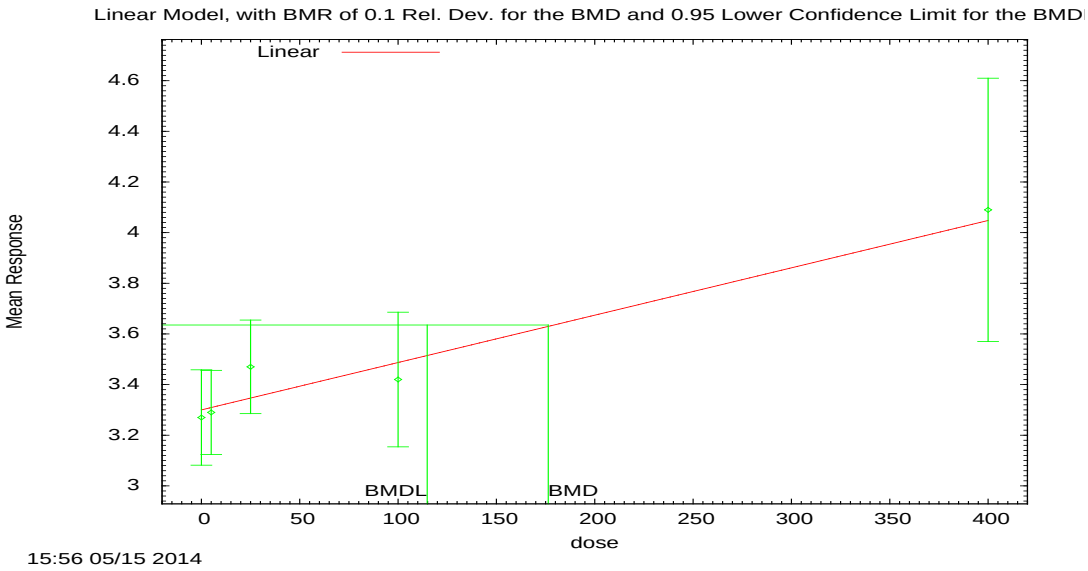


Figure C-2. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Polynomial Model. (Version: 2.17; Date: 01/28/2013)

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

A modeled variance is fit.

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 176.354

BMDL at the 95% confidence level = 114.829

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	−13.8218	−1.41289
rho	9.65704	0
beta_0	3.30477	3.30246
beta_1	0.00187393	0.00193902

Table of Data and Estimated Values of Interest

Dose	*N*	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	15	3.27	3.3	0.34	0.32	−0.421
5	15	3.29	3.31	0.3	0.325	−0.288
25	14	3.47	3.35	0.32	0.343	1.29
100	15	3.42	3.49	0.48	0.418	−0.669
400	13	4.09	4.05	0.86	0.859	0.15

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	17.455074	6	−22.910149
A2	29.755425	10	−39.51085
A3	28.583571	7	−43.167142
fitted	28.027315	4	−48.05463
R	6.041664	2	−8.083328

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	47.4275	8	<0.0001
Test 2	24.6007	4	<0.0001
Test 3	2.34371	3	0.5042
Test 4	1.11251	3	0.7741

Table C-4. Summary of BMD modeling results for increased absolute kidney weight in female S-D rats exposed to ETBE by daily gavage for 26 weeks (Miyata et al., 2013; JPEC, 2008d); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.369	−168.25	406	271	The Exponential (M4) model was selected based on lowest BMDL.
Exponential (M4)	**0.670**	**−168.60**	**224**	**56.9**	
Exponential (M5)	0.865	−167.37	error[c]	0	
Hill	0.986	−169.37	error[c]	error[c]	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.382	−168.34	402	263	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.425), selected model in bold; scaled residuals for selected model for doses 0, 5, 25, 100, and 400 mg/kg-day were 0.2257, 0.2206, −0.737, 0.3806, and −0.08999, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]BMD or BMDL computation failed for this model.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space), and the model reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

1

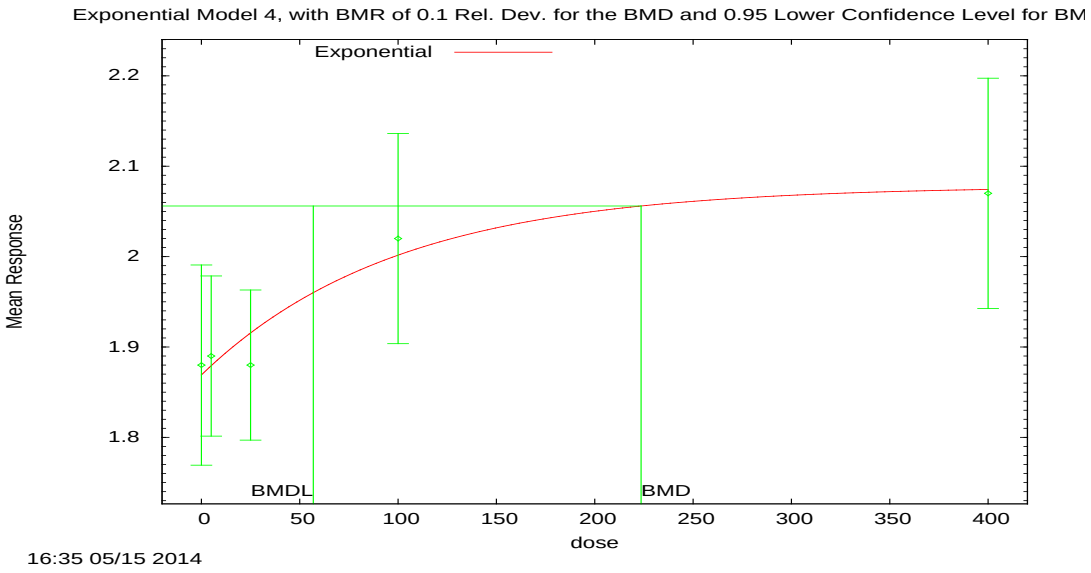


Figure C-3. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Exponential Model. (Version: 1.9; Date: 01/29/2013)

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 = 223.57

BMDL at the 95% confidence level = 56.8917

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
ln alpha	−3.35462	−3.36529
rho(S)	n/a	0
a	1.86911	1.786
b	0.0100557	0.00368689
c	1.11181	1.21697
d	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	15	1.88	1.869	0.2	0.1869	0.2257
5	15	1.89	1.879	0.16	0.1869	0.2206
25	15	1.88	1.916	0.15	0.1869	−0.737
100	15	2.02	2.002	0.21	0.1869	0.3806
400	15	2.07	2.074	0.23	0.1869	−0.08999

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	88.69837	6	−165.3967
A2	90.62918	10	−161.2584
A3	88.69837	6	−165.3967
R	82.20147	2	−160.4029
4	88.29837	4	−168.5967

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	16.86	8	0.03165
Test 2	3.862	4	0.4251
Test 3	3.862	4	0.4251
Test 6a	0.8	2	0.6703

Table C-5. Summary of BMD modeling results for increased absolute kidney weight in P0 male S-D rats exposed to ETBE by daily gavage for a total of 18 wk beginning 10 wk before mating until after weaning of the pups (Gaoua, 2004a); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.155	−38.410	551	423	The Hill model is selected based on lowest BMDL.
Exponential (M4)[c]	0.727	−40.012	255	123	
Exponential (M5)[c]	0.727	−40.012	255	123	
Hill	**0.811**	**−40.077**	**244**	**94.0**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.199	−38.902	517	386	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.119), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-day were −0.0247, 0.14, −0.181, and 0.0657, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]The Exponential (M4) model and the Exponential (M5) model appear equivalent; however, differences exist in digits not displayed in the table.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space, and the model reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

1

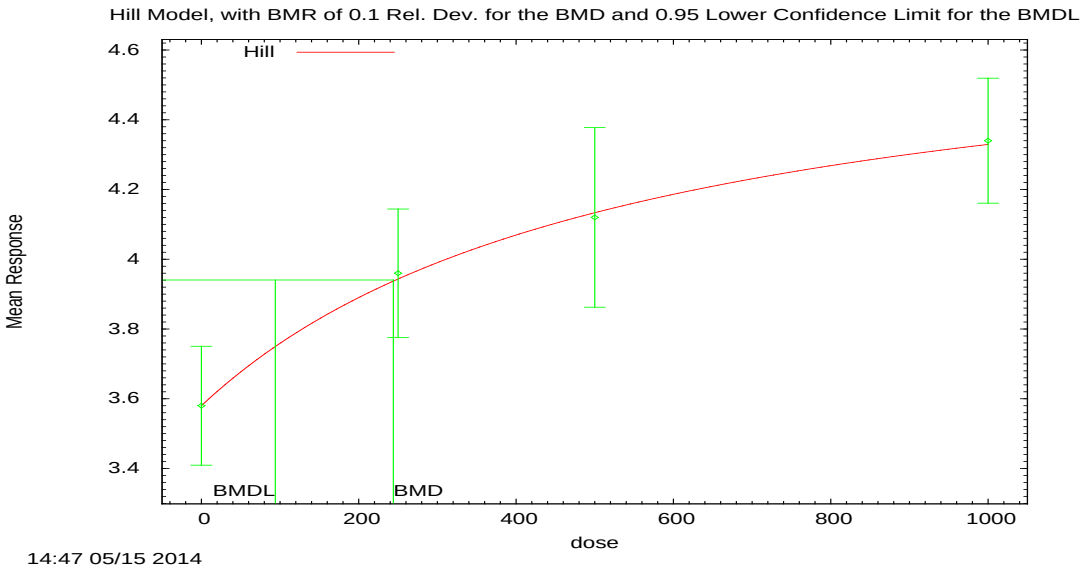


Figure C-4. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Hill Model. (Version: 2.17; Date: 01/28/2013)

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

A constant variance model is fit.

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 243.968

BMDL at the 95% confidence level = 93.9617

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.227462	0.236804
rho	n/a	0
intercept	3.58236	3.58
v	1.16337	0.76
n	1	0.647728
k	548.322	250

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	25	3.58	3.58	0.413	0.477	−0.0247
250	25	3.96	3.95	0.446	0.477	0.14
500	25	4.12	4.14	0.624	0.477	−0.181
1,000	25	4.34	4.33	0.434	0.477	0.0657

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	24.067171	5	−38.134342
A2	26.992591	8	−37.985183
A3	24.067171	5	−38.134342
fitted	24.038627	4	−40.077253
R	9.48179	2	−14.963581

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	35.0216	6	<0.0001
Test 2	5.85084	3	0.1191
Test 3	5.85084	3	0.1191
Test 4	0.057089	1	0.8112

Table C-6. Summary of BMD modeling results for increased absolute kidney weight in P0 female S-D rats exposed to ETBE by daily gavage for a total of 18 wk beginning 10 wk before mating until after weaning of the pups (Gaoua, 2004a); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)	**0.625**	**−214.58**	**1,734**	**1,030**	Exponential (M2) model is selected based on lowest AIC; however, BMDL is higher than the maximum dose.
Exponential (M3)	0.416	−212.86	1,458	1,040	
Exponential (M4)	0.327	−212.56	1,774	1,032	
Exponential (M5)	N/A[b]	−211.39	error[c]	0	
Hill	0.715	−213.39	error[c]	error[c]	
Power	0.418	−212.87	1,470	1,041	
Polynomial 3°	0.400	−212.81	1,409	1,035	
Polynomial 2°	0.400	−212.81	1,409	1,037	
Linear	0.619	−214.56	1,774	1,032	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.391), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-day were 0.5052, −0.7974, 0.1844, and 0.1033, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

