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Toxicological Review of Ethyl Tertiary Butyl Ether

[CASRN 637-92-3]

In Support of Summary Information on the Integrated Risk Information System (IRIS)

Supplemental Information

September 2014

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Integrated Risk Information System
National Center for Environmental Assessment
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Washington, DC

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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
CYP450	cytochrome P450
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
GI	gastrointestinal
HBA	2-hydroxybutyrate
KO	knockout
JPEC	Japan Petroleum Energy Center
MN	micronucleus, micronucleated
MNPCE	micronucleated polychromatic erythrocyte
MTBE	methyl tertiary butyl ether
MPD	2-methyl-1,2-propane diol
PCE	polychromatic erythrocytes
POD	point of departure
RET	reticulocyte
SD	standard deviation
TAME	methyl tertiary butyl ether
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
American Conference of Governmental Industrial Hygienists	Threshold limit value: 20.9 mg/m^3

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

B.1. CHEMICAL PROPERTIES

Table B-1. Chemical identity and physicochemical properties of ETBE.

Characteristic or property	Value	Reference
Chemical name	2-ethoxy-2-methylpropane 2-methyl-2-ethoxypropane	National Library of Medicine
Synonyms	ethyl tert-butyl ether ethyl tert-butyl oxide methyl-2-ethoxypropane tert-butyl ethyl ether ETBE	National Library of Medicine
Chemical formula	$C_6H_{14}O$	National Library of Medicine
CASRN (Chemical Abstracts Service Registry Number)	637-92-3	National Library of Medicine
Molecular weight	102.17	National Library of Medicine
Melting point	-94°C	Drogos and Diaz (2001)
Boiling point	67–73°C	Drogos and Diaz (2001)
Density at 25°C	0.73–0.74 g/cm^3 @ 25°C	Drogos and Diaz (2001)
Water solubility	7,650–26,000 mg/L	Drogos and Diaz (2001)
Partition coefficients: Log oil/water Log Kow	 1.48 1.74	 Montgomery (1994) Drogos and Diaz (2001)
Vapor pressure	130–152 mm Hg @ 25°C	Drogos and Diaz (2001)
Henry's law constant	2.7 × 10^{-3} atm-m^3/mol @ 25°C	Drogos and Diaz (2001)
Odor Detection threshold Recognition threshold	 0.013 ppm (0.054 mg/m^3) 0.024 ppm (0.1 mg/m^3)	Vetrano (1993)
Taste detection threshold (in water)	0.047 ppm (47 µg/L)	Vetrano (1993)
Odor detection threshold (in water)	0.049 ppm (49 µg/L)	Vetrano (1993)
Odor detection threshold (in water)	0.005 ppm (5 µg/L)	Vetrano (1993)
Conversion factors	1 ppm = 4.18 mg/m^3 1 mg/m^3 = 0.24 ppm 1 mg/m^3 = 102,180 mmol/L	ppm = mg/m^3 × 24.45 m^3/mole ÷ molecular weight in g/mol mmol/L = mg/m^3 ÷ molecular

Characteristic or property	Value	Reference
		weight in mg/mmol ÷ 1,000 L/m^3

B.2. TOXICOKINETICS

B.2.1. Absorption

B.2.1.1. Human Studies

Most of the available human data on the uptake of ETBE were obtained from volunteers. Nihlén et al. (1998a) 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 by 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 its primary metabolite, *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 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 a 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. (1998a) 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.

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 received 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. (1998a).

B.2.1.2. Animal Studies

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. However, the Japan Petroleum Energy Center (JPEC) conducted an oral dosing study of the absorption of ETBE in rats after single and repeated dosing for 14 days (JPEC, 2008d, e). 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 JPEC (2008e), 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 (2008d). 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 2nd 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 as well as 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 eq 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. 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 (2008d), the C_{max} was achieved 6 hours after the first exposure and increased until it reached a steady state around the 5th day of exposure. After the last exposure on Day 14, the C_{max}, of 6,660 ± 407 ng eq 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, over 90% of the administered dose was absorbed.

Dekant et al. (2001) published a review article that presented an overview of their studies of the toxicokinetics of ETBE, methyl tertiary butyl ether (MTBE), and methyl tertiary butyl ether (TAME) in both humans and rats following inhalation exposure at 4 ppm (16.7 mg/m^3 ETBE and TAME; 14.4 mg/m^3 MTBE) and 40 ppm (167.1 mg/m^3 ETBE and TAME; 144.2 mg/m^3 MTBE), respectively [see also Amberg et al. (2000); Bernauer et al. (1998)]. In addition, MTBE and TAME were administered to humans in aqueous solution at 5 and 15 mg, respectively. The authors assumed 100% absorption of MTBE and TAME following ingestion. Table B-2 presents a synopsis of their findings. A comparison of the MTBE, TAME, and ETBE data may provide some insight relative to uptake of ETBE following ingestion.

A comparison of the percentage of oral dose excreted versus the percentage of inhalation dose excreted suggests that the assumption of 100% absorption was correct for MTBE, but most likely not for TAME. If air:blood partition coefficients were the only determinants of inhalation uptake, one would expect the dose received for ETBE to be lower than those for both MTBE and TAME because the air:blood partition coefficient for ETBE (11.7) is lower than that of MTBE (17.7) and TAME (17.9) (Nihlén et al., 1995), and the uptake of ETBE is lower than that of MTBE based on the data from this laboratory. If the log octanol:water partition coefficients (log K_{ow}) were the only determinants (approximately 1.1 for MTBE, 1.48–1.74 for ETBE, and 1.55 for TAME [Table B-3; Drogos and Diaz (2001)]), then values for ETBE and TAME should be similar. Data in Table B-3 support the latter hypothesis, but there are limited data for the evaluation of either hypothesis. On a body-weight basis, doses were about 500 times higher in rats than in humans, although exposures were delivered under entirely identical conditions in the two species [e.g., Amberg et al. (2000)].

No studies investigating dermal absorption of ETBE were identified. However, because dermal absorption of homologous organic substances is thought to be a function of the octanol:water partition coefficient, ETBE may be assumed to penetrate rat skin relatively well. For humans, Potts and Guy (1992) have proposed an equation (3-1) to calculate the dermal permeability coefficient, K_p:

$$\log K_p \text{ (cm/sec)} = -6.3 + 0.71 \times \log k_{ow} - 0.0061 \times \text{(molecular weight)} \quad (3\text{-}1)$$

Table B-2. Plasma radioactivity after a single oral or intravenous dose of [14C]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 ± 1007	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 (2008d)

Using the log k_{ow} [identified as K_{oct} in Potts and Guy (1992)] values for ETBE (0.95–2.2) and MTBE (0.55–1.91) from Drogos and Diaz (2001) and converting cm/second values to cm/hour, K_p values yielded are 0.0020–0.016 cm/hour for ETBE and 0.0012–0.012 cm/hour for MTBE. These calculations predict that the dermal absorption rate of ETBE in humans would be 1.3–1.7 times that of MTBE. The K_p for MTBE (i.e., 0.028 cm/hour) calculated by Prah et al. (2004) was approximately

twice as high as the K_p derived using equation 3-1. However, the data from Prah et al. (2004) were derived from human subjects exposed to a single concentration, and the authors themselves highlighted the importance of experimental variables such as temperature and exposure concentration for dermal absorption.

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. (1998a) calculated the net respiratory uptake of ETBE in humans to be 26% compared with 38% for MTBE, which, as the authors point out, parallels the lower blood:air partition coefficient for ETBE (11.7) compared with MTBE (17.7). 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 (JPEC, 2008d, e) studies 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. Although comparison of log k_{ow} values suggests that dermal absorption rates for ETBE would be higher than that of MTBE, no data are available on dermal absorption of ETBE.

B.2.2. Distribution

In vivo data on the tissue distribution of ETBE in humans are not available. Nihlén et al. (1995) measured the partitioning of ETBE and *tert*-butanol in air into human blood, saline, or oil inside of sealed vials, and the human tissue partitioning coefficients were estimated based upon the relative water and fat contents in human tissues, including brain, fat, liver, kidney, lung, and muscle. Blood samples were obtained from 10 human donors (5 males, 5 females). 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 for humans and rats, respectively. Nihlén et al. (1995) also estimated oil:water partition (log k_{ow}) coefficients and obtained values of -0.56 for *tert*-butanol and 1.36 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) also used these coefficients and air:oil partition coefficients to calculate human blood:tissue 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	465	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

Nihlén et al. (1998a) exposed eight healthy male volunteers (average age: 29 years) to 21, 104, and 209 mg/m^3 ETBE by inhalation for 2 hours. The volunteers performed light physical exercise during exposure. Profiles of ETBE, *tert*-butanol, and acetone were established for blood throughout exposure and for up to 22 hours thereafter. The same laboratory conducted studies with MTBE using the same experimental protocol. Net uptake of MTBE was 38% of the dose (compared with 26% net uptake for ETBE), and net exhalation of MTBE was 28% of the net uptake for MTBE (compared with 31% net exhalation for ETBE) (Nihlén et al., 1998b). The results may reflect the difference in blood:air partition coefficients between MTBE and ETBE (18 and 12, respectively) (Nihlén et al., 1995), suggesting that MTBE has a higher tendency to partition into human blood and tissues and is less likely to be eliminated by exhalation compared with ETBE. Therefore, the high volume of distribution for ETBE in humans, 6.4 L/kg, as compared to 3.9 L/kg for MTBE (Nihlén et al., 1998a) is indicative of the higher partition coefficients for blood:tissue for ETBE relative to MTBE, particularly the over 2-fold greater blood:fat partition coefficient (11.6 and 4.98 for ETBE and MTBE, respectively).

The JPEC (2008d, e) 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 percent distribution of radioactivity in tissues was lower with the higher dose. Tissue radioactivity levels were at a maximum 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 7th 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 percent radioactivity found in red blood cells was estimated to be 20–27% within 72 hours of administration, and little was found bound to plasma proteins.

B.2.3. 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. On the basis of structures of the metabolites elucidated, ETBE is initially metabolized by cytochrome P450 (CYP) enzymes via oxidative deethylation by the addition of a hydroxy 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 may be the lead enzyme for this step in rats. Acetaldehyde is oxidized to acetic acid and eventually to carbon dioxide (CO_2). *tert*-Butanol can be sulfated, glucuronidated, and excreted into urine, or it can undergo further oxidation to form 2-methyl-1,2-propane diol (MPD), 2-hydroxybutyrate (HBA), acetone, and formaldehyde. It should be noted that these metabolites have been identified in human or rat liver extracts for ETBE, MTBE, and *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.

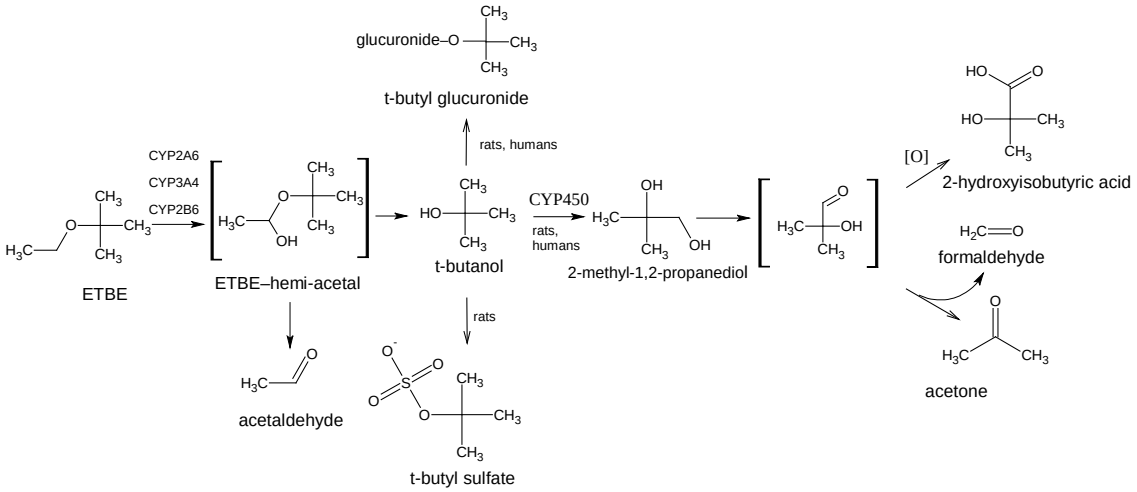


Figure B-1. Proposed metabolism of ETBE.

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 and their toxic effects. The metabolism model correctly predicted cleavage into *tert*-butanol and acetaldehyde and that *tert*-butanol would undergo glucuronidation and sulfation. However, for the further metabolism of *tert*-butanol, 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 HBA, which have been found in vivo.

B.2.3.1. Metabolism in Humans

Metabolism of ETBE in Humans in Vivo

Nihlén et al. (1998a) 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.

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 13 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 HBA, were also assayed.

At an exposure level of 170 mg/m^3, the peak concentration of *tert*-butanol in blood was 13.9 ± 2.2; the peak concentration was 1.8 ± 0.2 µM at 18.8 mg/m^3. The time courses of metabolite appearance in urine after 170 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:HBA) 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 both human liver microsomes and 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 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. Corresponding activities with 1 mM MTBE as the substrate were 6.1 and 0.7 nmol/min-nmol, respectively.

Hong et al. (1999a) obtained 15 human liver microsome samples and used them to compare metabolic activities with ETBE, MTBE, and TAME as the substrates. They found that the metabolism of all three substrates was highly correlated with certain CYP isozymes. The highest degree of correlation was found for CYP2A6, which also displayed the highest turnover numbers. The 15 samples displayed very large interindividual variations in metabolic activities, with turnover numbers for ETBE ranging from 179–3,130 pmol/minute-mg protein. Michaelis constant (K_m) values, estimated in three human liver microsomal samples using MTBE, ranged from 28–89 µM, with maximum substrate turnover velocity (V_{max}) values ranging from 215–783 pmol/minute-mg protein. The V_{max}/K_m ratios, however, varied only between 7.7 and 8.8.