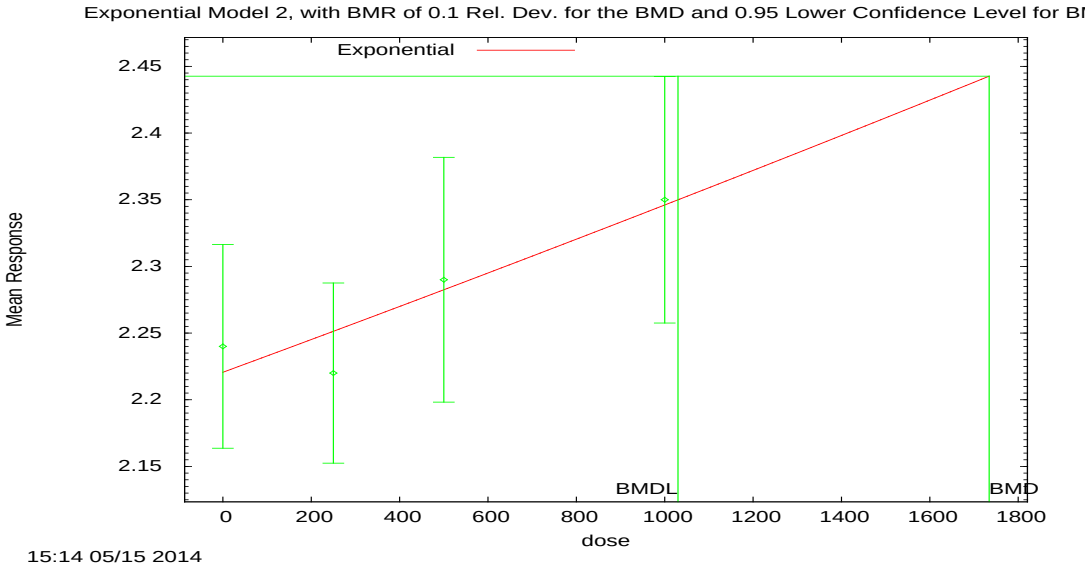


Figure C-5. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Exponential Model. (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a × exp(sign × b × dose)
A constant variance model is fit

Benchmark Dose Computation.
BMR = 10% Relative deviation
BMD = 1,734.24
BMDL at the 95% confidence level = 1,030.08

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
ln alpha	−3.29773	−3.30752
rho(S)	n/a	0
a	2.22057	2.22078
b	0.0000549578	0.0000546688
c	0	0
d	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	25	2.24	2.221	0.185	0.1923	0.5052
250	24	2.22	2.251	0.16	0.1923	−0.7974
500	22	2.29	2.282	0.207	0.1923	0.1844
1,000	25	2.35	2.346	0.224	0.1923	0.1033

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	110.761	5	−211.522
A2	112.2635	8	−208.5269
A3	110.761	5	−211.522
R	107.4777	2	−210.9553
2	110.2909	3	−214.5817

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	9.572	6	0.1439
Test 2	3.005	3	0.3909
Test 3	3.005	3	0.3909
Test 4	0.9403	2	0.6249

Table C-7. Summary of BMD modeling results for absolute kidney weight in F1 male Sprague-Dawley rats exposed to ETBE by gavage in a two-generation study (Gaoua, 2004b); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)	6.30×10^{-4}	89.912	232	175	Of the models that provided an adequate fit and a valid BMDL estimate, the Polynomial 3° model was selected based on lowest AIC.
Exponential (M3)	0.129	79.474	335	256	
Exponential (M4)	<0.0001	98.039	263	179	
Exponential (M5)	N/A[b]	82.504	347	267	
Hill	N/A[b]	82.509	347	267	
Power	0.0680	80.504	347	267	
Polynomial 3°	**0.374**	**77.965**	**318**	**235**	
Polynomial 2°	0.0943	79.973	330	251	
Linear	<0.0001	96.039	263	179	

[a]Modeled variance case presented (BMDS Test 2 *p*-value = <0.0001), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-day were −0.584, 0.717, 0.225, and −0.837, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.

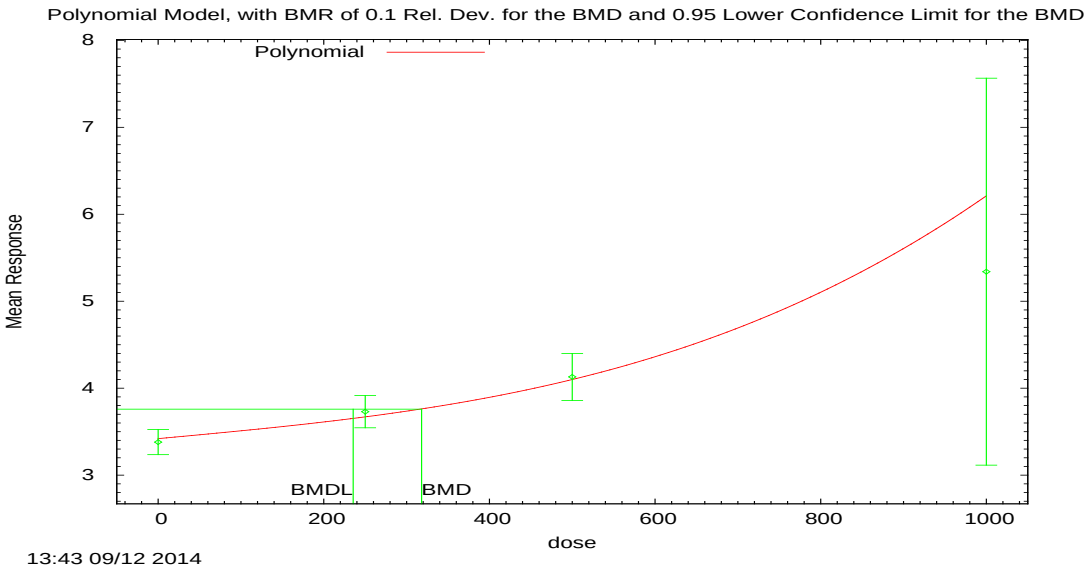


Figure C-6. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Polynomial Model. (Version: 2.19; Date: 06/25/2014)

The form of the response function is: Y[dose] = beta_0 + beta_1 × dose + beta_2 × dose^2 + …

A modeled variance is fit.

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 318.084

BMDL at the 95% confidence level = 235.491

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
ln alpha	−13.8779	2.02785
rho	9.40248	0
beta_0	3.41732	3.38
beta_1	0.000881597	0.00138667
beta_2	2.232×10^{-28}	0
beta_3	1.90507×10^{-9}	6.93333×10^{-9}

Table of Data and Estimated Values of Interest

Dose	*N*	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	24	3.38	3.42	0.341	0.313	−0.584
250	25	3.73	3.67	0.449	0.436	0.717
500	24	4.13	4.1	0.64	0.734	0.225
1,000	25	5.34	6.2	5.39	5.16	−0.837

Likelihoods of Interest

Model	Log(likelihood)	# Params	AIC
A1	−146.32249	5	302.644981
A2	−32.521507	8	81.043013
A3	−33.58656	6	79.17312
fitted	−33.982384	5	77.964768
R	−149.897277	2	303.794554

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	234.752	6	<0.0001
Test 2	227.602	3	<0.0001
Test 3	2.13011	2	0.3447
Test 4	0.791648	1	0.3736

Table C-8. Summary of BMD modeling results for absolute kidney weight in F1 female Sprague-Dawley rats exposed to ETBE by gavage in a two-generation study (Gaoua, 2004b); BMR = 10% relative deviation

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model selection
	p-value	AIC			
Exponential (M2)	**0.311**	**−180.23**	**978**	**670**	**Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential (M2) model was selected based on lowest AIC.**
Exponential (M3)	0.147	−178.46	1,016	679	
Exponential (M4)	0.121	−178.16	980	654	
Exponential (M5)	N/A[b]	−176.44	1,019	613	
Hill	N/A[b]	−176.44	1,019	611	
Power	0.145	−178.44	1,019	666	
Polynomial 3°	0.184	−178.80	1,001	684	
Polynomial 2°	0.159	−178.58	1,002	673	
Linear	0.301	−180.16	980	654	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.159), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1,000 mg/kg-day were −0.05426, 0.8898, −1.173, and 0.3711, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.

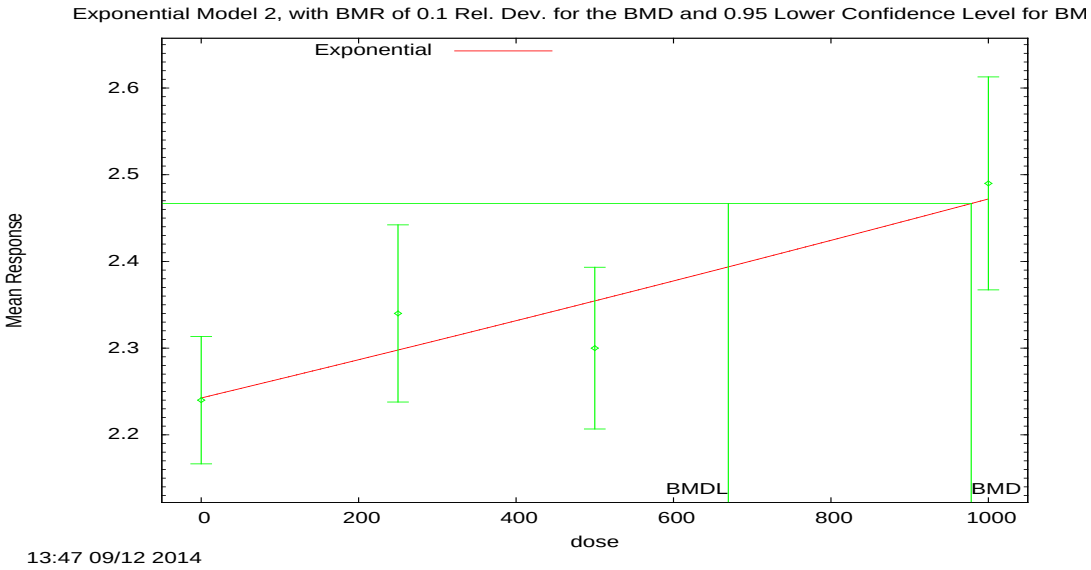


Figure C-7. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Exponential Model. (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a × exp(sign × b × dose)
A constant variance model is fit.

Benchmark Dose Computation.
BMR = 10% Relative deviation
BMD = 978.157
BMDL at the 95% confidence level = 669.643

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
ln alpha	−2.91989	−2.94397
rho(S)	n/a	0
a	2.24252	2.24321
b	0.0000974385	0.000096475
c	0	0
d	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	25	2.24	2.243	0.178	0.2322	−0.05426
250	24	2.34	2.298	0.242	0.2322	0.8898
500	25	2.3	2.354	0.226	0.2322	−1.173
1,000	23	2.49	2.472	0.284	0.2322	0.3711

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	94.28268	5	−178.5654
A2	96.87585	8	−177.7517
A3	94.28268	5	−178.5654
R	87.16418	2	−170.3284
2	93.11474	3	−180.2295

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	19.42	6	0.003505
Test 2	5.186	3	0.1587
Test 3	5.186	3	0.1587
Test 4	2.336	2	0.311

Table C-9. Summary of BMD modeling results for increased absolute kidney weight in P0 male S-D rats exposed to ETBE by daily gavage for 16 weeks beginning 10 weeks prior to mating (Fujii et al., 2010); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.668	−41.247	648	479	The Hill model was selected based on lowest BMDL. (BMDLs were greater than threefold difference.)
Exponential (M4) Exponential (M5)[c]	0.600	−39.779	438	163	
Hill	**0.613**	**−39.799**	**435**	**139**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.700	−41.342	625	448	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.102), selected model in bold; scaled residuals for selected model for doses 0, 100, 300, and 1,000 mg/kg-day were −0.202, 0.399, −0.232, and 0.0344, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space), and the model reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

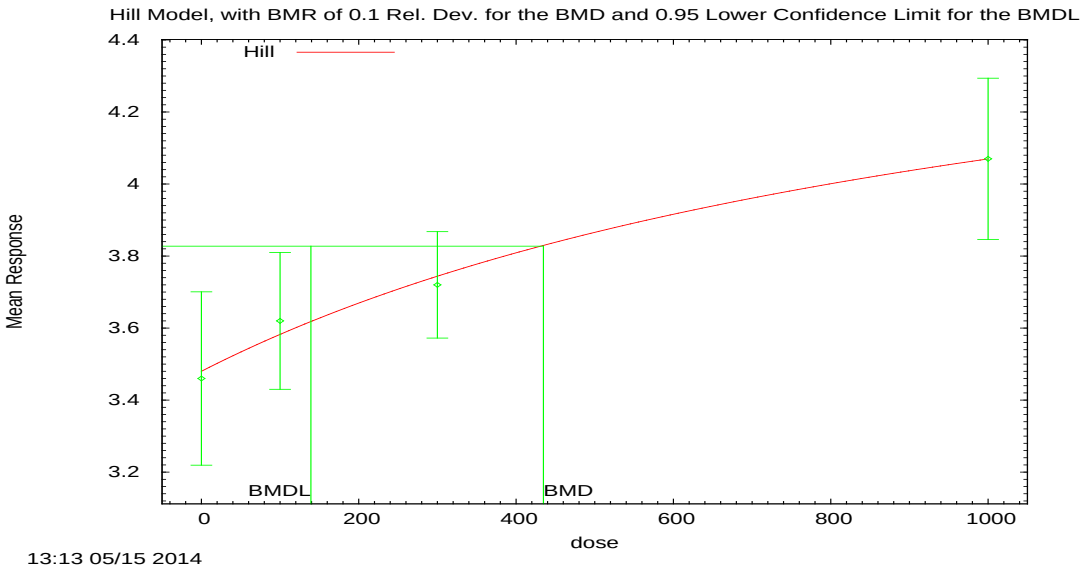


Figure C-8. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Hill Model. (Version: 2.17; Date: 01/28/2013)

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

A constant variance model is fit.

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 434.715

BMDL at the 95% confidence level = 139.178

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.223598	0.2327
rho	n/a	0
intercept	3.47949	3.46
v	1.24601	0.61
n	1	0.27452
k	1,122	1,610

Table of Data and Estimated Values of Interest

Dose	*N*	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	24	3.46	3.48	0.57	0.473	−0.202
100	24	3.62	3.58	0.45	0.473	0.399
300	24	3.72	3.74	0.35	0.473	−0.232
1,000	24	4.07	4.07	0.53	0.473	0.0344

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	24.027112	5	−38.054223
A2	27.13071	8	−38.26142
A3	24.027112	5	−38.054223
fitted	23.899392	4	−39.798783
R	14.313578	2	−24.627156

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	25.6343	6	0.0002604
Test 2	6.2072	3	0.102
Test 3	6.2072	3	0.102
Test 4	0.25544	1	0.6133

Table C-10. Summary of BMD modeling results for increased absolute kidney weight in P0 female S-D rats exposed to ETBE by daily gavage for 17 weeks beginning 10 weeks prior to mating until lactation day 21 (Fujii et al., 2010); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (mg/kg-day)	$BMDL_{10RD}$ (mg/kg-day)	Basis for Model Selection
	p-value	AIC			
Exponential (M2)	0.483	−199.73	1,135	781	Polynomial 2° is selected based on most parsimonious model with lowest AIC.
Exponential (M3)	0.441	−198.60	1,089	826	
Exponential (M4)	0.217	−197.67	1,144	771	
Exponential (M5)	N/A[b]	−196.66	error[c]	0	
Hill	N/A[b]	−196.66	error[c]	error[c]	
Power	0.441	−198.60	1,092	823	
Polynomial 3°[d] Polynomial 2°	**0.743**	**−200.60**	**1,094**	**905**	
Linear	0.467	−199.67	1,144	771	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.103), selected model in bold; scaled residuals for selected model for doses 0, 100, 300, and 1,000 mg/kg-day were 0.499, −0.579, 0.0914, and −0.00282, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.
[d]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space), and the model reduced to the Polynomial 2° model.

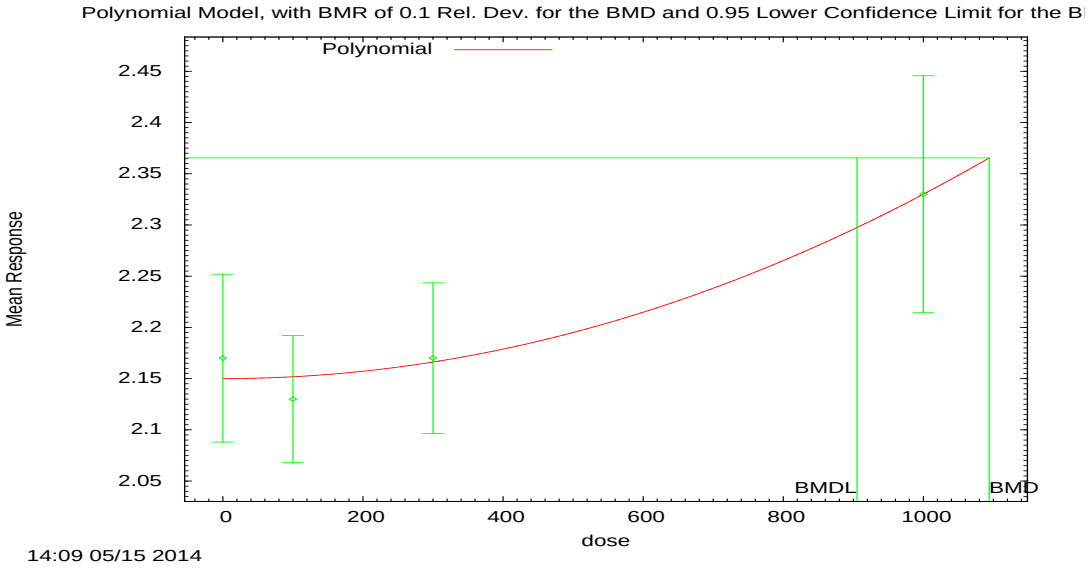


Figure C-9. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-day.

Polynomial Model. (Version: 2.17; Date: 01/28/2013)
The form of the response function is: Y[dose] = beta_0 + beta_1 × dose + beta_2 × dose^2 + …
A constant variance model is fit.

Benchmark Dose Computation.
BMR = 10% Relative deviation
BMD = 1,093.86
BMDL at the 95% confidence level = 905.267

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.0323691	0.0337309
rho	n/a	0
beta_0	2.1504	2.15624
beta_1	7.16226×10^{-28}	0
beta_2	1.79719×10^{-6}	0

Table of Data and Estimated Values of Interest

Dose	*N*	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	21	2.17	2.15	0.18	0.18	0.499
100	22	2.13	2.15	0.14	0.18	−0.579
300	23	2.17	2.17	0.17	0.18	0.0914
1,000	19	2.33	2.33	0.24	0.18	−0.00282

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	103.595625	5	−197.191249
A2	106.684319	8	−197.368637
A3	103.595625	5	−197.191249
fitted	103.298361	3	−200.596722
R	96.89324	2	−189.78648

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	19.5822	6	0.003286
Test 2	6.17739	3	0.1033
Test 3	6.17739	3	0.1033
Test 4	0.594528	2	0.7428

Inhalation Exposure Endpoints

Table C-11. Summary of BMD modeling results for urothelial hyperplasia of the renal pelvis in male F344 rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5 d/wk, for 104 wk (JPEC, 2010b); BMR = 10% extra risk

Model[a]	Goodness of Fit		BMC_{10Pct} (mg/m^3)	$BMCL_{10Pct}$ (mg/m^3)	Basis for Model Selection
	p-value	AIC			
Gamma	**0.874**	**164.37**	**2,734**	**1,498**	**Of the models that provided an adequate fit and a valid BMCL estimate, the Gamma model was selected based on lowest AIC.**
Logistic	0.146	166.30	4,329	3,522	
LogLogistic	0.814	164.40	3,010	1,831	
Probit	0.202	165.59	4,059	3,365	
LogProbit	0.633	164.57	3,050	1,896	
Weibull	0.758	164.44	2,623	1,478	
Multistage 3°	0.565	164.69	2,386	1,412	
Multistage 2°	0.565	164.69	2,386	1,422	
Quantal-Linear	0.269	165.16	1,541	1,227	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 2,089, 6,268, and 20,893 mg/m^3 were 0.036, −0.107, 0.104, and −0.040, respectively. Exposure concentrations were converted from 0, 500, 1,500, and 5,000 ppm to mg/m^3 using the calculation mg/m^3 = (102.17, molecular weight of ETBE) × ppm ÷ 24.45.

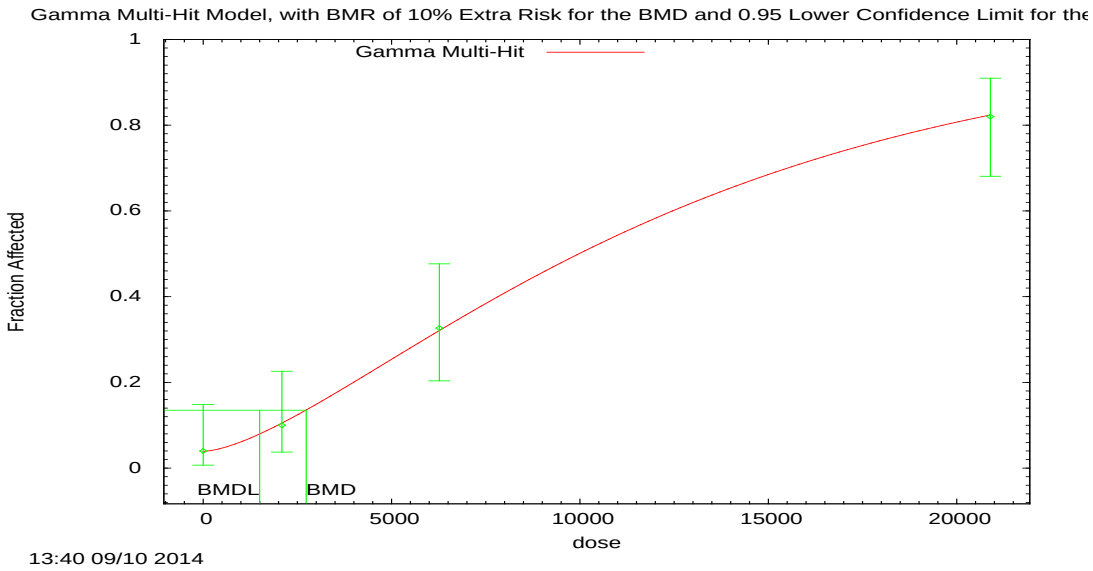


Figure C-10. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in mg/m³.

Gamma Model. (Version: 2.16; Date: 2/28/2013)

The form of the probability function is:

P[response] = background + (1-background) × CumGamma[slope×dose,power], where CumGamma(.) is the cumulative Gamma distribution function.

Power parameter is restricted as power > = 1.

Benchmark Dose Computation.