As part of CYP inhibition studies in the same paper, human liver microsomes were co-incubated with MTBE, ETBE, or TAME in the presence of chemicals or specific antibodies to inhibit either CYP2A6 or CPY2E1. For chemical inhibition, coumarin was dissolved in 2 µL of methanol and added to the liver microsomes prior to initiation of the reaction. For antibody inhibition, monoclonal antibodies against human CPY2A6 and CYP2E1 were preincubated with liver microsomes prior to incubation with the rest of the reaction mixture. Methanol alone caused approximately 20% inhibition of MTBE, ETBE, and TAME. Coumarin, a CYP2A6 substrate, caused a significant dose-dependent inhibition of all three oxidants with a maximal inhibition of ETBE of 99% at 100-µM coumarin. Antibodies against CYP2A6 inhibited metabolism of MTBE, ETBE, and TAME by 75–95%. In contrast, there was no inhibition by the antibody against CYP2E1. The same anti-CYP2E1 antibody inhibited over 90% of CPY2E1 activity assayed as *N*-nitrosodimethylamine in the liver microsomes.

In the same paper, these authors introduced several specific human CYPs into human β-lymphoblastoid cells and measured metabolic activities with ETBE and MTBE as the substrates. They established a correlation ranking for ETBE metabolism (relative to *tert*-butanol) by 10 human CYP isozymes: 2A6 > 3A4 ≈ 2B6 ≈ 3A4/5 » 2C9 > 2E1 ≈ 2C19 » 1A2 ≈ 2D6 ≈ 1A2. They characterized

the correlation with CYP2A6 as high, 3A4; 3A5, and 2B6 as good; 2C9, 2E1, and 2C19 as poor; and the remaining three CYP activities as not correlated with ETBE metabolism. They also reported direct enzyme activities toward ETBE as the substrate (in pmol *tert*-butanol formed per minute per pmol CYP enzyme): 2A6–1.61; 2E1–0.34; 2B6–0.18; and 1A2–0.13. CYPs 1B1, 2C8, 2C9, 2C19, and 2D6 were not investigated. CYP1A2, which showed activity toward ETBE, did not metabolize MTBE to *tert*-butanol. CYP4A11 showed considerable activity toward MTBE but very low activity toward ETBE and TAME. CYP3A4 and 1A1 did not metabolize ETBE or MTBE in this system but displayed considerable activity toward TAME. The authors concluded that CYP2A6 is the major enzyme responsible for the oxidative metabolism of MTBE, ETBE, and TAME 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 MTBE, ETBE, or TAME.

Le Gal et al. (2001) used similar human cytochrome preparations as Hong et al. (1999a) (i.e., from deceased human donors) or genetically modified human β-lymphoblastoid cells to elucidate the metabolism of ETBE, MTBE, and TAME. They identified as primary metabolites formaldehyde from MTBE and TAME, acetaldehyde from ETBE, tertiary amyl-alcohol from TAME, and *tert*-butanol from ETBE and MTBE. The human microsomes showed higher catalytic activity toward MTBE and TAME at 0.5 mM compared with ETBE, but very similar activities at substrate concentrations of 10 mM. Le Gal et al. (2001) confirmed the wide interindividual variation of activities previously reported by Hong et al. (1999a) and Hong et al. (1997b). Using MTBE as the substrate, they found a highly significant correlation with CYP2A6 activities and a lesser, but still significant, correlation with CYP3A4 activities. No correlations could be established for 1A1, 1A2, or 2E1 activities. However, using substrate concentrations of 0.5 and 10 mM, they found that 2A6 and 3E4, but not 2E1 or 2B6, had high activity at 0.5 mM, while 2E1 and 2B6 displayed considerable activity at 10 mM. Using the average levels and the turnover numbers of various CYPs in human liver, they concluded that fuel oxygenate ethers were predominantly metabolized by CYP2A6, with considerable contribution from CYP3A4. CYP2E1, they concluded, did not play a significant role in human metabolism of these substances.

B.2.3.2. Metabolism in Animals

Metabolism of ETBE in Animals In Vivo

Bernauer et al. (1998) studied the metabolism and excretion of [^{13}C]-ETBE, MTBE, and *tert*-butanol in rats. F344 rats, 2/sex, were exposed via inhalation to 2,000 ppm (8,400 mg/m^3) ETBE or 2,000 ppm (7,200 mg/m^3) MTBE for 6 hours; three male F344 rats received 250 mg/kg *tert*-butanol by gavage. Urine was collected for 48 hours. The metabolic profiles for ETBE and MTBE were essentially identical, with excretion of MPD > HBA > *tert*-butanol-sulfate > *tert*-butanol-glucuronide. Oral administration of *tert*-butanol produced a similar metabolite profile, with HBA > *tert*-butanol-sulfate > MPD » *tert*-butanol-glucuronide ≈ *tert*-butanol. *tert*-Butanol could not be detected in urine when ETBE or MTBE were administered by inhalation. 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 coincident with the 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 much lower than in humans: 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. Similar to humans, rats excreted mostly HBA in urine, followed by MPD and *tert*-butanol. The molar ratios for total urinary excretion of *tert*-butanol:MPD:HBA 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 metabolism of MTBE and ETBE in humans and rats. They reported that, at a high exposure level (8,400 mg/m^3 ETBE; 7,200 mg/m^3 MTBE), rats predominantly excreted the glucuronide of *tert*-butanol in urine, which, at low levels (16.7 mg/m^3 or 167.1 mg/m^3 ETBE; 14.4 or 144.2 mg/m^3 MTBE), had been barely detectable. They concluded that, at high exposure levels, the normally rapid metabolism of *tert*-butanol to MPD and HBA became saturated, forcing more of the initial metabolite of ETBE or MTBE through the glucuronidation pathway. The apparent final metabolite of ETBE was HBA, although this substance can undergo further metabolism to acetone. The latter process appeared to play a minor role in the overall metabolism of ETBE or MTBE. The authors also pointed out that many metabolites of the fuel oxygenate ethers, such as formaldehyde, acetaldehyde, *tert*-butanol, HBA, or acetone, occur naturally in normal mammalian physiology, providing a highly variable background that needs to be accounted for in metabolic experiments.

The JPEC (2008d, e) 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 both single and 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. These data indicate that ETBE is quickly metabolized to *tert*-butanol, which is then metabolized to *tert*-butanol glucuronide, 2-methyl-1,2-propanediol, and finally to 2-hydroxyisobutyrate.

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	% 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 (2008d, e) 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	% 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.

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

Source: JPEC (2008d, e) unpublished reports

Metabolism of ETBE in Animal Tissues in Vitro

Using isolated rat liver microsomes, Hong et al. (1997a) found that metabolism occurred only in the presence of an NADPH- (nicotinamide adenine dinucleotide phosphate) regenerating system and that the metabolic activity was inhibited by 80% after treating the microsomal preparation with carbon monoxide, indicating CYP involvement. In another study investigating potential target tissues for ETBE toxicity, Hong et al. (1997a) studied the metabolic activities of olfactory mucosa, respiratory epithelium, liver, lung, and olfactory bulb from rats. They prepared microsomes, added an NADPH-regenerating system, and evaluated enzyme kinetics at various substrate concentrations. In olfactory mucosa, the authors derived K_m values of 125 and 111 µM for ETBE and MTBE, with corresponding V_{max} values of 11.7 and 10.3 nmol/minute-mg protein, respectively. Addition of TAME to the reaction mixture exerted a concentration-dependent inhibition of ETBE or MTBE metabolism. Coumarin, a CYP2A6 substrate, also inhibited ETBE metabolism. These results indicated that rat olfactory mucosa, on a per-weight basis, has 37 times the capacity of liver to metabolize fuel oxygenate ethers, and hence, has the capacity for first-pass metabolism.

Hong et al. (1999b) used CYP2E1 knockout mice to investigate whether this enzyme plays a major role in fuel oxygenate ether metabolism. They compared the ether-metabolizing activity of liver microsomes (30 minutes at 37°C and 1 mM ether) 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 (nmol/minute-mg protein) were 0.51 ± 0.24 for CP2E1 knockout mice, 0.70 ± 0.12 for C57BL/6N mice, and 0.66 ± 0.14 for 129/Sv mice. The MTBE-metabolizing activities (nmol/minute-mg protein) were 0.54 ± 0.17 for CP2E1 knockout mice, 0.67 ± 0.16 for C57BL/6N mice, and 0.74 ± 0.14 for 129/Sv mice. The TAME-metabolizing activities (nmol/minute-mg protein) were 1.14 ± 0.25 for CP2E1 knockout mice, 1.01 ± 0.26 for C57BL/6N mice, and 0.76 ± 0.25 for 129/Sv mice. Mice that did not express any CYP2E1 did not differ from wild-type animals in their ability to metabolize ETBE, MTBE, or TAME, suggesting that CYP2E1 is unlikely to be important in the metabolism of ETBE. Turini et al. (1998) investigated the influence of ETBE exposure on hepatic microsomal enzyme activities (as measured using CYP isozyme-specific substrates) and the effects of specific enzyme induction on ETBE metabolism in male Sprague-Dawley rats. Moderate doses of ETBE (200 or 400 mg/kg) administered intraperitoneally for 4 days did not induce any hepatic CYPs. However, ETBE (2 mL/kg) administered by gavage as a 50% corn oil solution for 2 days almost doubled activities of 3A1/2 and 2B1, doubled 2E1, and induced CYP2B1/2 sixfold. CYP1A1/2 activity was slightly reduced after 2 days of ETBE (2 mL/kg) by gavage. The authors also estimated kinetic constants for various CYPs in rats and found the following K_m or V_{max} values: controls (2C forms predominant), 6.3 mM/0.93 nmol/minute-mg protein; 2A/2B induced, 4.1 mM/3.8 nmol/minute-mg protein; 2E1 induced, 4.7 mM/1.6 nmol/minute-mg protein; 3A induced, 4.4 mM/1.4 nmol/minute-mg protein; and 1A induced, not determined/0.9 nmol/minute-mg protein. Using a system with reconstituted CYPs, the authors

found that CYP2B1 displayed the lowest K_m (2.3 mM), and the highest turnover number (56 nmol/minute-nmol CYP) and concluded that this isoform was the principal CYP to metabolize ETBE in the rat.

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

B.2.4. Elimination

B.2.4.1. Elimination in Humans

Nihlén et al. (1998a) 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, *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 accounted for even less: 0.12, 0.061, and 0.056% of the dose was retained after 20.9, 104, and 209 mg/m^3 exposures, 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 [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).

These data suggest complex toxicokinetics for ETBE in humans. The first phase of elimination from blood likely indicates uptake into highly perfused tissues. The other phases may indicate uptake into less perfused tissues and fat, as well as 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.

Amberg et al. (2000) exposed six volunteers (three males and three females, 28 ± 2 years old) to 18.8 and 170 mg/m^3 of ETBE, respectively. 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 and at 4 or 6 hours after exposure,

and thereafter every 6 hours for 48 hours. Parent ETBE and *tert*-butanol were determined in blood and urine. Two further metabolites of *tert*-butanol, HBA and MPD, were also determined in urine.

At 170 mg/m^3, the peak concentration of ETBE in blood was 12.1 ± 4.0 µM, while 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. At the high exposure concentration, two elimination half-lives were found for ETBE, 1.1 ± 0.1 and 6.2 ± 3.3 hours. *tert*-Butanol displayed only one half-life, 9.8 ± 1.4 hours. At the low exposure concentration, only the short half-life for ETBE could be measured at 1.1 ± 0.2 hours, while that for *tert*-butanol was 8.2 ± 2.2 hours. The predominant urinary metabolite identified was HBA, excreted in urine at 5–10 times the amount of MPD and 12–18 times the amount of *tert*-butanol (note: urine samples had been treated with acid before analysis to cleave conjugates). Excretion of unchanged ETBE in urine was minimal. The time courses of urinary elimination after 170 and 18.8 mg/m^3, respectively, were similar, but relative urinary levels of HBA after 18.8 mg/m^3 were higher, while those for MPD were lower, as compared to 170 mg/m^3. HBA in urine showed a broad maximum at 12–30 hours after exposure to both concentrations, with a slow decline thereafter. MPD in urine peaked at 12 and 18 hours after 170 and 18.8 mg/m^3, respectively, while *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. Individual variations were large, but the authors did not report gender differences in the toxicokinetics of ETBE. Based on the dose estimates presented in Section B.2.3.1, 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.

B.2.4.2. Elimination in Animals

Amberg et al. (2000) exposed F344 NH rats, 5/sex/dose concurrent with the volunteers in the same exposure chamber. Urine was collected for 72 hours following exposure. Similar to humans, rats excreted mostly HBA 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 HBA. 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 three male F344 rats received 250 mg/kg *tert*-butanol by gavage. Urine was collected for 48 hours. The metabolic profiles for ETBE and MTBE were essentially identical, with relative excreted amounts of MPD > HBA > *tert*-butanol-sulfate > *tert*-butanol-glucuronide. Oral administration of *tert*-butanol produced a similar metabolite profile, with relative amounts of HBA > *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.

Sun and Beskitt (1995b) investigated the pharmacokinetics of [^{14}C]-ETBE in F344 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 (1995b) report for "Total" column in Table B-6 or for the specific tissues in Table B-7. Of the three animals per sex exposed concurrently, two were used in the further study, while the third was kept as a spare. One animal/sex was placed into a metabolic cage and monitored for up to 118 hours. 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 were combusted in a sample oxidizer and analyzed by liquid scintillation spectrometry.