BMR = 10% Extra risk

BMD = 2,734.41

BMDL at the 95% confidence level = 1,497.7

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
Background	0.0390054	0.0576923
Slope	0.000121504	0.000132454
Power	1.59019	1.84876

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	*p*-value
Full model	−79.1741	4			
Fitted model	−79.1867	3	0.0253512	1	0.8735
Reduced model	−124.987	1	91.626	3	<.0001

AIC = 164.373

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0.039	1.95	2	50	0.036
2,089	0.1046	5.231	5	50	−0.107
6,268	0.3196	15.659	16	49	0.104
20,893	0.8222	41.109	41	50	−0.04

χ^2 = 0.03; d.f = 1; *p*-value = 0.8737

Table C-12. Summary of BMD modeling results for increased absolute kidney weight in male S-D rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5 d/wk for 13 wk (IPEC, 2008b); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (ppm)	$BMDL_{10RD}$ (ppm)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.168	−43.014	1,105	750	Of the models that provided an adequate fit and a valid BMDL estimate, the Hill model was selected based on lowest BMDL (BMDLs differed by more than 3).
Exponential (M4)	0.200	−42.943	380	1.73	
Exponential (M5)	0.200	−42.943	380	2.61	
Hill	**0.294**	**−43.484**	**264**	**15.4**	
Power[c] Polynomial 3°[d] Polynomial 2°[e] Linear	0.178	−43.133	1,071	703	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.506), selected model in bold; scaled residuals for selected model for doses 0, 150, 500, and 1,500 ppm were −0.13, 0.54, −0.8, 0.38, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[d]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space), and the model reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[e]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

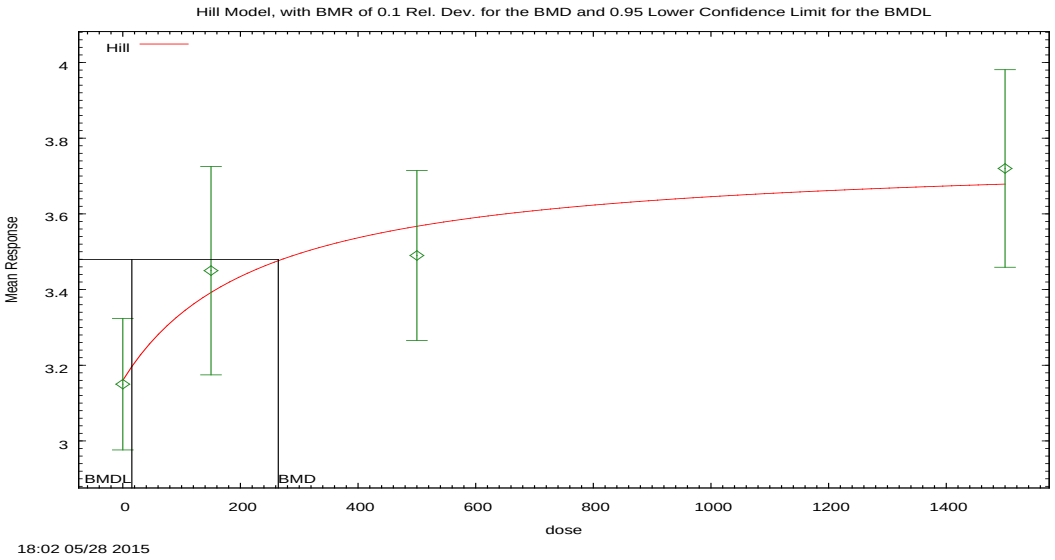


Figure C-11. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

Hill Model. (Version: 2.17; Date: 01/28/2013)
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 deviation
BMD = 264.371
BMDL at the 95% confidence level = 15.4115

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.101559	0.109774
rho	n/a	0
intercept	3.16295	3.15
v	0.600878	0.57
n	1	0.169179
k	237.864	157.5

Table of Data and Estimated Values of Interest

Dose	*N*	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	10	3.15	3.16	0.24	0.32	−0.129
150	10	3.45	3.4	0.38	0.32	0.542
500	10	3.49	3.57	0.31	0.32	−0.795
1,500	10	3.72	3.68	0.36	0.32	0.381

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	26.293887	5	−42.587775
A2	27.46147	8	−38.922941
A3	26.293887	5	−42.587775
fitted	25.742228	4	−43.484456
R	19.334386	2	−34.668772

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	16.2542	6	0.01245
Test 2	2.33517	3	0.5058
Test 3	2.33517	3	0.5058
Test 4	1.10332	1	0.2935

Table C-13. Summary of BMD modeling results for increased absolute kidney weight in female S-D rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5 d/wk for 13 wk (JPEC, 2008b); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMD_{10RD} (ppm)	$BMDL_{10RD}$ (ppm)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.8	−135.38	6,790	4,046	The Linear model is selected based on lowest AIC; however, the BMD is higher than the maximum dose.
Exponential (M4)	0.731	−133.76	error[c]	0	
Exponential (M5)	0.760	−132.29	error[c]	0	
Hill	0.760	−132.29	error[c]	error[c]	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	**0.806**	**−135.40**	**6,840**	**3,978**	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.623), selected model in bold; scaled residuals for selected model for doses 0, 150, 500, 1,500, and 5,000 ppm were −0.0742, 0.0535, −0.578, 0.774, and −0.176, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]BMD or BMDL computation failed for this model.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space), and the model reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

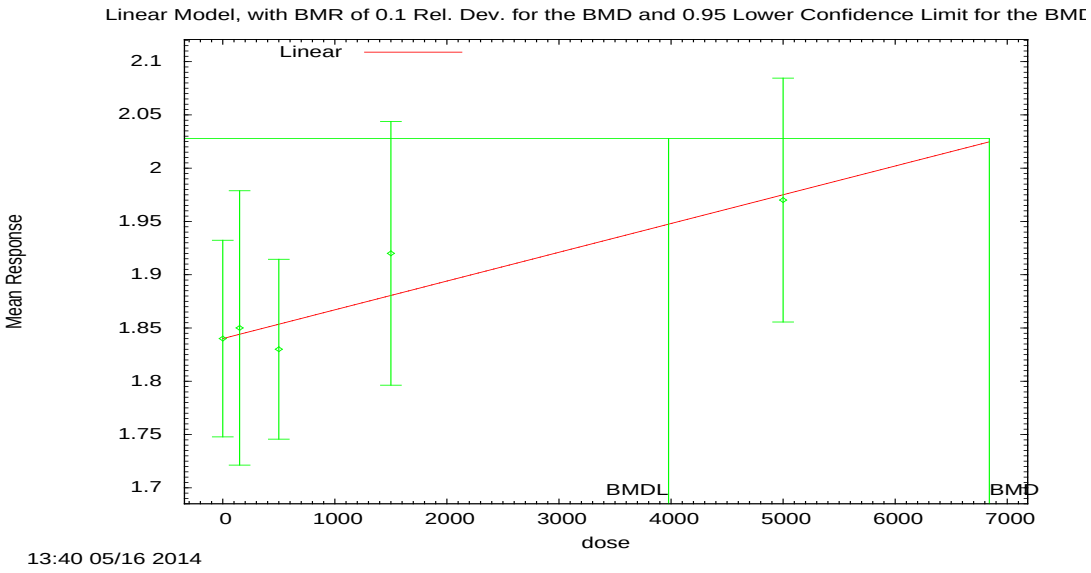


Figure C-12. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

Polynomial Model. (Version: 2.17; Date: 01/28/2013)

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 = 6,840.02

BMDL at the 95% confidence level = 3,978.09

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.021752	0.0236988
rho	n/a	0
beta_0	1.84346	1.84346
beta_1	0.0000269511	0.0000269511

Table of Data and Estimated Values of Interest

Dose	*N*	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	10	1.84	1.84	0.129	0.147	−0.0742
150	10	1.85	1.85	0.18	0.147	0.0535
500	10	1.83	1.86	0.118	0.147	−0.578
1,500	10	1.92	1.88	0.173	0.147	0.774
5,000	10	1.97	1.98	0.16	0.147	−0.176

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	71.192285	6	−130.384569
A2	72.502584	10	−125.005168
A3	71.192285	6	−130.384569
fitted	70.701239	3	−135.402478
R	67.96809	2	−131.93618

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	9.06899	8	0.3365
Test 2	2.6206	4	0.6232
Test 3	2.6206	4	0.6232
Test 4	0.982091	3	0.8056

Table C-14. Summary of BMD modeling results for increased absolute kidney weight in male F344 rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5 d/wk, for 13 wk (Medinsky et al., 1999; Bond et al., 1996b); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMC_{10RD} (ppm)	$BMCL_{10RD}$ (ppm)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.184	−129.97	3,107	2,439	The Hill model was selected based on lowest BMDL.
Exponential (M4) Exponential (M5)[c]	0.199	−129.71	1,798	808	
Hill	**0.224**	**−129.89**	**1,667**	**603**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.208	−130.22	2,980	2,288	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.540), selected model in bold; scaled residuals for selected model for doses 0, 500, 1,750, and 5,000 ppm were −0.441, 0.91, —0.635, and 0.166, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space), and the model reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

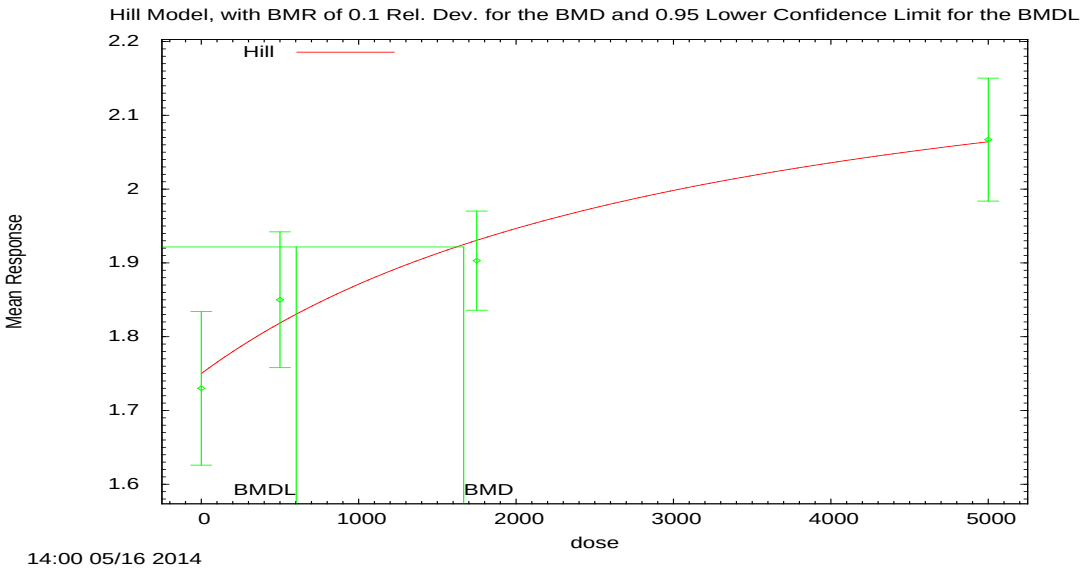


Figure C-13. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

Hill Model. (Version: 2.17; Date: 01/28/2013)

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

A constant variance model is fit.

Benchmark Dose Computation.

BMR = 10% Relative deviation

BMD = 1,666.92

BMDL at the 95% confidence level = 603.113

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.0160221	0.0170425
rho	n/a	0
intercept	1.74684	1.73
v	0.521534	0.337
n	1	0.225826
k	3,309.8	1,856.13

Table of Data and Estimated Values of Interest

Dose	*N*	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	11	1.73	1.75	0.155	0.127	−0.441
500	11	1.85	1.82	0.137	0.127	0.91
1,750	11	1.9	1.93	0.1	0.127	−0.635
5,000	11	2.07	2.06	0.124	0.127	0.166

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	69.681815	5	−129.36363
A2	70.76062	8	−125.521241
A3	69.681815	5	−129.36363
fitted	68.943332	4	−129.886663
R	55.026208	2	−106.052416

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	31.4688	6	<0.0001
Test 2	2.15761	3	0.5403
Test 3	2.15761	3	0.5403
Test 4	1.47697	1	0.2242

Table C-15. Summary of BMD modeling results for increased absolute kidney weight in female F344 rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5 d/wk, for 13 wk (Medinsky et al., 1999; Bond et al., 1996b); BMR = 10% relative deviation from the mean

Model[a]	Goodness of Fit		BMC_{10RD} (ppm)	$BMCL_{10RD}$ (ppm)	Basis for Model Selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0630	−187.67	2,706	2,275	The Exponential (M4) model was selected as the most parsimonious model of adequate fit.
Exponential (M4) Exponential (M5)[c]	**0.956**	**−191.20**	**1,342**	**816**	
Hill	N/A[d]	−189.20	1,325	741	
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	0.0928	−188.45	2,552	2,111	

[a]Constant variance case presented (BMDS Test 2 *p*-value = 0.428), selected model in bold; scaled residuals for selected model for doses 0, 500, 1,750, and 5,000 ppm were −0.0252, 0.043, −0.02385, and 0.004872, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary), and the model reduced to the Exponential (M4) model.
[d]No available degrees of freedom to calculate a goodness-of-fit value.
[e]For the Power model, the power parameter estimate was 1, and the model reduced to the Linear model.
[f]For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space), and the model reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space), and the model reduced to the Linear model.
[g]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space), and the model reduced to the Linear model.

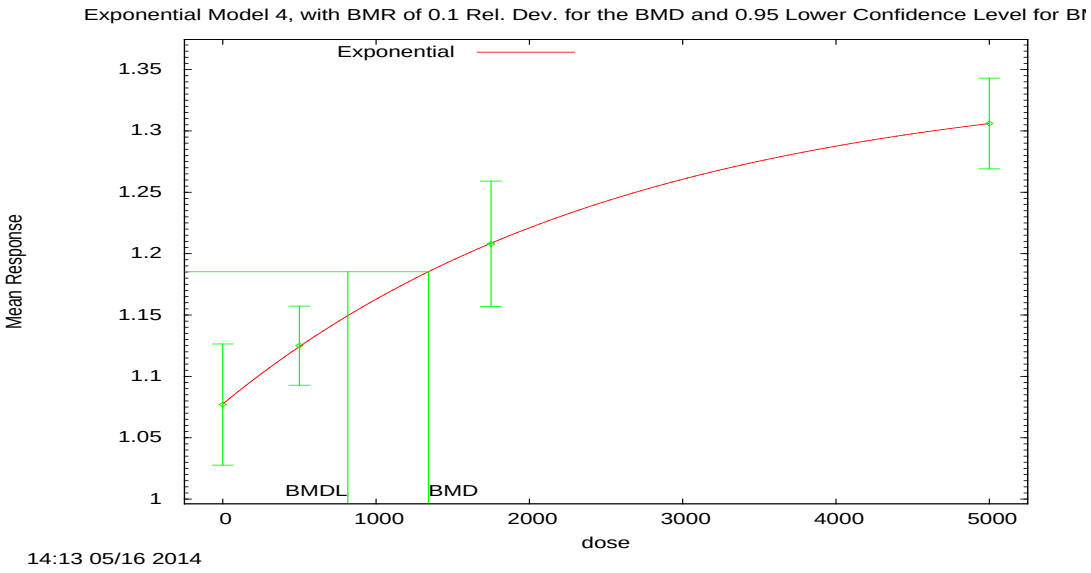


Figure C-14. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

Exponential Model. (Version: 1.9; Date: 01/29/2013)

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 = 1,341.66

BMDL at the 95% confidence level = 815.742

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
ln alpha	−5.63259	−5.63266
rho(S)	n/a	0
a	1.07748	1.02315
b	0.000383798	0.000348471
c	1.24847	1.34027
d	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	1.077	1.077	0.069	0.05983	−0.0252
500	11	1.125	1.124	0.048	0.05983	0.043
1,750	11	1.208	1.208	0.076	0.05983	−0.02385
5,000	11	1.306	1.306	0.055	0.05983	0.004872

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	99.60217	5	−189.2043
A2	100.9899	8	−185.9798
A3	99.60217	5	−189.2043
R	75.30605	2	−146.6121
4	99.60063	4	−191.2013

Tests of Interest

Test	−2*log(Likelihood Ratio)	Test df	*p*-value
Test 1	51.37	6	<0.0001
Test 2	2.775	3	0.4276
Test 3	2.775	3	0.4276
Test 6a	0.003077	1	0.9558

C.1.2. Cancer Endpoints

For the multistage cancer models, the coefficients were restricted to be non-negative (beta's ≥ 0). For each endpoint, multistage cancer models were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 *p*-value < 0.05[3] 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 in the vicinity of 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. If the BMDL estimates were "sufficiently close," that is, differed by more than threefold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

[3]A significance level of 0.05 is used for selecting cancer models because the model family (multistage) is selected a priori *Benchmark Dose Technical Guidance Document*, U.S. EPA (2012).

The incidence of liver tumors in male F344 rats was found to be statistically significantly increased following a 2-year inhalation exposure; hepatocellular adenomas and a single hepatocellular carcinoma in the high-dose group were combined in modeling the data set. The data were modeled using two different exposure metrics: administered concentration as ppm, and administered concentration as mg/m^3.

Table C-16. Cancer endpoints selected for dose-response modeling for ETBE

Species/Sex Endpoint	Doses and Effect Data				
Hepatocellular adenomas and carcinomas in male rats JPEC (2010b)	Exposure Concentration (ppm)	0	500	1,500	5,000
	Exposure Concentration (mg/m^3)	0	2,089	6,268	20,893
	Incidence/Total	0/50	2/50	1/49	10/50

Modeling Results

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

Table C-17. Summary of BMD modeling results for hepatocellular adenomas and carcinomas in male F344 rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5d/wk, for 104 wk; modeled with doses as administered exposure concentration in ppm (JPEC, 2010b); BMR = 10% extra risk

Model[a]	Goodness of Fit			BMC_{10Pct} (ppm)	$BMCL_{10Pct}$ (ppm)	Basis for Model Selection
	p-value	Scaled Residuals	AIC			
Three	0.0991	−0.030, 1.382, −0.898, and 0.048	84.961	2,942	1,735	Multistage 1° was selected based on lowest AIC.
Two	0.264	0.000, 1.284, −1.000, and 0.137	83.093	2,756	1,718	
One	**0.490**	**0.000, 1.009, −1.144, and 0.309**	**81.208**	**2,605**	**1,703**	

[a]Selected model in bold.

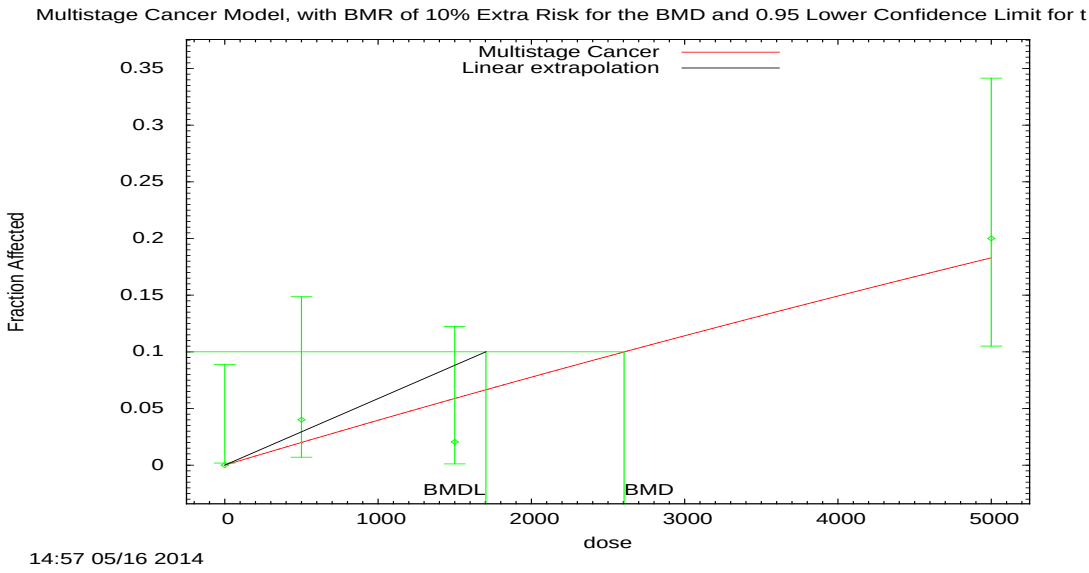


Figure C-15. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in ppm.

Multistage Model. (Version: 3.4; Date: 05/02/2014)
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 = 2,604.82
BMDL at the 95% confidence level = 1,703.47
BMDU at the 95% confidence level = 4,634.52
Collectively, (1,703.47, 4,634.52) 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	0
Beta(1)	4.04483×10^{-4}	4.38711×10^{-4}

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	*p*-value
Full model	−38.2989	4			
Fitted model	−39.6042	1	2.61063	3	0.4556
Reduced model	−48.0344	1	19.4711	3	0.0002184

AIC = 81.2084

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0	0	0	50	0
500	0.02	1.001	2	50	1.009
1,500	0.0589	2.885	1	49	−1.144
5,000	0.1831	9.155	10	50	0.309

χ^2 = 2.42; d.f = 3; *p*-value = 0.4898

Table C-18. Summary of BMD modeling results for hepatocellular adenomas and carcinomas in male F344 rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5d/wk, for 104 wk; modeled with doses as mg/m^3 (IPEC, 2010b); BMR = 10% extra risk

Model[a]	Goodness of Fit			BMD_{10Pct} (mg/m^3)	$BMDL_{10Pct}$ (mg/m^3)	Basis for Model Selection
	p-value	Scaled Residuals	AIC			
Three	0.0991	−0.040, 1.382, −0.897, and 0.048	84.961	12,300	7,251	Multistage 1° was selected based on lowest AIC
Two	0.264	0.000, 1.284, −1.000, and 0.137	83.093	11,514	7,179	
One	**0.490**	**0.000, 1.009, −1.144, and 0.309**	**81.209**	**10,884**	**7,118**	

[a]Selected model in bold.

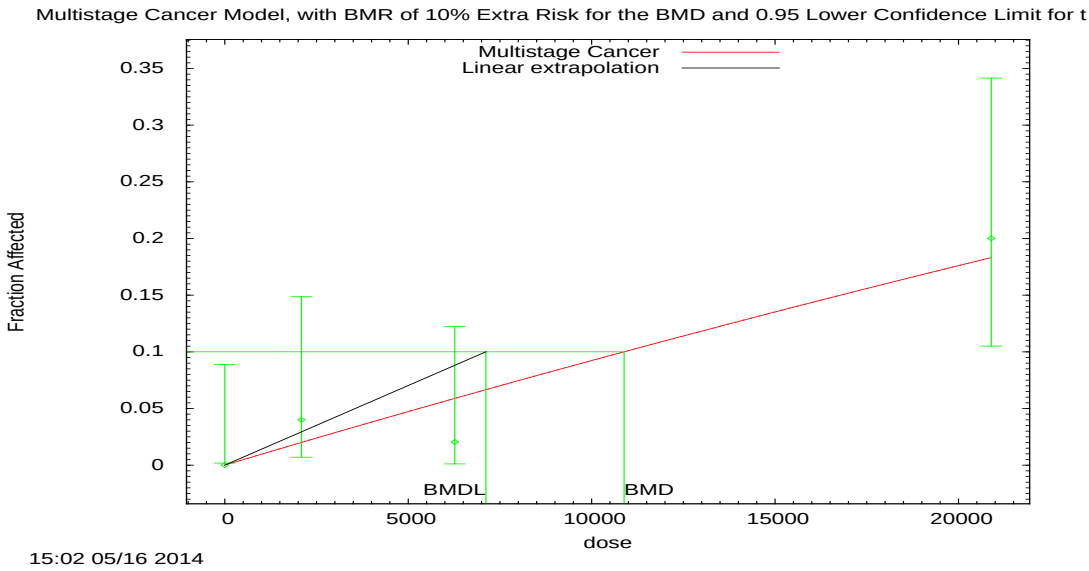


Figure C-16. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in mg/m³.

Multistage Model. (Version: 3.4; Date: 05/02/2014)
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 = 10,884.4
BMDL at the 95% confidence level = 7,118.08
BMDU at the 95% confidence level = 19,366.3
Collectively, (7,118.08, 19,366.3) 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	0
Beta(1)	9.6799×10^{-6}	1.04989×10^{-4}

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	*p*-value
Full model	−38.2989	4			
Fitted model	−39.6044	1	2.61098	3	0.4556
Reduced model	−48.0344	1	19.4711	3	0.0002184

AIC = 81.2087

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0	0	0	50	0
2,089	0.02	1.001	2	50	1.009
6,268	0.0589	2.885	1	49	−1.144
20,893	0.1831	9.155	10	50	0.309

χ^2 = 2.42; d.f = 3; *p*-value = 0.4897

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

The Toxicological Review of ethyl tertiary butyl ether (ETBE) was released for a 60-day public comment period on September 1, 2016. Public comments on the assessment were submitted to EPA by:

- Japan Petroleum Energy Center (posted November 1 and November 3, 2016),
- Exponent, Inc. on behalf of LyondellBasell (posted October 24, 2016),
- LyondellBasell (posted October 20, 2016 and November 3, 2016),
- American Chemistry Council (posted October 28, 2016),
- Tox Strategies on behalf of LyondellBasell (posted October 24, 2016), and
- American Petroleum Institute (posted November 1, 2016).