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	37	1	60	2	9.9
3,130	36	1	62	2	17.5
4,180	42	1	56	2	22.1
7,310	58	2	38	3	56.9
10,400	52	2	45	2	56.2
20,900[d]	63 (51)	2 (1)	34 (44)	1 (3)	97.5 (116)
CD-1 Mouse[e]					
2,090	10	1	74	16	6.38

Exposure level (mg/m^3)	Volatile organics[a]	Exhaled CO_2[a]	Urine[a]	Feces[a]	Total[b]
3,130	28	2	60	10	7.9
4,180	29	2	64	6	12.8
7,310	42	2	46	10	13.7
10,400	42	2	47	10	22.7
20,900[d]	44 (37)	5 (2)	39 (57)	12 (2)	18.9 (28)

[a]Percent of total eliminated radioactivity; mean of one male and one female.
[b]In mg [^{14}C]-ETBE equivalents.

Sources: [c]Sun and Beskitt (1995b); [d]values in parentheses: Borghoff (1996); [e]Sun and Beskitt (1995b)

Table B-7. 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/m3)	F344 Rata,		CD-1 Mousea,	
	Bloodb	Kidneyc	Bloodb	Liverc
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.
[b]In mg [^{14}C]-ETBE equivalents per gram blood.
[c]In mg [^{14}C]-ETBE equivalents.

Sources: Sun and Beskitt (1995b).

During 96 hours in metabolic cages, approximately 60% of the eliminated radioactivity was recovered from urine, and approximately 38% was recovered from exhaled organic volatiles. 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, while exhalation of organic volatiles increased to 63%. Exhalation of $^{14}CO_2$ increased marginally, from 1% at 2,090 mg/m^3 to 2% at 20,900 mg/m^3, while fecal elimination remained fairly constant at about 2% throughout the exposure concentrations. A compilation of these results, together with results from mice from a parallel study (Sun and Beskitt, 1995b), is given in Table B-6. The authors concluded

that the metabolic pathways leading to urinary excretion of ETBE degradation products became saturated at an exposure concentration of approximately 7,310 mg/m^3.

The time course of elimination indicated that exhalation of organic volatiles was essentially complete by 24 hours, while 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. Levels of radioactivity in blood and kidneys after increasing exposure concentrations of [^{14}C]-ETBE are shown in Table B-7 (again combined with the mouse data from the parallel study). The major finding was that radioactivity levels increased up to 10,450 mg/m^3 but leveled off in kidney and fell considerably in blood at 20,900 mg/m^3. To the authors, these data were indicative of saturation of the absorption pathway at around 10,450 mg/m^3. However, it is noteworthy that total elimination of ETBE-derived radioactivity increased steadily from 2,090 to 20,900 mg/m^3 (Table B-6). The authors reported no deaths following 6 hours of ETBE exposure. The findings of Sun and Beskitt (1995a), unpublished report, at 20,900 mg/m^3 were essentially confirmed by Borghoff (1996) (unpublished report) in a pilot study that used the identical species, experimental protocol, materials, and methods but was conducted at a different laboratory later.

In a parallel study with an identical experimental protocol, Sun and Beskitt (1995b), in an unpublished report, exposed CD-1 mice (3/sex/dose) to 2,090, 3,130, 4,180, 7,310, 10,450, and 20,900 mg/m^3 [^{14}C]-ETBE. The only difference from the rat study in the Sun and Beskitt (1995a) unpublished report was that, instead of kidneys, livers were harvested from mice. The corresponding results from this study are shown in Tables B-6 and B-7, jointly with the results from the rat study.

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 (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 were considerably higher, as reported, 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 and/or had a higher metabolic capacity. 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 and metabolic capacities of mice had become saturated. Judging from the data in Table B-67, saturation of blood and liver had occurred already at 7,310 mg/m^3. The authors reported no deaths following 6 hours of ETBE exposure. It may be noted here that Sun and Beskitt (1995a, b) did not state any estimates for absorbed dose. The data in Table B-6, however, indicate that, given the rapid exhalation of [^{14}C]-ETBE-derived material, any attempt to estimate a level of inhalation absorption following a 6-hour exposure without respiratory elimination control would be futile.

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) in their unpublished reports; however, in this pilot study, only three male F344 rats and three male CD-1 mice were used per experiment, with the only exposure level 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. 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, while the balance of radioactivity after 96 hours in metabolic cages came to 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 animals 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, HBA, 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. HBA was detected in urine of both species but could not be quantified. MPD was not detected. These results may be interpreted as suggesting that mice metabolize and, hence, eliminate ETBE faster than rats.

Unpublished reports by the JPEC (2008d) 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.2.5. Physiologically based pharmacokinetic models

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

Many PBPK models have been developed for the structurally related substance, MTBE, in rats and humans (Borghoff et al., 2010; Leavens and Borghoff, 2009; Blancato et al., 2007; Kim et al., 2007; Rao and Ginsberg, 1997; Borghoff et al., 1996). These MTBE models can be modified for ETBE by using the available toxicokinetic data described above. EPA's model evaluation and use for the dose-response modeling in this assessment can be found below.

The U.S. Environmental Protection Agency (EPA) evaluated a PBPK model of ETBE and its principle metabolite *tert*-butanol that was developed for humans exposed while performing physical work (Nihlén and Johanson, 1999). As previously mentioned, the Nihlén and Johanson model is not appropriate for rodents or for oral exposures, precluding cross-species or route-to-route extrapolations. Thus, EPA developed a PBPK model for ETBE and its metabolite, *tert*-butanol, in the rat. This section present details on this model and applicability to this assessment.

A PBPK model for ETBE and *tert*-butanol in rats was developed in acslX (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, Alabama) by adapting information from the many PBPK models that were developed in rats and humans for MTBE and the metabolite *tert*-butanol that is common to both MTBE and ETBE (Borghoff et al., 2010; Leavens and Borghoff, 2009; Blancato et al., 2007; Kim et al., 2007; Rao and Ginsberg, 1997; Borghoff et al., 1996). A brief description highlighting the similarities and differences in the Blancato et al. (2007) and Leavens and Borghoff (2009) models is given, followed by an evaluation of the MTBE models and the assumptions adopted from MTBE models or modified in the ETBE model.

The Blancato et al. (2007) model is an update of the earlier Rao and Ginsberg (1997) model, and the Leavens and Borghoff (2009) model is an update of the Borghoff et al. (1996) model. Both the Blancato et al. (2007) and Leavens and Borghoff (2009) models are flow-limited models that

predict amounts and concentrations of MTBE and *tert*-butanol in blood and six tissue compartments: liver, kidney, fat, brain, and rapidly and slowly perfused tissues. These tissue compartments are linked through blood flow, following an anatomically accurate, typical, physiologically based description (Andersen, 1991). The parent (MTBE) and metabolite (*tert*-butanol) models are interlinked by the metabolism of MTBE to *tert*-butanol in the liver. Routes of exposure included in the models are oral and inhalation for MTBE; Leavens and Borghoff (2009) included inhalation exposure to *tert*-butanol. Oral doses are assumed to be 100% bioavailable and 100% absorbed from the gastrointestinal tract represented with a first-order rate constant. Following inhalation of MTBE or *tert*-butanol, the chemical is assumed to directly enter the systemic blood supply, and the respiratory tract is assumed to be at a pseudo-steady state. Metabolism of MTBE by CYP450s to formaldehyde and *tert*-butanol in the liver is described with two Michaelis-Menten equations representing high- and low-affinity enzymes. *tert*-Butanol is either conjugated with glucuronide or sulfate or further metabolized to acetone through 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate; both of these processes are described by a single Michaelis-Menten equation in the models. All model assumptions are valid for *tert*-butanol and were applied to the EPA-developed *tert*-butanol PBPK model, except for the separate brain compartment. The brain compartment was lumped with the compartment for other richly perfused tissues in the EPA *tert*-butanol PBPK model.

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

MTBE and *tert*-butanol binding to α_{2u}-globulin in the kidneys of male rats was incorporated in the PBPK model of MTBE by Leavens and Borghoff (2009). Binding to α_{2u}-globulin is one hypothesized mode of action for the observed kidney effects in MTBE-exposed animals. For a detailed description of the role of α_{2u}-globulin and other modes of action in kidney effects, see the kidney MOA section of the main volume (see Section 1.1.1). Binding of MTBE to α_{2u}-globulin was applied to sex differences in kidney concentrations of MTBE and *tert*-butanol in the Leavens and Borghoff (2009) model but acceptable estimates of MTBE and *tert*-butanol pharmacokinetics in the blood are predicted in other models that did not consider α_{2u}-globulin binding. Moreover, as discussed below, the Leavens and Borghoff (2009) model did not adequately fit the available *tert*-butanol i.v. dosing data, adding uncertainty to the binding parameters they estimated. Given the lack of ETBE concentration data in kidney tissue following ETBE exposure, binding to

α_{2u}-globulin could not be applied to the ETBE PPBK model. However, this binding does not significantly affect blood concentrations, so this data gap is not considered critical to estimating systemic concentration of ETBE.

B.2.5.1. Evaluation and Modification of Existing *tert*-butanol submodels

The Blancato et al. (2007) and Leavens and Borghoff (2009) models were evaluated by comparing predictions from the *tert*-butanol portions of the models with the *tert*-butanol i.v. data of Poet and Borghoff (1997) (Figure B-2). Neither model adequately represented the *tert*-butanol blood concentrations. Modifications of model assumptions for alveolar ventilation, explicit pulmonary compartments, and induction of metabolism of *tert*-butanol did not significantly improve model fits to the data.

A) B)

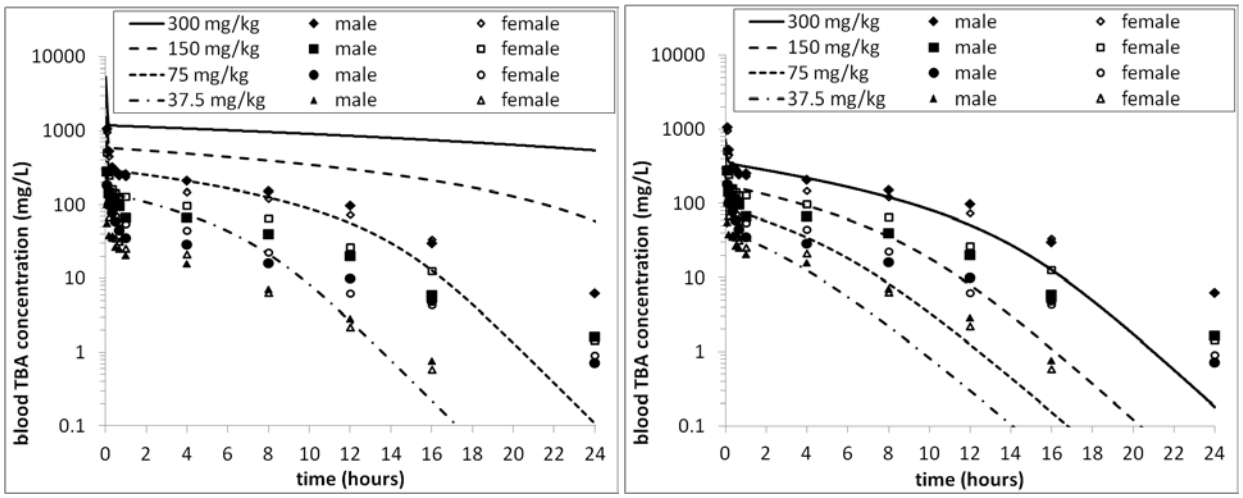


Figure B-2. Comparison of the *tert*-butanol portions of existing MTBE models with *tert*-butanol blood concentrations from i.v. exposure by Poet and Borghoff (1997).