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

Comments Related to Kidney Effects

Comment [LyondellBasell]: The selection of urothelial hyperplasia as the key endpoint reflecting a potential human kidney hazard from ETBE exposure is inappropriate because urothelial hyperplasia is associated with chronic progressive nephropathy (CPN). In addition, CPN should not be considered relevant to humans because it is rat-specific with no known human counterpart.

EPA Response: Section 1.21 shows that urothelial hyperplasia is weakly correlated with CPN. CPN is a common and well-established constellation of age-related lesions in the kidney of rats, and there is no known counterpart to CPN in aging humans. However, CPN is not a specific diagnosis but is a spectrum of lesions. These individual lesions or processes (tubular degeneration/regeneration and dilatation, glomerular sclerosis and atrophy, interstitial fibrosis and inflammation, etc.) could certainly occur in a human kidney. Because they happen to occur as a group in the aged rat kidney does not necessarily make them rat-specific individually if there is a treatment effect for one or more of them. In addition, exacerbation of one or more of these processes likely reflects some type

of cell injury/cytotoxicity, which is relevant to the human kidney. Different federal agencies have considered CPN exacerbation not confounded by α_{2u}-globulin to be a basis for reference values. For instance, FDA also used CPN in their draft calculation of PDEs for MIBK (http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm467089.pdf). EPA considers CPN exacerbation to be relevant for human health.

Comment [LyondellBasell]: Dismissal of α_{2u}-globulin nephropathy as an operative MOA for ETBE is not scientifically justified. Multiple studies reported that ETBE induced hyaline droplets, and one group observed that those droplets had angular profiles characteristic of accumulating α_{2u}-globulin (Cohen et al., 2011). Granular casts were observed in a 13-week study by two independent groups (indicative of cell exfoliation), and linear papillary mineralization by several groups. Increased tubule cell proliferation was reported to be sustained over a period of 1 to 13 weeks. Development of renal tubule hyperplasia is not a necessary histopathological step for identifying α_{2u}-globulin nephropathy. When it does occur, it is an outcome of that histopathological sequence. Thus, the absence of tubule hyperplasia (or renal tubule tumors) does not rule out an α_{2u}-globulin MOA.

EPA Response: EPA does not discount the evidence for ETBE induction of hyaline droplets or α_{2u}-globulin. Granular casts were observed in one experiment observed by two independent sets of pathologists, which does not offer an explanation of why other studies failed to observe them despite similar durations and doses. Tubule cell proliferation was reported in both male and female rats, which supports a non-α_{2u}-globulin mechanism for this effect. EPA agrees that absence of one lesion does not rule out α_{2u}-globulin; however, absence of several lesions may. The criteria for establishing an α_{2u}-globulin mechanism does not offer alternative criteria for weak inducers of α_{2u}-globulin to explain or allow for absence of evidence in the histopathological sequence.

Comments Related to Liver Effects

Regarding the Possible Mode of Action

Comment [American Petroleum Institute]: The draft review concludes that a mode of action (MOA) for the high-dose male rat liver tumors could not be established, and in the absence of information to indicate otherwise, the liver tumors induced by ETBE are considered to be relevant to humans. We encourage the Agency to fully review the mode of action research of the Japan Petroleum Energy Center (JPEC) provided in comments to EPA docket for ETBE. Interpretations from the JPEC research program differ from those of EPA and conclude that the mode of action for ETBE high-dose liver tumors in male rats is unlikely to be relevant to humans. The basis for this difference in data interpretation is not clear in the draft IRIS review document. Although EPA states that data are inadequate to conclude that ETBE induces liver tumors via a PPARα MOA, a CAR/PXR MOA, or an acetaldehyde-mediated mutagenicity, the rationale underlying these Agency conclusions is not

clearly described in sufficient detail to understand EPA's views regarding the shortcomings of the data set or how it could be improved.

EPA Response: The draft was modified in section 1.2.2 to clarify the rationale for why the data are inadequate to establish a conclusion for these proposed MOAs. Specifically, all positive evidence related to the 10 key characteristics of cancer were grouped and summarized in Table 1-13. This summary of the evidence provides a more holistic approach for organizing and further discussing cancer MOAs and is a more transparent presentation of potentially informative data gaps. In addition, several gaps in the receptor-mediated effects data were explicitly noted such as evidence in only one species, lack of any studies in PPAR KO mice, lack of dose-response concordance between receptor-mediated gene changes and tumors, and lack of any receptor-mediated data outside of the 1- and 2-week time-points, which preclude establishing temporal associations. These data gaps led to the conclusion that the receptor-mediated MOA data are inadequate to establish conclusions.

Comment [Japan Petroleum Energy Center]: Cellular hypertrophy was likely a result of microsome proliferation and increased synthesis of microsomal cytochrome P450 enzymes. Significant increase of hydroxyl radical levels by Week 2 of ETBE exposure accompanied the accumulation of 8-OHdG in the nucleus and P450 isoenzymes CYP2B1/2, CYP3A1/2 etc., and increase of peroxisomes in the cytoplasm of hepatocytes. Examination of rat livers after 14 days of ETBE treatment showed the high levels of concordance between induction of 8-OHdG and apoptosis (ssDNA), which were inversely correlated with low cell proliferation. Increased 8-OHdG formation is caused by developing oxidative stress and/or apoptotic degradation of DNA. Continuous P450 and hydroxyl radical elevation by high dose ETBE was coordinated with enhanced cell proliferation at Day 3, followed by cell cycle arrest (low cell proliferation) and apoptosis at Day 14 (Week 2), and regenerative cell proliferation at Day 28, as a continuing response to liver damage occurred at Day 14 (Week 2). Adaptive response to liver damage at Day 14 (Week 2) firstly include activation of repair mechanisms, which contributes to protection of tissue against reactive oxygen species-induced cell death (such as increase of DNA repair enzymes), and lastly, regenerative cell proliferation. Elevation of P450 has been proven to be associated with generation of reactive oxygen species (ROS), which damage proteins and DNA. If the balance between the generation of ROS and activity of repair system enzymes is disturbed, severe damage occurs on cellular and molecular levels, what was reported to result in promotion of carcinogenesis if the damaged cell was not eliminated by apoptosis. ETBE at high dose induced significant generation of hydroxyl radicals, thus, the long-term exposure could result in promotion of hepatocarcinogenesis in spontaneously initiated hepatocytes. Therefore, centrilobular hypertrophy is likely to be associated with hepatocarcinogenesis.

EPA Response: The data do not provide evidence of a gradual increase in hydroxyl radicals because radicals were only increased at one dose at both time points measured. Two time points are insufficient data to establish a temporal trend in radical species formation, and a single 14-day data-point that reports apoptosis and oxidative stress occurring at one dose is insufficient data to establish either dose or temporal associations for a MOA. Thus, the conclusion that the available evidence is inadequate appears to be the most appropriate for the database at this time.

Comments Related to Cancer Weight of Evidence

Comment [LyondellBasell]: The draft assessment inappropriately considers the ETBE two-stage tumor promotion studies. The animal experimental data indicates that ETBE might be acting as a promoter of mutagen induced liver tumors when administered at high doses of ETBE (1,000 mg/kg-day), a dose level that also exceeds metabolic saturation. This promotional activity has clear thresholds, with no evidence of promotion at a 300 mg/kg-day dose that is near or at an oral dose reflecting onset on nonlinear toxicokinetics. Thus, the available data supports the conclusion that the liver tumors observed following inhalation exposure of ETBE are most likely the result of the promotion of spontaneously initiated cells and as such have clear threshold dose-response relationships. Evidence of promotion of multi-mutagen-initiated tumorigenicity was observed in thyroid at oral gavage doses of 300 and 1,000 mg/kg-day and in colon only at the high dose. No evidence of tumor promotion was observed in kidney, forestomach, urinary bladder, or urethra. In a later single mutagen-initiated study, ETBE-induced liver promotion was restricted to the high dose of 1,000 mg/kg-day, while the incidence of kidney adenomas was increased at 500 and 1,000 mg/kg-day (however, combined adenoma/carcinoma incidence was not altered at any dose). It is important to note, however, that these studies do not provide meaningful evidence of ETBE carcinogenicity. Both assay designs were developed as screening assays of potential carcinogenicity hazard (but not risk), and were validated for target organ predictability against existing apical animal cancer bioassays. In the case of ETBE, however, which has two high quality apical rat carcinogenicity studies conducted by two routes of administration, the possibility of nonhepatic tumorigenicity (kidney, thyroid, colon) as suggested in the initiation-promotion assays was not confirmed in two apical animal bioassays. This combined evidence indicates that nonhepatic carcinogenicity identified in the two-stage carcinogenicity assays is not relevant to ETBE carcinogen assessment, other than to provide possible supporting evidence that high doses of ETBE exceeding metabolic saturation may have tumor-promoting activity. Such promotion responses are generally regarded as threshold-based MOA events.

EPA Response: The two stage carcinogenicity bioassay data provide several instances of increased tumors or preneoplastic lesions at doses below 1,000 mg/kg-day. These organ sites were in the forestomach, thyroid, and kidney (see Tables 1-4, 1-17, 1-18). These data indicate that ETBE has the potential to induce tumorigenic responses below 1,000 mg/kg-day. Furthermore, no MOA was

identified for liver tumors so it is not possible to conclude that the induction of liver tumors by ETBE at one dose is also not operative at lower doses.

Comment [LyondellBasell]: The conclusion that the 5,000 ppm inhalation exposure concentration was an excessively high test concentration in rats is further evidenced by the Saito et al. (2013) study that reported that male and female body weights were significantly decreased to 75 and 78%, respectively, of controls at the terminal 104-week sacrifice. This severe weight loss exceeds the 10% body weight loss recommended for achievement of a maximum tolerated dose. The potential that excessive toxicity was uniquely associated with the high dose (5,000 ppm) exposure condition under which nonlinear toxicokinetics were apparent is further evidenced by the observation that male and female terminal body weights were a nonstatistically significant 94 and 91% of controls, respectively, in next lower (1,500 ppm) ETBE exposure. In addition, the significantly increased incidence of preneoplastic eosinophilic and basophilic liver foci was limited to the 5,000 ppm treatment group, indicating that tumorigenic responses would be unlikely at the 1,500 ppm mid-dose. These findings further indicate that the high-dose-specific ETBE male rat liver tumors were secondary to use of an excessively high top bioassay dose.

EPA Response: Although loss of body weight occurred in the 2-year study, this was not the case for the two-stage initiation-promotion cancer bioassays, which observed increased tumors at multiple organ sites and multiple doses. This suggests that the increase in tumors were not related to a maximum tolerated dose as indicated by loss of body weight.

Comments Related to Reproductive and Developmental Effects

Comment [LyondellBasell]: Numerous detailed questions and specific concerns related to the organization, presentation, and interpretation of rodent evidence relevant to the determination of reproductive and developmental effects following ETBE exposure were received.

EPA Response: EPA appreciates the detailed comments regarding evidence summarized in evidence tables and exposure-response arrays and discussed in the associated synthesis text. The reproductive effects discussion and associated tables and figures (see Section 1.2.3) has now been reorganized and revised to separately present and evaluate evidence relevant to male and female reproductive effects, including an expanded and more detailed presentation and discussion of all the pertinent endpoints reported in the identified literature. Likewise, the discussion of evidence for developmental effects (see Section 1.2.4) has been revised to more clearly present and discuss all pertinent endpoints reported in the assembled database. Revisions emphasized a transparent consideration of all available data, integrated into a conclusion statement for each possible effect: male reproductive effects, female reproductive effects, and developmental effects.

Comments Related to the Physiologically Based Pharmacokinetic Model and Toxicokinetics

Comment [LyondellBasell]: Numerous questions and concerns related to specific aspects of PBPK modeling described by Salazar et al. (2015) and as implemented in public comment draft were received.

EPA Response: EPA has adopted the newly available Borghoff et al. (2016) model, as summarized in Appendix B.1.5–B.1.6 and U.S. EPA (2017).

Comment [LyondellBasell]: Several comments noted that the results of Sun and Beskitt (1995a, b) and a preliminary study by Borghoff (1996) were presented and discussed, while the more comprehensive study of Borghoff and Asgharian (1996) was not.

EPA Response: A summary of Borghoff and Asgharian (1996) has been added to the ADME discussion in Appendix B; furthermore, results from Borghoff and Asgharian (1996) are now incorporated into the ADME/TK review, and model simulations are compared to those in U.S. EPA (2017).

Comment [LyondellBasell]: The section of the draft assessment does not describe the interpretative implications of the finding that liver tumors were only observed at an inhalation dose level exhibiting nonlinear toxicokinetic behavior. As implied by the title of this section of the draft assessment ("Toxicokinetic Considerations Relevant to Liver Toxicity and Tumors"), such data should be a key consideration in the overall MOA evaluation. Importantly, in the Supplementary Information provided for the draft assessment it is stated that: "A review of the data demonstrating the percentage of recovered radiolabel via various routes of elimination demonstrate, in the rat and mouse, a pattern indicative of metabolic saturation occurring at inhaled concentrations above 4,180 mg/m3 [1,000 ppm]" (p. B-19). This key conclusion is not carried over to the main draft assessment document.

EPA Response: Metabolic saturation would only lead to a disproportionate increase in toxicity if it is the parent chemical, ETBE in this case, that is the proximate agent. Figure 11, panels A and C, in Borghoff et al. (1996) shows that extent of nonlinearity in the blood AUC of ETBE in the dose range evaluated is modest; there is not a sudden sharp inflection upward of AUC versus exposure at 1,000 ppm. Further, the MOA analysis indicates a probability that it is not parent ETBE that is the proximate agent, leading to the choice of ETBE metabolic rate as a measure of internal dose for route-to-route extrapolation. And while Figure 13-A of Borghoff et al. (1996) shows that the metabolic rate is predicted to be approaching saturating (becoming flatter) in the range of 5,000 ppm, it is close to linear at 1/5th that level. This result is not consistent with an argument

that toxicity appears due to a significant shift in toxicokinetics at 1,000 ppm, let alone increase disproportionately with exposure at higher levels.

REFERENCES FOR APPENDICES

Amberg, A; Rosner, E; Dekant, W. (1999). Biotransformation and kinetics of excretion of methyl-tert-butyl ether in rats and humans. Toxicol Sci. 51: 1-8.

Amberg, A; Rosner, E; Dekant, W. (2000). Biotransformation and kinetics of excretion of ethyl tert-butyl ether in rats and humans. Toxicol Sci. 53: 194-201. http://dx.doi.org/10.1093/toxsci/53.2.194.

ATSDR (Agency for Toxic Substances and Disease Registry). (1996). Toxicological profile for methyl-tert-butyl ether [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/ToxProfiles/tp91.pdf.

Banton, MI; Peachee, VL; White, KL; Padgett, EL. (2011). Oral subchronic immunotoxicity study of ethyl tertiary butyl ether in the rat. J Immunotoxicol. 8: 298-304. http://dx.doi.org/10.3109/1547691X.2011.598480.

Bernauer, U; Amberg, A; Scheutzow, D; Dekant, W. (1998). Biotransformation of 12C- and 2-13C-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and tert-butyl alcohol in rats: Identification of metabolites in urine by 13C nuclear magnetic resonance and gas chromatography/mass spectrometry. Chem Res Toxicol. 11: 651-658. http://dx.doi.org/10.1021/tx970215v.

Bond, JA; Medinsky, MA; Wolf, DC; Cattley, R; Farris, G; Wong, B; Janszen, D; Turner, MJ; Sumner, SCJ. (1996a). Ethyl tertiary butyl ether (ETBE): ninety-day vapor inhalation toxicity study in CD-1(R) mice. Bond, JA; Medinsky, MA; Wolf, DC; Cattley, R; Farris, G; Wong, B; Janszen, D; Turner, MJ; Sumner, SCJ.

Bond, JA; Medinsky, MA; Wolf, DC; Dorman, DC; Cattley, R; Farris, G; Wong, B; Morgan, K; Janszen, D; Turner, MJ; Sumner, SCJ. (1996b). Ethyl tertiary butyl ether (ETBE): ninety-day vapor inhalation toxicity study with neurotoxicity evaluations in Fischer 344 rats [TSCA Submission] (pp. 1-90). (89970000047). Research Triangle Park, NC: Chemical Industry Institute of Toxicology under contract to ARCO Chemical Company. http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/1332F4B209355DC785256F9E006B7EA0/$File/89970000047.pdf.

Borghoff, SJ. (1996). Ethyl tertiary-butyl ether: Pilot/methods development pharmacokinetic study in male F-344 rats & male cd-1 mice after single nose-only inhalation exposure, w/cvr ltr dated 7/29/96. (TSCATS/444664). Chemical Industry Institute of Toxicology (CIIT).

Borghoff, SJ; Asgharian, B. (1996). Ethyl tertiary-butyl ether (ETBE): Pharmacokinetic study in male and female CD-1 mice after single inhalation exposure and male and female F-344 rats after single and repeated inhalation exposure. (CIIT Protocol 95026). La Palma, CA: ARCO Chemical Company.

Borghoff, SJ; Murphy, JE; Medinsky, MA. (1996). Development of physiologically based pharmacokinetic model for methyl tertiary-butyl ether and tertiary-butanol in male Fisher-344 rats. Fundam Appl Toxicol. 30: 264-275. http://dx.doi.org/10.1006/faat.1996.0064.

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

α2u-globulin in male rats and high-exposure nonlinear kinetics to toxicity and cancer outcomes. J Appl Toxicol. http://dx.doi.org/10.1002/jat.3412.

Cederbaum, AI; Cohen, G. (1980a). Oxidative demethylation of t-butyl alcohol by rat liver microsomes. Biochem Biophys Res Commun. 97: 730-736.

Cederbaum, AI; Cohen, G. (1980b). Oxidative demethylation of tert-butyl alcohol by rat-liver microsomes. Biochem Biophys Res Commun. 97: 730-736. http://dx.doi.org/10.1016/0006-291X(80)90325-3.

Cohen, SM; Hard, GC; Regan, KS; Seely, JC; Bruner, RH. (2011). Pathology working group review of selected histopathologic changes in the kidneys of rats assigned to toxicology and carcinogenicity studies of ethyl tertiary butyl ether (ETBE): Japan Bioassay Research Center studies no.: 0065 and 0691 [Unpublished report] (pp. 1-30). Research Triangle Park, NC: Research Pathology Associates under contract to Lyondell Chemical Company.

Dekant, W; Bernauer, U; Rosner, E; Amberg, A. (2001). Toxicokinetics of ethers used as fuel oxygenates [Review]. Toxicol Lett. 124: 37-45. http://dx.doi.org/10.1016/s0378-4274(00)00284-8.

Drogos, DL; Diaz, AF. (2001). Oxygenates in Gasoline Appendix A: Physical properties of fuel oxgenates and addititves. In ACS Symposium Series. Washington, DC: American Chemical Society. http://dx.doi.org/10.1021/bk-2002-0799.ch018.

Elmore, SA. (2006). Enhanced histopathology of the spleen [Review]. Toxicol Pathol. 34: 648-655. http://dx.doi.org/10.1080/01926230600865523.

Fujii, S; Yabe, K; Furukawa, M; Matsuura, M; Aoyama, H. (2010). A one-generation reproductive toxicity study of ethyl tertiary butyl ether in rats. Reprod Toxicol. 30: 414-421. http://dx.doi.org/10.1016/j.reprotox.2010.04.013.

Gaoua, W. (2004a). Ethyl tertiary butyl ether (ETBE): Prenatal developmental toxicity study by the oral route (gavage) in rats (pp. 1-280). (CIT Study No. 24860 RSR). unpublished study for Totalfinaelf on behalf of the ETBE Producers' Consortium. An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

Gaoua, W. (2004b). Ethyl tertiary butyl ether (ETBE): Two-generation study (reproduction and fertility effects) by the oral route (gavage) in rats. (CIT Study No. 24859 RSR). unpublished study for Totalfinaelf on behalf of the ETBE Producers' Consortium. An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

Hagiwara, A; Doi, Y; Imai, N; Nakashima, H; Ono, T; Kawabe, M; Furukawa, F; Tamano, S; Nagano, K; Fukushima, S. (2011). Medium-term multi-organ carcinogenesis bioassay of ethyl tertiary-butyl ether in rats. Toxicology. 289: 160-166. http://dx.doi.org/10.1016/j.tox.2011.08.007.

Hong, JY; Wang, YY; Bondoc, FY; Lee, M; Yang, CS; Hu, WY; Pan, J. (1999a). Metabolism of methyl tert-butyl ether and other gasoline ethers by human liver microsomes and heterologously expressed human cytochromes P450: Identification of CYP2A6 as a major catalyst. Toxicol Appl Pharmacol. 160: 43-48. http://dx.doi.org/10.1006/taap.1999.8750.

Hong, JY; Wang, YY; Bondoc, FY; Yang, CS; Gonzalez, FJ; Pan, Z; Cokonis, CD; Hu, WY; Bao, Z. (1999b). Metabolism of methyl tert-butyl ether and other gasoline ethers in mouse liver microsomes lacking cytochrome P450 2E1. Toxicol Lett. 105: 83-88. http://dx.doi.org/10.1016/s0378-4274(98)00389-0.

Hong, JY; Wang, YY; Bondoc, FY; Yang, CS; Lee, M; Huang, WQ. (1997a). Rat olfactory mucosa displays a high activity in metabolizing methyl tert-butyl ether and other gasoline ethers. Toxicol Sci. 40: 205-210. http://dx.doi.org/10.1093/toxsci/40.2.205.

Hong, JY; Yang, CS; Lee, M; Wang, YY; Huang, WQ; Tan, Y; Patten, CJ; Bondoc, FY. (1997b). Role of cytochromes P450 in the metabolism of methyl tert-butyl ether in human livers. Arch Toxicol. 71: 266-269.

Johanson, G; Nihlén, A; Löf, A. (1995). Toxicokinetics and acute effects of MTBE and ETBE in male volunteers. Toxicol Lett. 82/83: 713-718. http://dx.doi.org/10.1016/0378-4274(95)03589-3.

JPEC (Japan Petroleum Energy Center). (2007a). Micronucleus test of 2-ethoxy-2-methylpropane (ETBE) using bone marrow in rats administered ETBE by gavage. (Study Number: 7049). Japan: Japan Industrial Safety and Health Association.

JPEC (Japan Petroleum Energy Center). (2007b). Micronucleus test of 2-ethoxy-2-methylpropane (ETBE) using bone marrow in rats administered ETBE intraperitoneally. (Study Number: 7048). Japan: Japan Bioassay Research Center, Japan Industrial Safety and Health Association.

JPEC (Japan Petroleum Energy Center). (2007c). Micronucleus test of ETBE using bone marrow of rats of the "13-week toxicity study of 2-ethoxy-2-methylpropane in F344 rats (inhalation study) [preliminary carcinogenicity study]". (Study Number: 7047). Japan Industrial Safety and Health Association.