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

Attempts to reoptimize model parameters in the *tert*-butanol submodels of Blancato et al. (2007) and Leavens and Borghoff (2009) to match blood concentrations from the i.v. dosing study were unsuccessful. To account for the *tert*-butanol blood concentrations after i.v. *tert*-butanol exposure, the model was modified by adding a pathway for reversible sequestration of *tert*-butanol in the blood. This could represent binding of *tert*-butanol to proteins in blood (see Figure B-32). The JPEC pharmacokinetic studies show that approximately 60% of the radiolabel in whole blood is in the plasma, providing some limited evidence for association of *tert*-butanol with components in blood. The PBPK model represented the rate of change of *tert*-butanol amount in the sequestered blood compartment (A_{blood2}) with the equation:

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

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

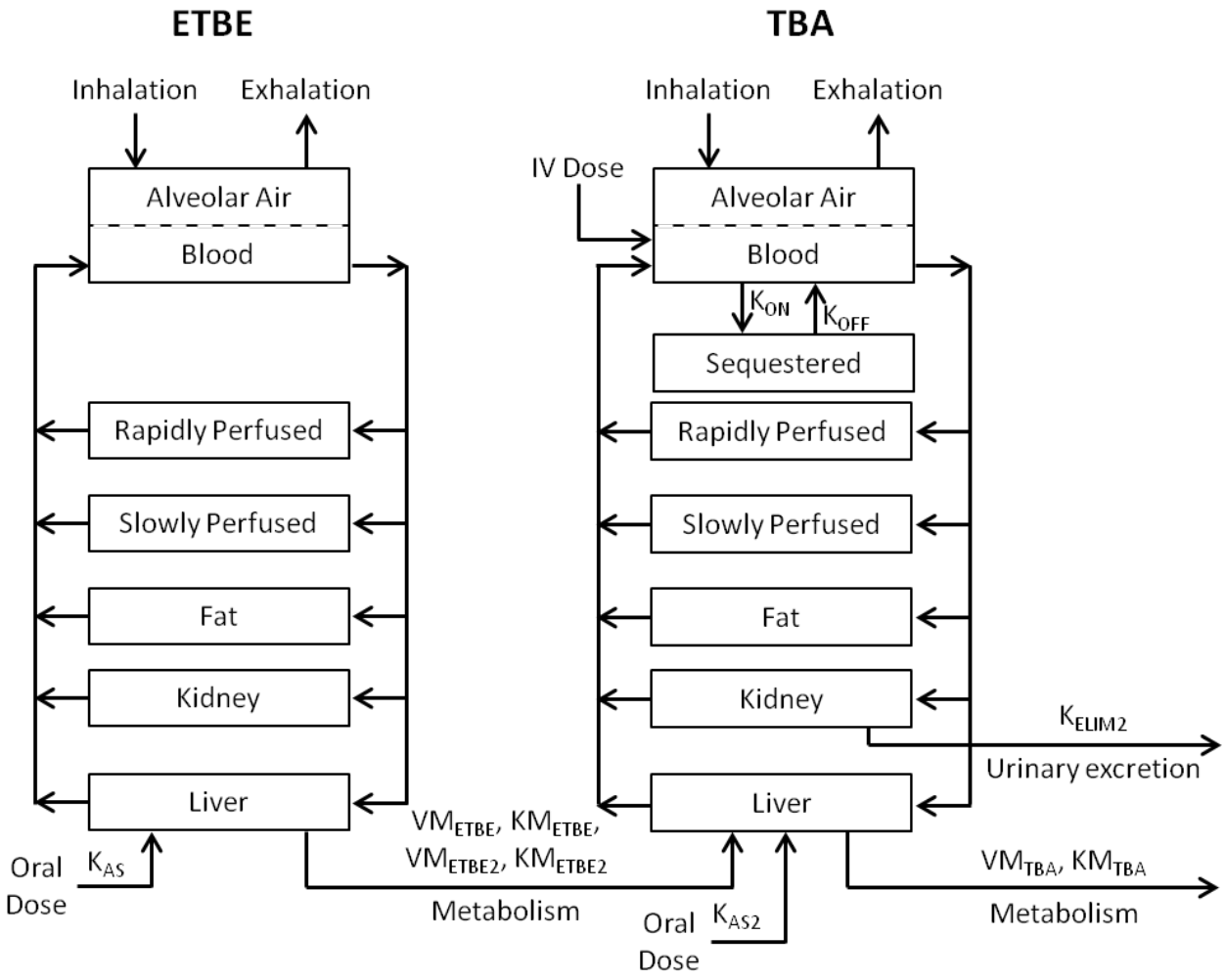


Figure B-3. Schematic of the PBPK model for ETBE and its major metabolite *tert*-butanol in rats.

Exposure can be via multiple routes including inhalation, oral, or i.v. dosing. Metabolism of ETBE and *tert*-butanol occur in the liver and are described by Michaelis-Menten equations with two pathways for ETBE and one for *tert*-butanol. ETBE and *tert*-butanol are cleared via exhalation, and *tert*-butanol is additionally cleared via urinary excretion. See Table B-8 for definitions of parameter abbreviations.

The physiologic parameter values were obtained from the literature (Brown et al., 1997) and are shown in Table B-8. *tert*-Butanol partition coefficients were obtained from literature where they were determined by the ratios of measured tissue:air and blood:air partition coefficients (Borghoff et al., 1996). The parameters describing rate constants of metabolism and elimination of *tert*-butanol were obtained from the literature (Blancato et al., 2007) and kept fixed because these have been optimized to *tert*-butanol blood concentrations measured after MTBE exposure, which is also metabolized to *tert*-butanol. The parameters describing *tert*-butanol absorption and *tert*-butanol sequestration in blood were estimated by optimizing the model to the blood *tert*-butanol time-course data simultaneously for rats exposed via i.v., inhalation, and oral routes (Leavens and Borghoff, 2009; Poet and Borghoff, 1997; ARCO, 1983).

The model parameters were estimated with the acslX optimization routine to minimize the log-likelihood function of estimated and measured *tert*-butanol concentrations. The Nedler-Mead algorithm was used with heteroscedasticity allowed to vary between 0 and 2. The predictions of the model with optimized parameters have a much improved fit to the *tert*-butanol blood concentrations after *tert*-butanol i.v. as shown in panel A of Figure B-4. Additionally, the model adequately estimates the *tert*-butanol blood concentrations after inhalation and oral gavage exposures. The optimized parameter values are shown in Table B-9. The ARCO (1983) study measured *tert*-butanol in plasma only, not whole blood like the Poet and Borghoff (1997) and Leavens and Borghoff (2009) studies. Based on the measurements of plasma and whole blood by JPEC (2008e), the concentration of *tert*-butanol in plasma is approximately 60% of the concentration in whole blood. The *tert*-butanol plasma concentrations measured by ARCO were increased (divided by 60%) to the expected concentration in whole blood for comparison with the PBPK model.

B.2.5.2. ETBE Model Parameterization and Fitting

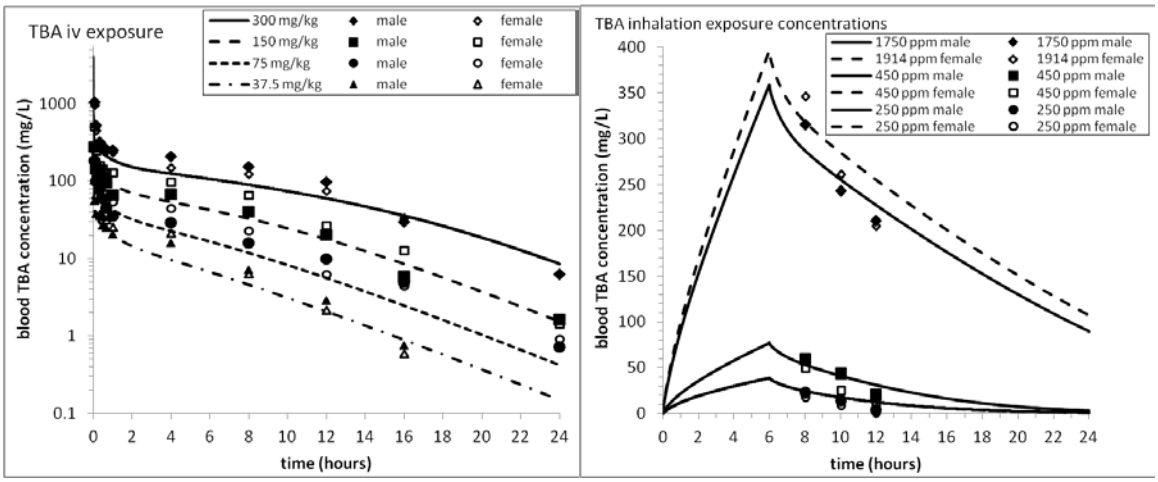
The ETBE submodel used the same physiological parameters as *tert*-butanol obtained from the literature Brown et al. (1997) shown in Table B-8. ETBE partition coefficients were obtained from literature Nihlén et al. (1995) where they were calculated for ETBE in tissues by relating measured blood:air, water:air, and oil:air partition coefficients to reported compositions of water and lipids in rat tissues. The parameters describing ETBE absorption and metabolism were optimized to fit the blood and urine time-course data for rats exposed to ETBE via oral and inhalation routes (JPEC, 2008e; Amberg et al., 2000; Borghoff, 1996). During the optimization, parameters describing *tert*-butanol were held constant. The model parameters were estimated with the acslX optimization routine in the same way as for the *tert*-butanol submodel. The optimized parameter values are shown in Table B-9. The predictions of the model with optimized parameters for ETBE oral gavage by JPEC (2008e) are shown in Figure B-5. This study measured *tert*-butanol in plasma only, not whole blood like the Amberg et al. (2000) and other *tert*-butanol studies. Based on the measurements of plasma and whole blood by JPEC (2008e), the concentration of *tert*-butanol in

Table B-8. PBPK model physiologic parameters and partition coefficients.

Body weight and organ volumes as fraction of body weight		
Body Weight (kg)	0.25	Brown et al. (1997)
Body fraction that is blood perfused (Fperf)	0.8995	Brown et al. (1997)
Liver	0.034	Brown et al. (1997)
Kidney	0.007	Brown et al. (1997)
Fat	0.07	Brown et al. (1997)
Rapidly perfused	0.04	Brown et al. (1997)
Slowly perfused	0.7485	[a]
Blood	0.074	Brown et al. (1997)
Cardiac output and organ blood flows as fraction of cardiac output		
Cardiac output (L/hr)	5.38	Brown et al. (1997)[b]
Alveolar ventilation (L/hr)	5.38	Brown et al. (1997)[c]
Liver	0.174	Brown et al. (1997)[d]
Kidney	0.141	Brown et al. (1997)
Fat	0.07	Brown et al. (1997)
Rapidly perfused	0.279	[e]
Slowly perfused	0.336	Brown et al. (1997)
Partition coefficients for ETBE		
Blood:air	11.7	Nihlén et al. (1995)
Liver:blood	1.68	Nihlén et al. (1995)
Fat:blood	12.3	Nihlén et al. (1995)
Rapidly perfused:blood	2.34	[f]
Slowly perfused:blood	1.71	[g]
Kidney:blood	1.42	Nihlén et al. (1995)
Partition coefficients for *tert*-butanol		
Blood:air	481	Borghoff et al. (1996)
Liver:blood	0.83	Borghoff et al. (1996)
Fat:blood	0.4	Borghoff et al. (1996)
Rapidly perfused:blood	0.83	Borghoff et al. (1996)
Slowly perfused:blood	1.0	Borghoff et al. (1996)
Kidney:blood	0.83	Borghoff et al. (2001)

[a] Fperf - Σ(other compartments)
[b] $15.2*BW^{0.75}$
[c] Alveolar ventilation is set equal to cardiac output
[d] sum of liver and gastrointestinal (GI) blood flows
[e] 1 - Σ(all other compartments)
[f] Set equal to brain tissue
[g] Set equal to muscle tissue

A) B)



C)

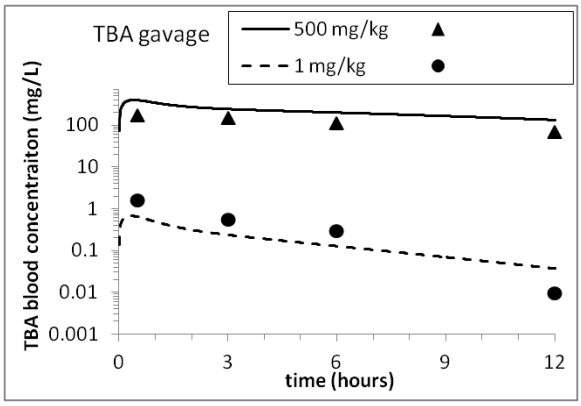


Figure B-4. Comparison of the EPA model predictions with measured *tert*-butanol blood concentrations for i.v., inhalation and oral gavage exposure to *tert*-butanol.

A) i.v. data from Poet and Borghoff (1997) B) inhalation data from Leavens and Borghoff (2009) and C) oral gavage data from ARCO (1983) with the optimized parameter values as shown in Table B-9.

Table B-9. Rate constants determined by optimization of the model with experimental data.

Parameter	Value	Source or Reference
tert-butanol rate constants		
Metabolism (VM_{TBA}; mg/kg-hr)[a]	8.0	Blancato et al. (2007)
Metabolism (KM_{TBA}; mg/L)	28.8	Blancato et al. (2007)
Urinary elimination (K_{ELIM2}; 1/hr)	0.5	Blancato et al. (2007)
tert-butanol sequestration rate constant (K_{ON}; L/hr)	0.148	Optimized
tert-butanol unsequestration rate constant (K_{OFF}; L/hr)	0.0134	Optimized
Absorption from gastrointestinal (GI) (K_{AS2}; 1/hr)	0.5	Optimized
ETBE rate constants		
Metabolism high affinity (VM_{ETBE}; mg/L-hr)	1.89	Optimized

Parameter	Value	Source or Reference
Metabolism high affinity (KM_{ETBE}; mg/L)	0.035	Optimized
Metabolism low affinity (VM_{ETBE2}; mg/L-hr)	15.2	Optimized
Metabolism low affinity (KM_{ETBE2}; mg/L)	10.0	Optimized
Absorption from GI (K_{AS}; 1/hr)	0. 5	Optimized

[a] scaled by $BW^{0.7}$ ($0.25^{0.7}$ = 0.379)

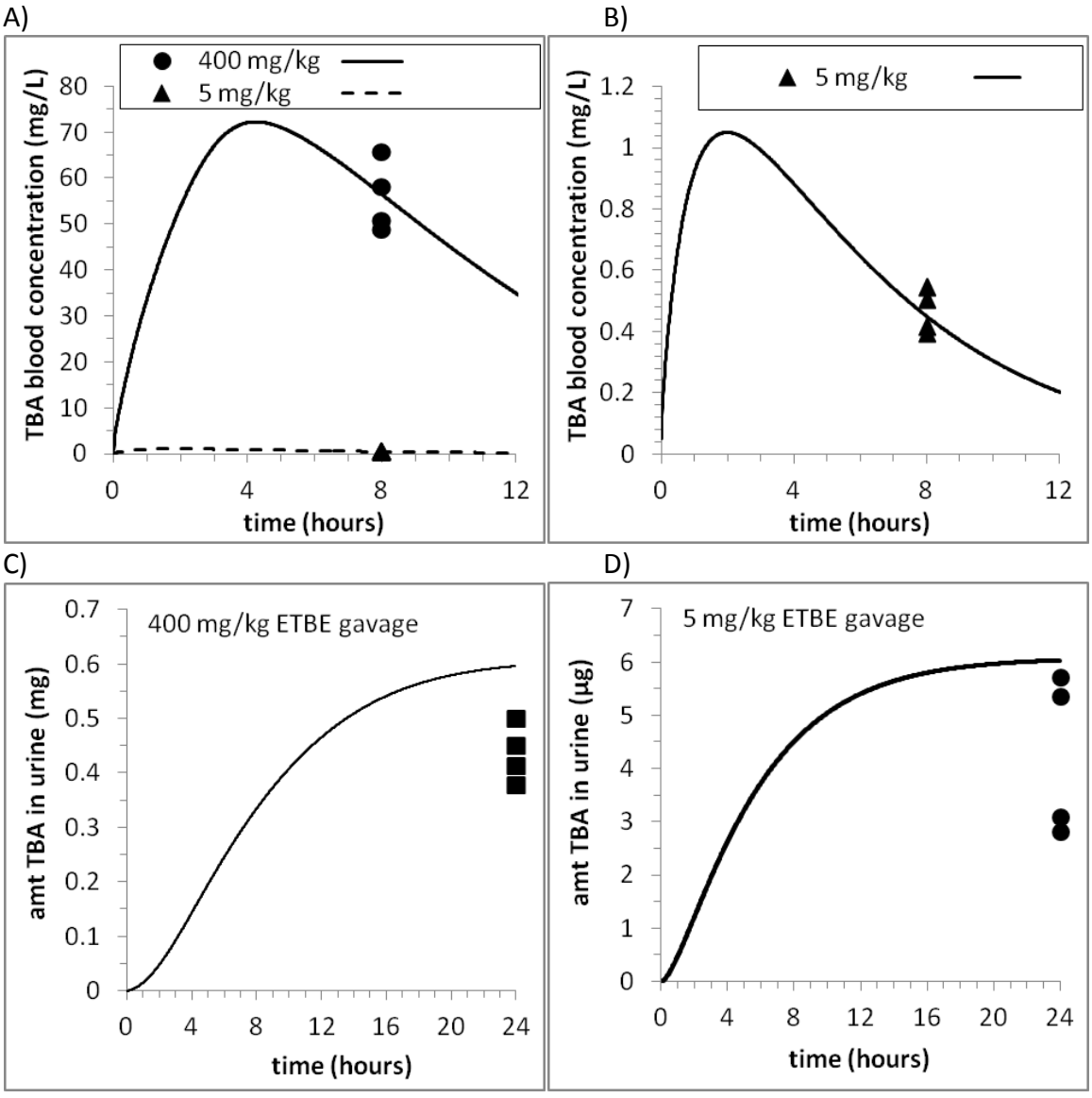


Figure B-5. Comparison of the EPA model predictions with measured amounts of *tert*-butanol after oral gavage of ETBE.

The data points show the measurements from the four individual rats in the JPEC (2008e) study. The concentrations of *tert*-butanol in blood are shown in A) for the 5- and 400-mg/kg doses, and B) for only the 5-mg/kg dose. The amount of *tert*-butanol in urine is shown in C) for the 400-mg/kg dose and in D) for the 5-mg/kg dose. The model predictions used the optimized parameter values as shown in Table B-9.

plasma is approximately 60% of the concentration in whole blood. The *tert*-butanol plasma concentrations measured by JPEC were increased (divided by 60%) to the expected concentration in whole blood for comparison with the PBPK model. The predictions of the model with optimized parameters are compared with amounts measured by Amberg et al. (2000) after ETBE inhalation in Figure B-6. While the fit of the model to the data for the 4-ppm exposure are sufficient, the prediction of the *tert*-butanol blood concentration after the 40-ppm exposure is higher than was measured. The *tert*-butanol blood concentration would be reduced if exposed animals were reducing their breathing rate or other breathing parameters but the exposure concentration of 40-ppm ETBE exposure is unlikely to be high enough to cause a change in breathing parameters because at the much higher ETBE concentration in the Chemical Industry Institute of Toxicology (CIIT) study (5000 ppm), changes in breathing were not noted and the model predictions fit measured concentrations well. The urinary elimination of *tert*-butanol is underestimated by the *tert*-butanol submodel (Figure B-6). The rate constant for *tert*-butanol urinary elimination (K_{ELIM2}) 0.5/hour was obtained from the literature (the same value was used by (Blancato et al., 2007; Rao and Ginsberg, 1997) and Leavens and Borghoff (2009), which is supported by multiple studies of MTBE and *tert*-butanol. To match the measured amount of *tert*-butanol in urine, the rate constant would need to be increased to 1.5/hour as shown in **Error! Reference source not found.** Urinary elimination of *tert*-butanol is the minor elimination route; elimination is primarily by metabolism and exhalation, so increasing urinary elimination does not noticeably change the fit to the *tert*-butanol blood concentrations. Additionally, increasing the urinary elimination rate worsens the model predictions for urinary elimination after oral gavage (Figure B-5); therefore, the rate constant obtained from literature (0.5/hour) was used for model predictions. The predictions of the model with optimized parameters are compared with the amounts of ETBE and *tert*-butanol exhaled after exposure to 5000-ppm ETBE as measured by CIIT in Figure B-7. The EPA model fits the measured amounts well.

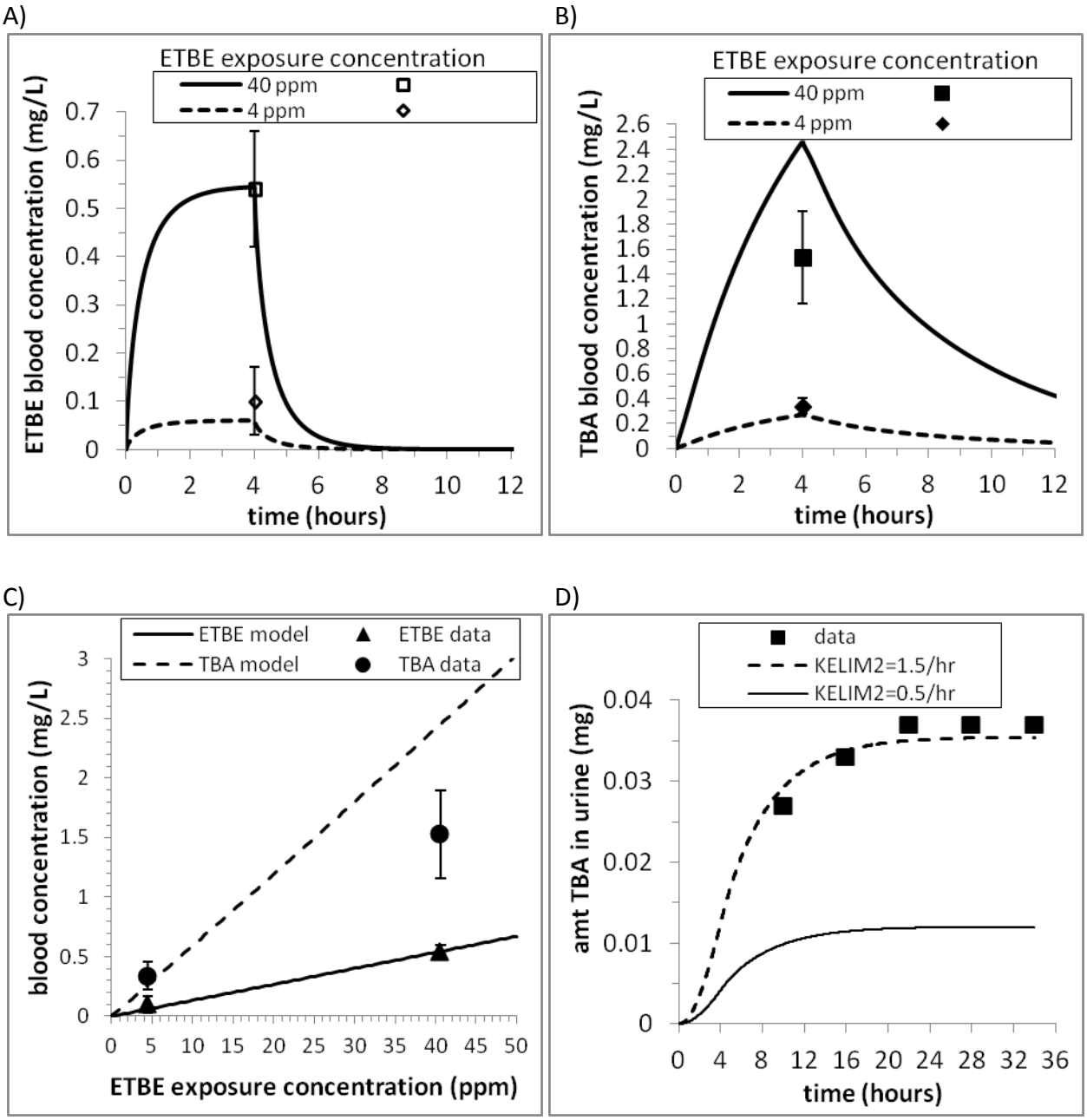


Figure B-6. Comparison of the EPA model predictions with measured amounts after a 4-hour inhalation exposure to 4 and 40 ppm ETBE.

Concentrations in blood are shown in A) for ETBE, B) for *tert*-butanol. In C) the measured ETBE and *tert*-butanol blood concentrations for exposures to 4 and 40 ppm ETBE are compared with model predictions of exposures from 0 to 50 ppm ETBE. The amount of *tert*-butanol in urine is shown in D) for the 40 ppm exposure for two values of K_{ELIM2}, the rate constant for *tert*-butanol urinary elimination. The value 0.5/hr was obtained from Blancato et al. (2007) and is used in all other EPA model predictions (e.g. Figure B-5). The increased rate constant 1.5/hr improves the fit of the model to urinary data. The 4 ppm exposure did not significantly increase the amount of urine over background. The data are from Amberg et al. (2000). The model predictions used the optimized parameter values as shown in Table B-9.

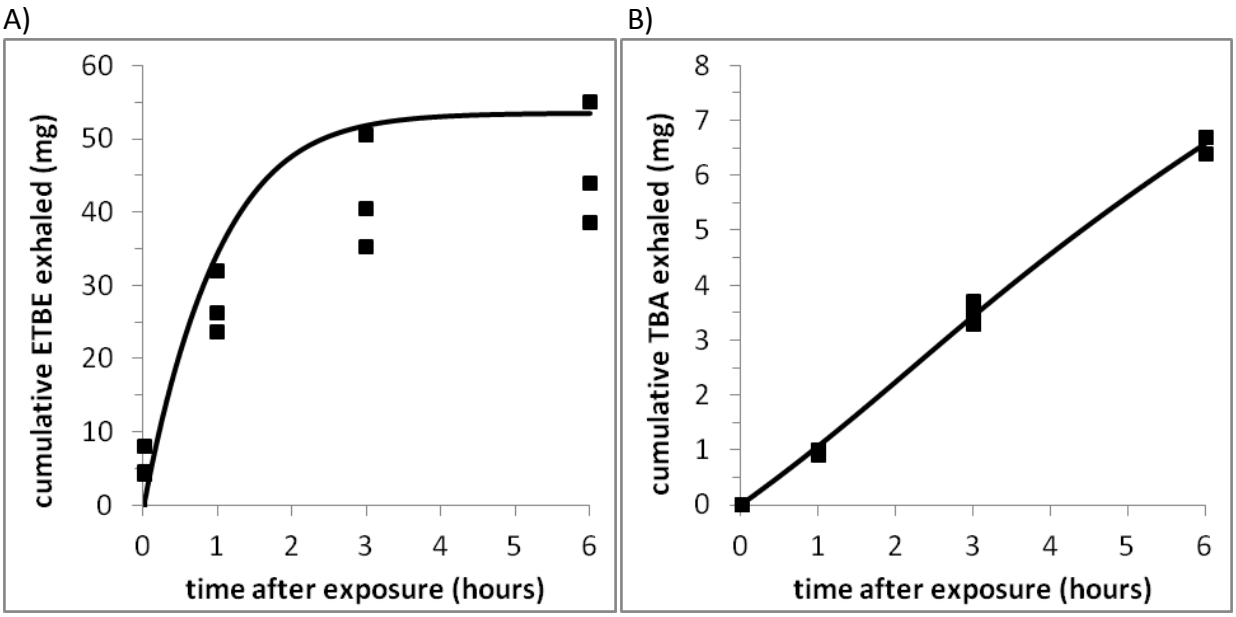


Figure B-7 . Comparison of the EPA model predictions with measured amounts of A) ETBE and B) *tert*-butanol in exhaled breath after a 6-hour inhalation exposure to 5000 ppm ETBE.

The data points show the individual measurements of the three rats in the ARCO (1983) study. The model predictions used the optimized parameter values as shown in Table B-9.