JPEC (Japan Petroleum Energy Center). (2007d). Micronucleus test of ETBE using bone marrow of rats of the "13-week toxicity study of 2-ethoxy-2-methylpropane in F344 rats (drinking water study) [preliminary carcinogenicity study]". (Study Number: 7046). Japan Bioassay Research Center, Japan Industrial Safety and Health Association.

JPEC (Japan Petroleum Energy Center). (2008a). A 90-day repeated dose toxicity study of ETBE by whole-body inhalation exposure in rats. (Study Number: B061829). Mitsubishi Chemical Safety Institute Ltd. An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

JPEC (Japan Petroleum Energy Center). (2008b). A 180-Day repeated dose oral toxicity study of ETBE in rats. (Study Number: D19-0002). Japan: Hita Laboratory, Chemicals Evaluation and Research Institute (CERI). An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

JPEC (Japan Petroleum Energy Center). (2008c). Medium-term mutli-organ carcinogenesis bioassay of 2-ethoxy-2-methylpropane (ETBE) in rats. (Study Number: 0635). Ichinomiya, Japan: DIMS Institute of Medical Science.

JPEC (Japan Petroleum Energy Center). (2008d). A one-generation reproduction toxicity study of ETBE in rats. (Study Number: SR07060). Safety Research Institute for Chemical Compounds.

JPEC (Japan Petroleum Energy Center). (2008e). Pharmacokinetic study in rats treated with [14c] ETBE repeatedly for 14 days. (P070497). Japan: Kumamoto Laboratory, Mitsubishi Chemical Safety Institute Ltd. An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

JPEC (Japan Petroleum Energy Center). (2008f). Pharmacokinetic study in rats treated with single dose of [14C] ETBE. (P070496). Japan: Kumamoto Laboratory, Mitsubishi Chemical Safety Institute Ltd. An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

JPEC (Japan Petroleum Energy Center). (2010a). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Drinking water study). (Study No: 0691). Japan Industrial Safety and Health Association, Japan Bioassay Research Center. An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

JPEC (Japan Petroleum Energy Center). (2010b). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Inhalation study). (Study No: 0686). Japan: Japan Industrial Safety and Health Association. An external peer review was conducted by EPA in November 2008 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 56-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.

Kaneko, T; Wang, P, -Y; Sato, A. (2000). Partition coefficients for gasoline additives and their metabolites. J Occup Health. 42: 86-87. http://dx.doi.org/10.1539/joh.42.86.

Le Gal, A; Dreano, Y; Gervaso, PG; Berthou, F. (2001). Human cytochrome P450 2A6 is the major enzyme involved in the metabolism of three alkoxyethers used as oxyfuels [Review]. Toxicol Lett. 124: 47-58. http://dx.doi.org/10.1016/s0378-4274(00)00286-1.

Leavens, TL; Borghoff, SJ. (2009). Physiologically based pharmacokinetic model of methyl tertiary butyl ether and tertiary butyl alcohol dosimetry in male rats based on binding to alpha2u-globulin. Toxicol Sci. 109: 321-335. http://dx.doi.org/10.1093/toxsci/kfp049.

Li, Q; Kobayashi, M; Inagaki, H; Hirata, Y; Hirata, K; Shimizu, T; Wang, R, -S; Suda, M; Kawamoto, T; Nakajima, T; Kawada, T. (2011). Effects of subchronic inhalation exposure to ethyl tertiary butyl ether on splenocytes in mice. Int J Immunopathol Pharmacol. 24: 837-847. https://doi.org/10.1177/039463201102400403.

Malarkey, DE; Bucher, JR. (2011). Summary report of the National Toxicology Program and Environmental Protection Agency-sponsored review of pathology materials from selected Ramazzini Institute rodent cancer bioassays [NTP]. Research Triangle Park: National Toxicology Program.

http://ntp.niehs.nih.gov/ntp/about_ntp/partnerships/international/summarypwg_report_ri_bioassays.pdf.

Maltoni, C; Belpoggi, F; Soffritti, M; Minardi, F. (1999). Comprehensive long-term experimental project of carcinogenicity bioassays on gasoline oxygenated additives: plan and first report of results from the study on ethyl-tertiary-butyl ether (ETBE). Eur J Oncol. 4: 493-508.

McComb, JA; Goldstein, DB. (1979). Quantitative comparison of physical dependence on tertiary butanol and ethanol in mice: Correlation with lipid solubility. Journal of Pharmacology and Experimental Therapeutics. 208: 113-117.

Medinsky, MA; Wolf, DC; Cattley, RC; Wong, B; Janszen, DB; Farris, GM; Wright, GA; Bond, JA. (1999). Effects of a thirteen-week inhalation exposure to ethyl tertiary butyl ether on Fischer-344 rats and CD-1 mice. Toxicol Sci. 51: 108-118. http://dx.doi.org/10.1093/toxsci/51.1.108.

Miyata, K; Koga, T; Aso, S; Hoshuyama, S; Ajimi, S; Furukawa, K. (2013). A subchronic (180-day) oral toxicity study of ethyl tertiary-butyl ether, a bioethanol, in rats. Drug Chem Toxicol. http://dx.doi.org/10.3109/01480545.2013.851690.

Nihlén, A; Löf, A; Johanson, G. (1995). Liquid/air partition coefficients of methyl and ethyl t-butyl ethers, t-amyl methyl ether, and t-butyl alcohol. J Expo Anal Environ Epidemiol. 5: 573-582.

Nihlén, A; Löf, A; Johanson, G. (1998). Controlled ethyl tert-butyl ether (ETBE) exposure of male volunteers: I Toxicokenetics. Toxicol Sci. 46: 1-10. http://dx.doi.org/10.1006/toxs.1998.2516.

Noguchi, T; Kamigaito, T; Katagiri, T; Kondou, H; Yamazaki, K; Aiso, S; Nishizawa, T; Nagano, K; Fukushima, S. (2013). Lack of micronucleus induction activity of ethyl tertiary-butyl ether in the bone marrow of F344 rats by sub-chronic drinking-water treatment, inhalation exposure, or acute intraperitoneal injection. J Toxicol Sci. 38: 913-924. http://dx.doi.org/10.2131/jts.38.913.

NSF International. (2003). t-Butanol: Oral Risk Assessment Document (CAS 75-65-0) (pp. 81). Ann Arbor, MI. http://www.documents.dgs.ca.gov/bsc/pex/exibit_nsf_t_butanol.pdf.

NTP (National Toxicology Program). (1996a). Genetic toxicity evaluation of 2-methyl-2-ethoxypropane (ETBE) (637-92-3) in micronucleus study A70000 in B6C3F1 mice. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

NTP (National Toxicology Program). (1996b). Genetic toxicity evaluation of 2-methyl-2-ethoxypropane (ETBE) (637-92-3) in micronucleus study A87074 in F344 rats. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

Potts RO, G, uy RH. (1992). Predicting skin permeability. Pharm Res. 9: 663-669.

Saito, A; Sasaki, T; Kasai, T; Katagiri, T; Nishizawa, T; Noguchi, T; Aiso, S; Nagano, K; Fukushima, S. (2013). Hepatotumorigenicity of ethyl tertiary-butyl ether with 2-year inhalation exposure in F344 rats. Arch Toxicol. 87: 905-914. http://dx.doi.org/10.1007/s00204-012-0997-x.

Salazar, KD; Brinkerhoff, CJ; Lee, JS; Chiu, WA. (2015). Development and application of a rat PBPK model to elucidate kidney and liver effects induced by ETBE and tert-butanol. Toxicol Appl Pharmacol. 288: 439-452. http://dx.doi.org/10.1016/j.taap.2015.08.015.

Spiteri, NJ. (1982). Circadian patterning of feeding, drinking and activity during diurnal food access in rats. Physiol Behav. 28: 139-147. http://dx.doi.org/10.1016/0031-9384(82)90115-9.

Sun, JD; Beskitt, JL. (1995a). Ethyl tertiary-butyl ether (ETBE): Pharmacokinetics after single and repeated inhalation exposures of mice, with cover letter dated 06/21/95 [TSCA

Submission]. (Project ID 94N1455). Export, PA: Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0557696.

Sun, JD; Beskitt, JL. (1995b). Ethyl tertiary-butyl ether (ETBE): Pharmacokinetics after single and repeated inhalation exposures of rats [TSCA Submission]. (Project ID 94N1454). Export, PA: Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0557695.

Suzuki, M; Yamazaki, K; Kano, H; Aiso, S; Nagano, K; Fukushima, S. (2012). No carcinogenicity of ethyl tertiary-butyl ether by 2-year oral administration in rats. J Toxicol Sci. 37: 1239-1246.

Turini, A; Amato, G; Longo, V; Gervasi, PG. (1998). Oxidation of methyl- and ethyl-tertiary-butyl ethers in rat liver microsomes: role of the cytochrome P450 isoforms. Arch Toxicol. 72: 207-214. http://dx.doi.org/10.1007/s002040050490.

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

U.S. EPA (U.S. Environmental Protection Agency). (2016). Model files for tert-butanol and ETBE.

U.S. EPA (U.S. Environmental Protection Agency). (2017). PK/PBPK model evaluation for the IRIS assessments of ethyl tertiary butyl ether (CASRN 637-92-3) and tert-butyl alcohol (CAS No. 75-65-0) (Draft) [EPA Report]. Washington, DC: U.S. Environmental Protection Agency, Pharmacokinetics Working Group.

Vergnes, JS. (1995). Ethyl tertiary butyl ether: In vitro chromosome aberrations assay in Chinese hamster ovary cells. (Project ID 94N1425). Export, PA: Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0557635.

Vergnes, JS; Kubena, MF. (1995a). Ethyl tertiary butyl ether: Bone marrow micronucleus test in mice. (Project ID 94N1426). Export, PA: Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=OTS0557636.

Vergnes, JS; Kubena, MF. (1995b). Ethyl Tertiary Butyl Ether: Mutagenic Potential in the Cho/hgprt Forward Mutation Assay [TSCA Submission]. (Project ID 94N1424). Export, PA: Bushy Run Research Center, Union Carbide Corporation under contract to ARCO Chemical Company.

Weng, Z; Ohtani, K; Suda, M; Yanagiba, Y; Kawamoto, T; Nakajima, T; Wang, RS. (2014). Assessment of the reproductive toxicity of inhalation exposure to ethyl tertiary butyl ether in male mice with normal, low active and inactive ALDH2. Arch Toxicol. 88: 1007-1021. http://dx.doi.org/10.1007/s00204-014-1192-z.

Weng, Z; Suda, M; Ohtani, K; Mei, N, an; Kawamoto, T; Nakajima, T; Wang, R. (2013). Subchronic exposure to ethyl tertiary butyl ether resulting in genetic damage in Aldh2 knockout mice. Toxicology. 311: 107-114. http://dx.doi.org/10.1016/j.tox.2013.06.005.

Weng, Z; Suda, M; Ohtani, K; Mei, N; Kawamoto, T; Nakajima, T; Wang, RS. (2012). Differential genotoxic effects of subchronic exposure to ethyl tertiary butyl ether in the livers of Aldh2 knockout and wild-type mice. Arch Toxicol. 86: 675-682. http://dx.doi.org/10.1007/s00204-011-0779-x.

Weng, ZQ; Suda, M; Ohtani, K; Mei, N; Kawamoto, T; Nakajima, T; Wang, RS. (2011). Aldh2 Knockout Mice Were More Sensitive to DNA Damage in Leukocytes due to Ethyl Tertiary Butyl Ether Exposure. Ind Health. 49: 396-399.

WHO (World Health Organization). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf.

Zeiger, E; Anderson, B; Haworth, S; Lawlor, T; Mortelmans, K. (1992). Salmonella mutagenicity tests: V Results from the testing of 311 chemicals. Environ Mol Mutagen. 19: 2-141. http://dx.doi.org/10.1002/em.2850190603.

Zhang, YP; Macina, OT; Rosenkranz, HS; Karol, MH; Mattison, DR. (1997). Prediction of the metabolism and toxicological profiles of gasoline oxygenates. Inhal Toxicol. 9: 237-254.