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

$$VmaxTBAIND = VmaxTBA*INDMAX(1-exp(-KIND*t))$$

where VmaxTBAIND is the maximum metabolic rate after accounting for enzyme induction, VmaxTBA is the metabolism rate constant from Table B-9 for both *tert*-butanol pathways, INDMAX is the maximum percent increase in VmaxTBA (124.9), and KIND is the rate constant for enzyme induction (0.3977/day). The increased *tert*-butanol metabolism better estimates the measured *tert*-butanol blood concentrations as can be seen in the comparison of the model predictions and experimental measurements shown in Figure B-8. The model better predicted blood concentrations in female rats than male rats. The male rats have lower *tert*-butanol blood concentrations after repeated exposures than female rats and this difference could indicate greater induction of *tert*-butanol metabolism in males or other physiologic changes such as ventilation, or urinary excretion. The current data for *tert*-butanol metabolism do not provide sufficient information for resolving this difference between male and female rats. The only repeat dose study

with ETBE was by oral gavage for 14 days at 5 mg/kg-day and *tert*-butanol blood concentrations did not decline after repeated doses JPEC (2010b). The internal dose of *tert*-butanol after repeated ETBE dosing in the JPEC (2010b) study was much lower than in the *tert*-butanol repeated dosing study (Leavens and Borghoff, 2009) and possibly the lower *tert*-butanol blood concentration wasn't sufficient to cause significant induction of *tert*-butanol metabolizing enzymes. The comparison of the model predictions and experimental measurements assuming no enzyme induction are shown in Figure B-9. An alternative explanation of the repeat dose studies is that some *tert*-butanol metabolism occurs in the respiratory tract and after inhalation exposure the induction of enzymes occurs more than after oral exposure.

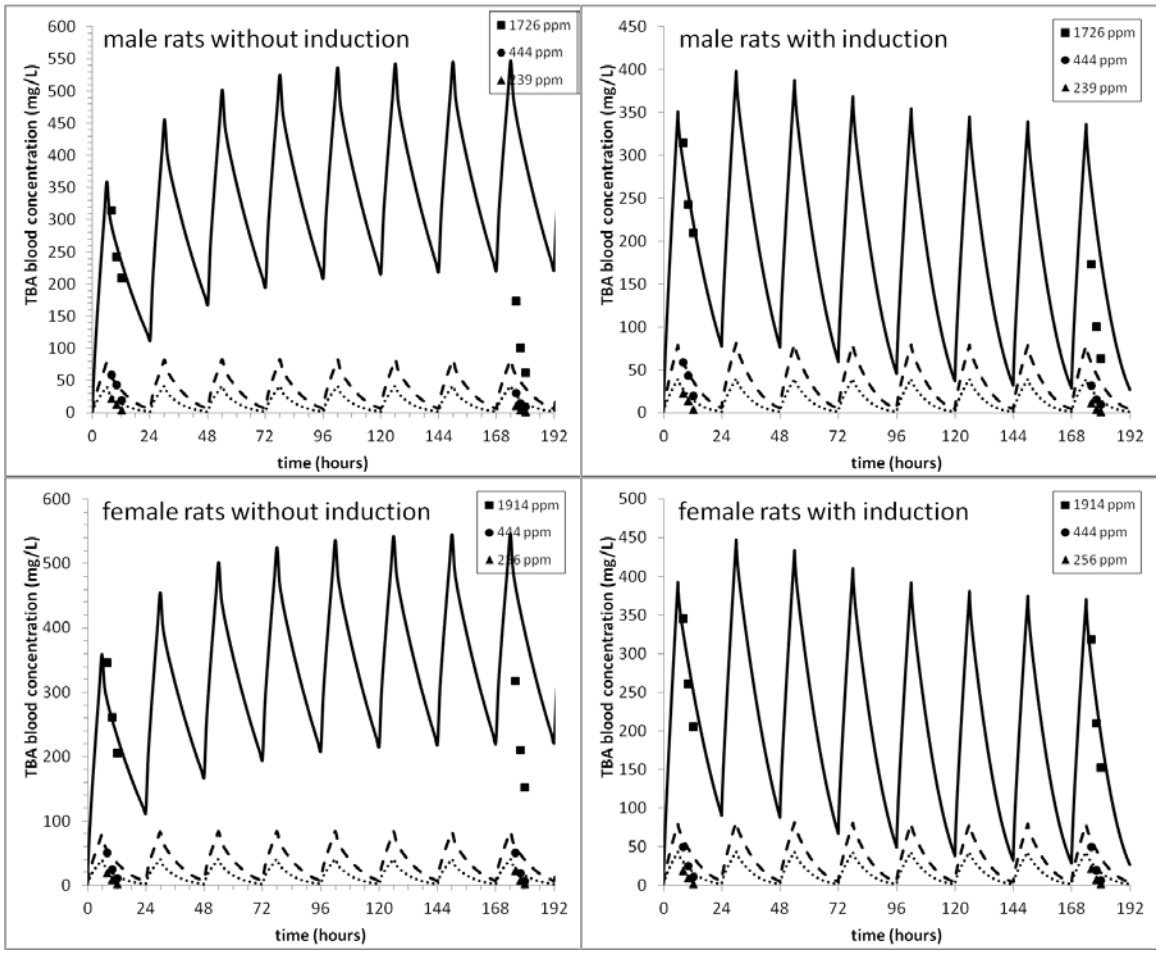


Figure B-8. Comparison of the EPA model predictions with measured amounts of *tert*-butanol in blood after repeated inhalation exposure to *tert*-butanol, 5 mg/kg-day ETBE oral gavage for up to 14 days in male rats.

The data show the individual measurements of the four rats in the JPEC (2010b) study. *tert*-Butanol blood concentrations are not well predicted by the model at the highest tert-butanol exposure concentration without enzyme induction.

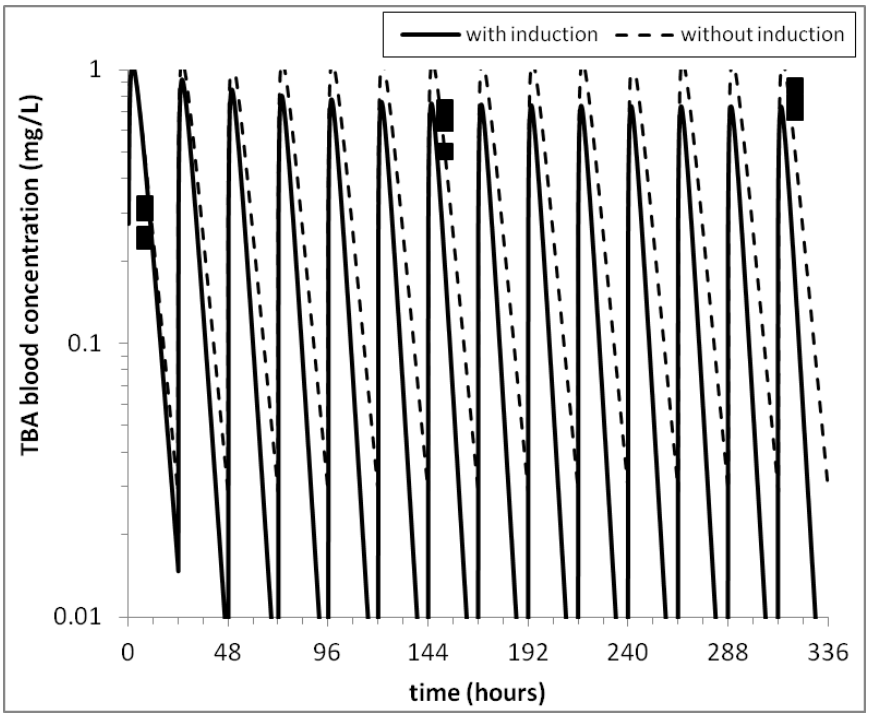


Figure B-9. Comparison of the EPA model predictions with measured amounts of *tert*-butanol in blood after 5 mg/kg-day ETBE oral gavage for up to 14 days in male rats.

The data show the individual measurements of the four rats in the JPEC (2010b) study. Adding enzyme induction to the model has a small effect on the predicted *tert*-butanol blood concentrations and the model predictions are closer to measured data when induction is not included.

B.2.5.3. Summary of the PBPK Model for ETBE

A PBPK model for ETBE and tert-butanol was developed by adapting previous models for MTBE and tert-butanol (Blancato et al. (2007); Leavens and Borghoff (2009)). Published tert-butanol models (or sub-models) do not adequately represent the tert-butanol blood concentrations measured in the i.v. study (Poet et al. 1997). The addition of a sequestered blood compartment for tert-butanol substantially improved the model fit. The alternative modification of changing to diffusion-limited distribution between blood and tissues also improved the model fit, but was considered less biologically plausible. Physiological parameters and partition coefficients were obtained from published measurements. The rate constants for tert-butanol metabolism and elimination were from a published PBPK model of MTBE with a tert-butanol subcompartment (Blancato et al. (2007)). Additional model parameters were estimated by calibrating to data sets for i.v., oral and inhalation exposures as well as repeated dosing studies for both ETBE and TBA. Although in one case (Ambert et al., 2000), the model modestly overpredicted the tert-butanol blood concentration by approximately 1.5-fold, overall, the model produced acceptable fits to multiple rat time-course datasets of ETBE and TBA blood levels following either inhalation or oral gavage exposures.

B.2.5.4. ETBE Model Application

The PBPK model described above was applied to conduct route-to-route extrapolation based on an equivalent internal dose. The selection of the appropriate internal dose metric depends on the endpoint and is discussed in the Volume 1, Section 2 Dose-Response Analysis. For simulating studies where ETBE or *tert*-butanol was administered in drinking water, the consumption was modeled as episodic, based on the pattern of drinking observed in rats by Spiteri (1982).

B.2.5.5. PBPK Model Code08

The PBPK acslX model code is made available electronically through EPA's Health and Environmental Research Online (HERO) database. All model files may be downloaded in a zipped workspace from HERO (www.epa.gov/hero).

B.3. GENOTOXICITY STUDIES

B.3.1.1. 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. One dose of 10,000 µg/plate was 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-10.

B.3.1.2. 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 5000µg/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 author, (Vergnes and Kubena (1995b) unpublished report) studied the clastogenic potential of ETBE in *in vitro* Chinese hamster ovary cells using chromosome aberration assay. The cells were exposed from 100 to 5000µg/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.

B.3.1.3. 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 (5animals/sex/group) were exposed to ETBE by inhalation at target concentrations of 0, 400, 2000, and 5000 ppm (0, 1671, 8357, and 20894 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 co-authors, 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, 2007a, b, c, d) published as Noguchi et al. (2013)].

The first two studies (oral and intraperitoneal injection) were part of an acute (2 days) exposure. In the first study, both male and female F344 rats (5animals/sex/dose group) were administered ETBE (99.3% pure) via gavage at doses of 0, 500, 1000, or 2000 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, 1000, or 2000 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 2000 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, 1600, 4000, or 10000 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, 1500, or 5000 ppm (0, 2089, 6268, or 20894 mg/m^3) 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.

Weng et al. conducted a number of 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, 1750 and 5000 ppm ETBE for 6hrs/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, while the increase was only found in 5000ppm exposure group for the knockout female mice. In the wild-type, significant DNA damage was seen only in males in the 5000 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), in addition to the DNA strand breaks, 8-hydroxyguanine DNA-glycosylase (hOGG1)-modified oxidative base modification, and 8-hydroxydeoxyguanosine, the authors performed *in vivo* micronucleus tests on what appear to be the same set of animals. The mice (wild-type and knockout, males and females) were exposed to 0, 500, 1750 and 5000 ppm ETBE for 6h/day, 5days/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 1750ppm and 5000 ppm exposure groups were significantly increased when compared with the control group. In the wild-type male mice, however, only the 5000 ppm group had a higher frequency of MN-RETs than that of control group. In female mice, there was no difference in the frequencies of MN-RETs between exposure groups and control group in wild-type mice. In the same exposure group (5000 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 6h/day, 5days/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-10. 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	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, 1000, 3000, 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, 1000, 3000, 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, 2000, 5000 ppm (0, 1670, 8360, 20900 mg/m^3)[b]	-		Whole body Inhalation, 6hrs/day, 5 days, 5 animals/ses/group	Vergnes and Kubena (1995a) (unpublished report)
Fisher 344 rats (male and female)	Bone Marrow Micronucleus test	0, 500, 1000, 2000 mg/kg/day	-		Oral gavage, 24h apart, 2 days, 5 animals/sex/group	JPEC (2007b) (unpublished report)
Fisher 344 rats (male and female)	Bone Marrow Micronucleus test	0, 250, 500, 1000, 2000 mg/kg/day	-		Intraperitoneal injection, 24h apart, 2 days, 5 animals/sex/group	Noguchi et al. (2013); JPEC (2007b),unpublished report
Fisher 344 rats (male and female)	Bone Marrow Micronucleus test	0, 1600, 4000, 10000 ppm (0, 101, 259, 626 mg/kg/day in males; 0, 120, 267, 629 mg/kg-d in females)[c]	-		Drinking water, 13 weeks, 10 animals/sex/group	Noguchi et al. (2013); JPEC (2007c), unpublished report

Species	Test System	Dose/Conc.	Results[a]		Comments	Reference
Fisher 344 rats (male and female)	Bone Marrow Micronucleus test	0, 500, 1500, 5000 ppm (0, 2090, 6270, 20900 mg/m3)[b]	-		Whole body inhalation, 6hrs/day, 5 days/week, 13 weeks. 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, 1750 and 5000 ppm	Male – WT/KO	+[d]/+	Whole body inhalation, 6hrs/day, 5 days/week, 13 weeks	Weng et al. (2011)
			Female WT/KO	-/+[d]		
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	DNA strand breaks (alkaline comet assay)	0, 500, 1750 and 5000 ppm	Male – WT/KO	+[d]/+	Whole body inhalation, 6hrs/day, 5 days/week, 13 weeks	Weng et al. (2012)
			Female WT/KO	-/+[d]		
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	Micronucleus assay, erythrocytes	0, 500, 1750 and 5000 ppm	Male* WT/KO	+[d]/+	Whole body inhalation, 6hrs/day, 5 days/week, 13 weeks	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, 6hrs/day, 5 days/week, 9 weeks	Weng et al. (2014)
C57BL/6 wild-type (WT) and *Aldh2* knockout (KO) mice	DNA strand breaks (alkaline comet assay); sperm	0, 500, 1750 and 5000 ppm	WT/KO	+/+	Whole body inhalation, 6hrs/day, 5 days/week, 13 weeks	Weng et al. (2014)

[fffa]+ = positive; – = negative; (+), equivocal

[b]4.18 mg/m^3 = 1ppm

[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 co-authors 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 and/or DNA strand breaks. Key studies in terms of chromosomal aberrations, DNA adducts etc 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.

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 the U.S. EPA's Benchmark Dose Software (BMDS, version 2.2). Sections C.1.1.1 and C.1.1.2 (non-cancer) and Section 0 (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 may 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. Non-cancer Endpoints

C.1.1.1. 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

[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.

mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p-value < 0.10 indicating inadequate fit). Other factors 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.

C.1.1.2. 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 three-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

Table C-1. Non-cancer 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-d)	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-d)	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 relative kidney weight Miyata et al. (2013);JPEC (2008b)	Male Sprague-Dawley rats	Dose (mg/kg-d)	0	5	25	100	400
		No. of animals	15	15	14	15	13
		Mean ± SD	0.52 ± 0.04	0.56 ± 0.05	0.55 ± 0.04	0.58 ± 0.07	0.63 ± 0.07
Increased absolute kidney weight Miyata et al. (2013);JPEC (2008b)	Female Sprague-Dawley rats	Dose (mg/kg-d)	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 relative kidney weight Miyata et al. (2013);JPEC (2008b)	Female Sprague-Dawley rats	Dose (mg/kg-d)	0	5	25	100	400
		No. of animals	15	15	15	15	15
		Mean ± SD	0.54 ± 0.06	0.58 ± 0.07	0.56 ± 0.04	0.6 ± 0.06	0.62 ± 0.06
Increased absolute kidney weight Gaoua (2004b)	P0 Male Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		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 relative kidney weight Gaoua (2004b)	P0 Male Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		No. of animals	25	25	25	25
		Mean ± SD	0.59628 ± 0.053	0.66246 ± 0.052	0.70569 ± 0.076	0.76341 ± 0.063
Increased absolute kidney weight Gaoua (2004b)	P0 Female Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		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

Endpoint, Study	Sex, Strain, Species	Doses and Effect Data				
Increased relative kidney weight Gaoua (2004b)	P0 Female Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		No. of animals	25	24	22	25
		Mean ± SD	0.70673 ± 0.11	0.77143 ± 0.198	0.74388 ± 0.16	0.72691 ± 0.06
Increased absolute kidney weight Gaoua (2004b)	F1 Male Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		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
Increased relative kidney weight Gaoua (2004b)	F1 Male Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		No. of animals	24	25	24	25
		Mean ± SD	0.57406 ± 0.043	0.63368 ± 0.046	0.68399 ± 0.068	0.90836 ± 0.958
Increased absolute kidney weight Gaoua (2004b)	F1 Female Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		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 relative kidney weight Gaoua (2004b)	F1 Female Sprague-Dawley rats	Dose (mg/kg-d)	0	250	500	1000
		No. of animals	25	24	25	23
		Mean ± SD	0.69219 ± 0.061	0.73338 ± 0.075	0.7305 ± 0.048	0.76202 ± 0.097
Increased absolute kidney weight Fujii et al. (2010); JPEC (2008c)	Male Sprague-Dawley rats	Dose (mg/kg-d)	0	100	300	1000
		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 (2008c)	Male Sprague-Dawley rats	Dose (mg/kg-d)	0	100	300	1000
		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 (2008c)	Female Sprague-Dawley rats	Dose (mg/kg-d)	0	100	300	1000
		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	Female Sprague-	Dose (mg/kg-d)	0	100	300	1000

Endpoint, Study	Sex, Strain, Species	Doses and Effect Data				
Fujii et al. (2010); JPEC (2008c)	Dawley rats	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	2090	6270	20900
		Incidence / Total	2 / 50	5 / 50	16 / 49	41 / 50
Increased absolute kidney weight JPEC (2008a)	Male Sprague-Dawley rats	Exposure concentration (ppm)	0	150	500	1500	5000
		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 relative kidney weight JPEC (2008a)	Male Sprague-Dawley rats	Exposure concentration (ppm)	0	150	500	1500	5000
		No. of animals	10	10	10	10	10
		Mean ± SD	0.584 ± 0.042	0.644 ± 0.064	0.638 ± 0.046	0.7 ± 0.073	0.726 ± 0.047
Increased absolute kidney weight JPEC (2008a)	Female Sprague-Dawley rats	Exposure concentration (ppm)	0	150	500	1500	5000
		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 relative kidney weight JPEC (2008a)	Female Sprague-Dawley rats	Exposure concentration (ppm)	0	150	500	1500	5000
		No. of animals	10	10	10	10	10
		Mean ± SD	0.545 ± 0.04	0.587 ± 0.056	0.583 ± 0.035	0.613 ± 0.06	0.656 ± 0.043
Increased absolute kidney weight Medinsky et al. (1999); Bond et al. (1996)	Male F344 rats	Exposure concentration (ppm)	0	500	1750	5000
		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. (1996)	Female F344 rats	Exposure concentration (ppm)	0	500	1750	5000
		No. of animals	10	11	11	11

Endpoint, Study	Sex, Strain, Species	Doses and Effect Data				
		Mean ± SD	1.077 ± 0.069	1.125 ± 0.048	1.208 ± 0.076	1.306 ± 0.055

C.1.1.3. 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 slight urothelial hyperplasia of the renal pelvis in male F344 rats exposed to ETBE in drinking water for 104 weeks (JPEC, 2010a); modeled with doses as mg/kg-d (calculated by study authors); BMR = 10% extra risk.

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	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	1.00E-03	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[d]	**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-d 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). The models in this row reduced to the Multistage 2° model.

[c] The Multistage 2° model may appear equivalent to the Quantal-Linear model, however differences exist in digits not displayed in the table.

[d] The Quantal-Linear model may appear equivalent to the Multistage 3° model, however differences exist in digits not displayed in the table. This also applies to the Multistage 2° model.

Data from JPEC (2010a)

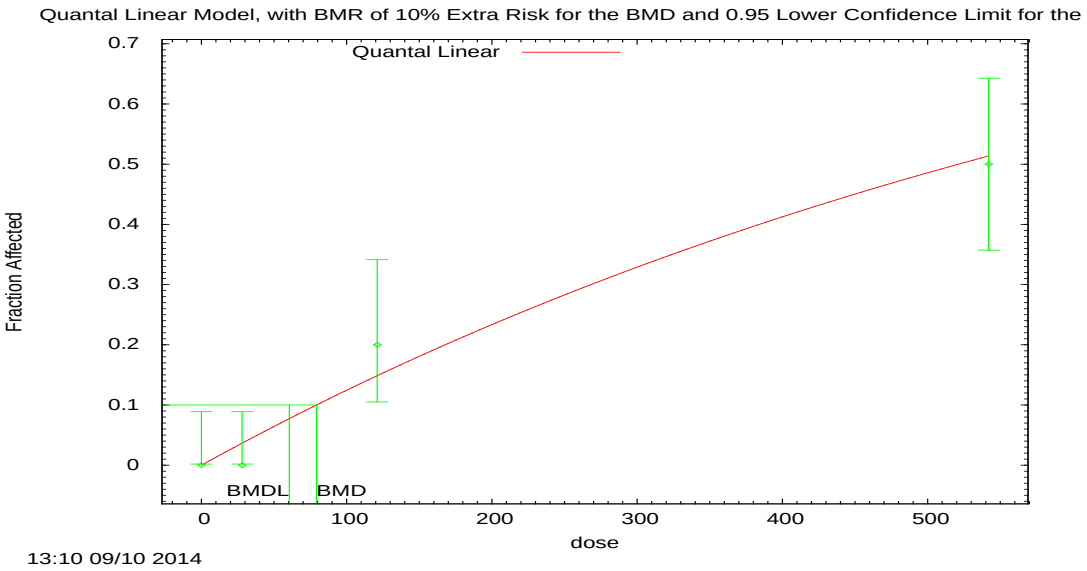


Figure C-1. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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

Chi^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 180 days (Miyata et al., 2013; JPEC, 2008c); BMR = 10% relative deviation from the mean.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.752	-47.963	186	126	The linear model was selected on the basis of 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-d 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). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] The Linear model may appear equivalent to the Polynomial 3° model, however differences exist in digits not displayed in the table.
[g] The Polynomial 3° model may appear equivalent to the Power model, however differences exist in digits not displayed in the table. This also applies to the Polynomial 2° model. This also applies to the Linear model.

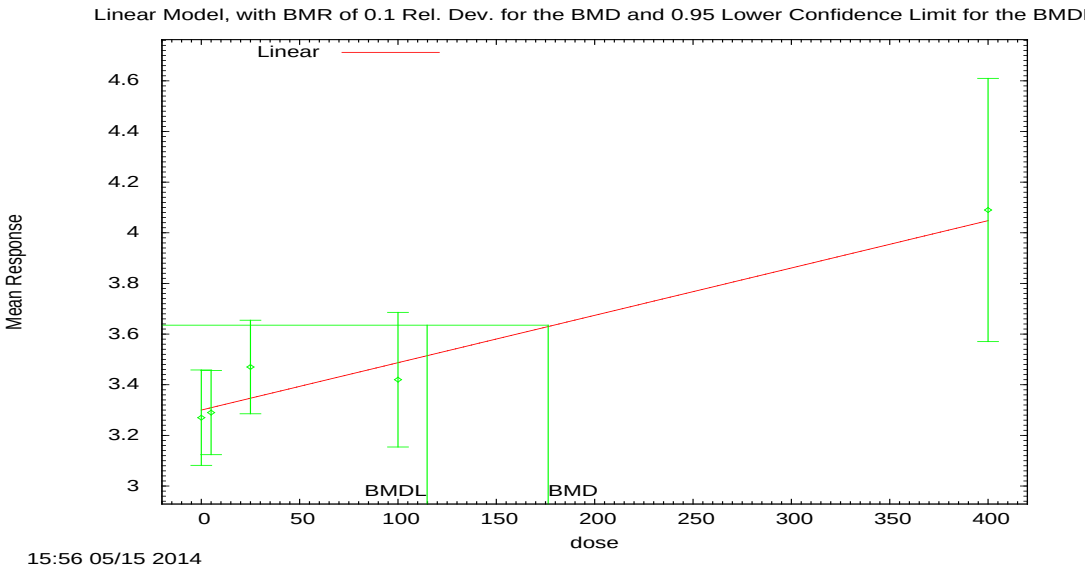


Figure C-2. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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
lα	-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

1
2
3

Table C-4. Summary of BMD modeling results for increased relative kidney weight in male S-D rats exposed to ETBE by daily gavage for 180 days (Miyata et al., 2013; JPEC, 2008c); BMR = 10% relative deviation from the mean.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)[b]	0.0262	-339.53	242	174	No model adequately fit the data.
Exponential (M3)[c]	0.0262	-339.53	242	174	
Exponential (M4) Exponential (M5)[d]	0.0472	-340.67	113	45.6	
Hill	0.0481	-340.71	112	47.2	
Power	<0.0001	-315.18	40000	4.00E-13	
Polynomial 3°[e] Polynomial 2°[f] Linear	0.03	-339.83	231	161	

[a] Modeled variance case presented (BMDS Test 2 *p*-value = 0.0648, BMDS Test 3 *p*-value = 0.596), no model was selected as a best-fitting model.

[b] The Exponential (M2) model may appear equivalent to the Exponential (M3) model, however differences exist in digits not displayed in the table.

[c] The Exponential (M3) model may appear equivalent to the Exponential (M2) model, however differences exist in digits not displayed in the table.

[d] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.

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

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

Table C-5. Summary of BMD modeling results for increased absolute kidney weight in female S-D rats exposed to ETBE by daily gavage for 180 days (Miyata et al., 2013; JPEC, 2008c); BMR = 10% relative deviation from the mean.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.369	-168.25	406	271	The Exponential (M4) model was selected on the basis of 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-d 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). The models in this row 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. The models in this row reduced to the Linear model.

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

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

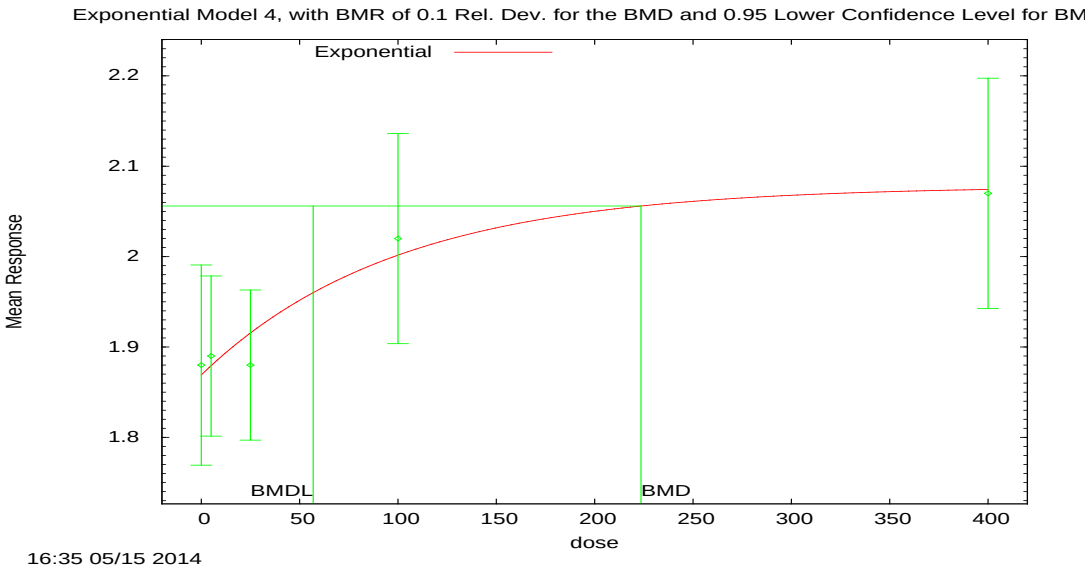


Figure C-3. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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
lnα	-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-6. Summary of BMD modeling results for increased relative kidney weight in female S-D rats exposed to ETBE by daily gavage for 180 days (Miyata et al., 2013; JPEC, 2008c); BMR = 10% relative deviation from the mean.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.111	-343.15	374	253	The Hill model is selected on the basis of lowest BMDL.
Exponential (M4) Exponential (M5)[c]	0.163	-343.53	170	41.1	
Hill	**0.158**	**-343.47**	**191**	**20.1**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.116	-343.25	369	244	

[a] Constant variance case presented (BMDS Test 2 *p*-value = 0.335), selected model in bold; scaled residuals for selected model for doses 0, 5, 25, 100, and 400 mg/kg-d were -0.917, 1.47, -0.738, 0.242, and -0.054, respectively.
[b] For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from (Miyata et al., 2013; JPEC, 2008c)

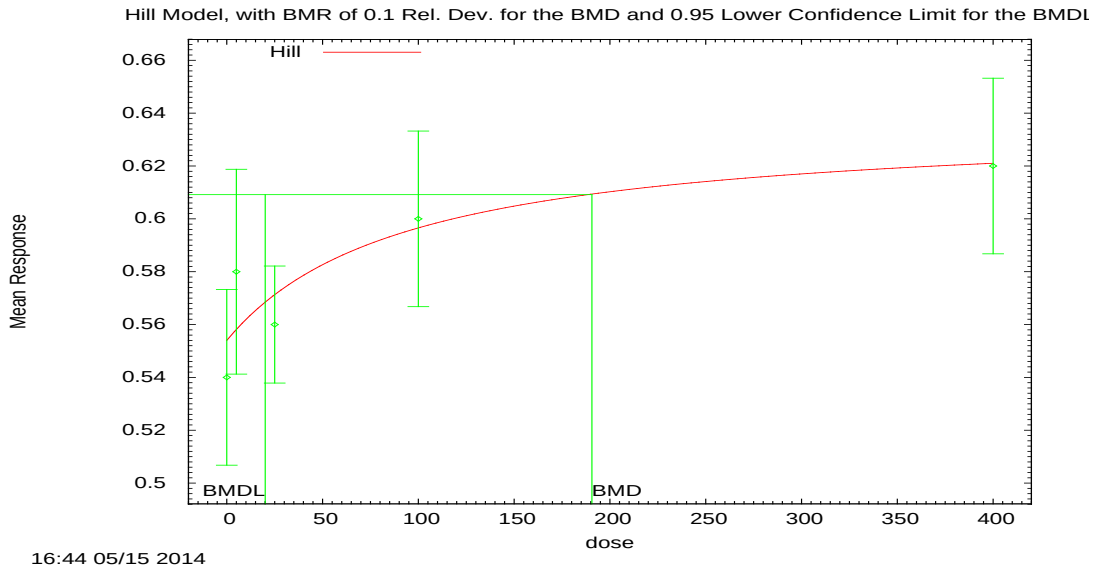


Figure C-4. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 190.577
BMDL at the 95% confidence level = 20.0557

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.00339206	0.00346
rho	n/a	0
intercept	0.553785	0.54
v	0.0828955	0.08
n	1	0.214814
k	94.6956	137.5

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	15	0.54	0.554	0.06	0.0582	-0.917
5	15	0.58	0.558	0.07	0.0582	1.47
25	15	0.56	0.571	0.04	0.0582	-0.738

100	15	0.6	0.596	0.06	0.0582	0.242
400	15	0.62	0.621	0.06	0.0582	-0.054

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	177.580484	6	-343.160967
A2	179.862753	10	-339.725506
A3	177.580484	6	-343.160967
fitted	175.736902	4	-343.473804
R	169.280788	2	-334.561576

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.1639	8	0.006724
Test 2	4.56454	4	0.335
Test 3	4.56454	4	0.335
Test 4	3.68716	2	0.1582

Table C-7. 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 weeks beginning 10 weeks 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.155	-38.410	551	423	The Hill model is selected on the basis of lowest BMDL.
Exponential (M4)[c]	0.727	-40.012	255	123	
Exponential (M5)[d]	0.727	-40.012	255	123	
Hill	**0.811**	**-40.077**	**244**	**94.0**	
Power[e] Polynomial 3°[f] Polynomial 2°[g] 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 1000 mg/kg-d were -0.0247, 0.14, -0.181, and 0.0657, respectively.
[b] For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c] The Exponential (M4) model may appear equivalent to the Exponential (M5) model, however differences exist in digits not displayed in the table.
[d] The Exponential (M5) model may appear equivalent to the Exponential (M4) model, however differences exist in digits not displayed in the table.
[e] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from Gaoua (2004a)

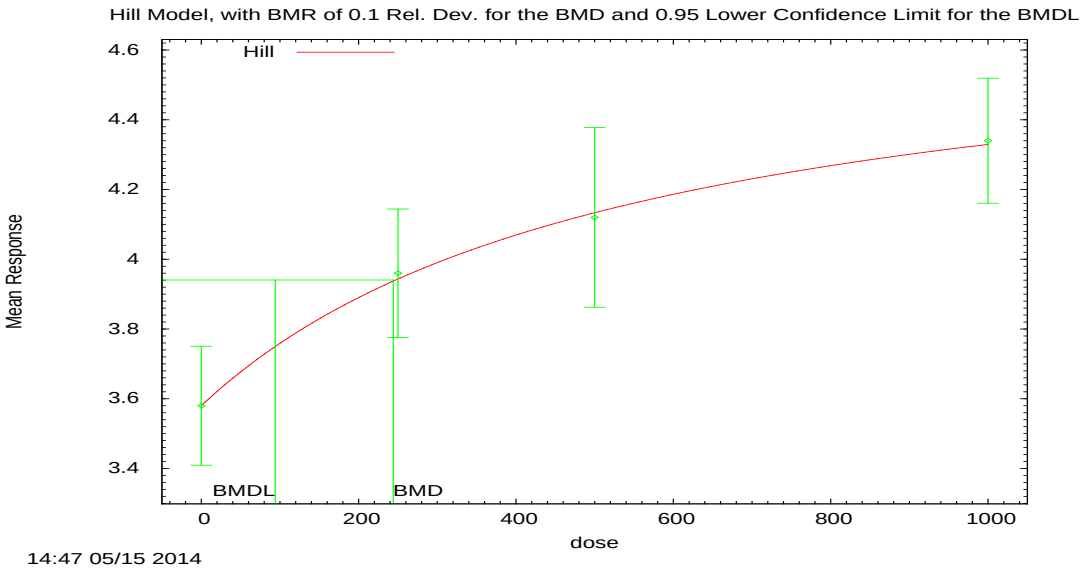


Figure C-5. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 243.968
BMDL at the 95% confidence level = 93.9617

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	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

1000	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-8. Summary of BMD modeling results for increased relative kidney weight in P0 male S-D rats exposed to ETBE by daily gavage for a total of 18 weeks beginning 10 weeks 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0632	-449.45	415	355	The Hill model was selected on the basis of lowest AIC.
Exponential (M4) Exponential (M5)[c]	0.871	-452.95	228	150	
Hill	**0.936**	**-452.97**	**224**	**137**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.127	-450.86	378	316	

[a] Constant variance case presented (BMDS Test 2 *p*-value = 0.180), selected model in bold; scaled residuals for selected model for doses 0, 250, 500, and 1000 mg/kg-d were -0.0131, 0.0533, -0.0566, and 0.0164, respectively.
[b] For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

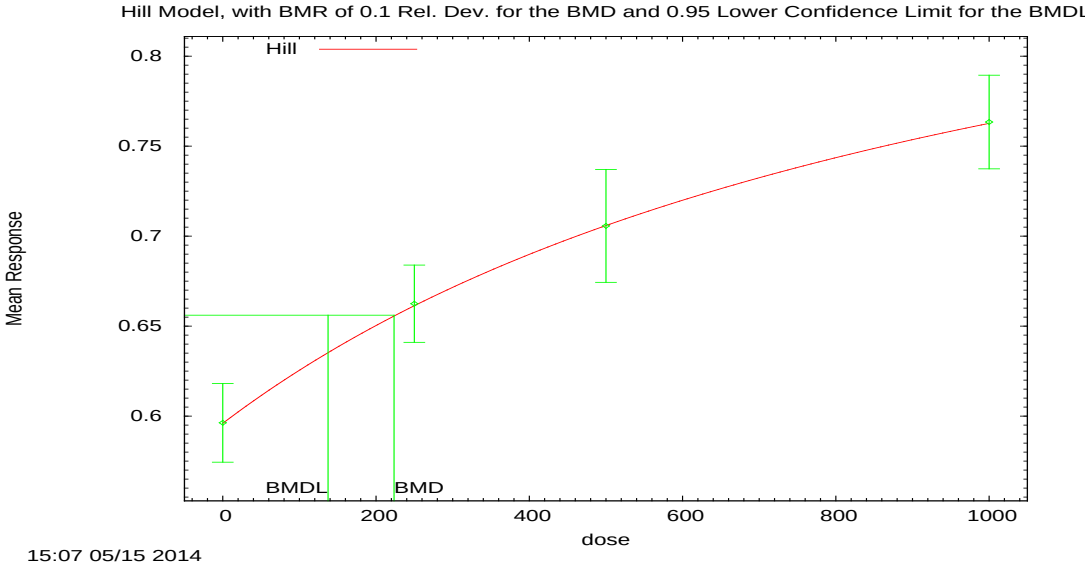


Figure C-6. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 223.505
BMDL at the 95% confidence level = 137.393

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.00366216	0.0038145
rho	n/a	0
intercept	0.596439	0.59628
v	0.345283	0.16713
n	1	0.221145
k	1070.38	649.462

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	25	0.596	0.596	0.053	0.0605	-0.0131
250	25	0.662	0.662	0.052	0.0605	0.0533
500	25	0.706	0.706	0.076	0.0605	-0.0566
1000	25	0.763	0.763	0.063	0.0605	0.0164

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	230.488384	5	-450.976768
A2	232.931535	8	-449.86307
A3	230.488384	5	-450.976768
fitted	230.48514	4	-452.97028
R	195.370878	2	-386.741756

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value

Test 1	75.1213	6	<0.0001
Test 2	4.8863	3	0.1803
Test 3	4.8863	3	0.1803
Test 4	0.0064882	1	0.9358

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Table C-9. 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 weeks beginning 10 weeks 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)	**0.625**	**-214.58**	**1734**	**1030**	Exponential (M2) model is selected on the basis of lowest AIC; however, BMDL is higher than the maximum dose.
Exponential (M3)	0.416	-212.86	1458	1040	
Exponential (M4)	0.327	-212.56	1774	1032	
Exponential (M5)	N/A[b]	-211.39	error[c]	0	
Hill	0.715	-213.39	error[c]	error[c]	
Power	0.418	-212.87	1470	1041	
Polynomial 3°	0.400	-212.81	1409	1035	
Polynomial 2°	0.400	-212.81	1409	1037	
Linear	0.619	-214.56	1774	1032	

[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 1000 mg/kg-d 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.

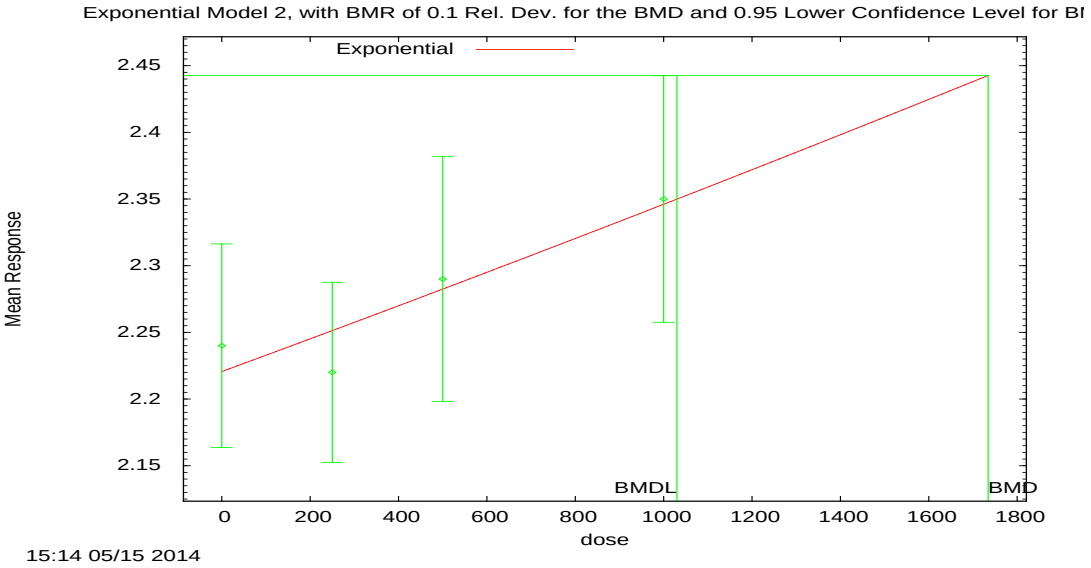

Figure C-7. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 1734.24
BMDL at the 95% confidence level = 1030.08

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
lnα	-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
1000	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

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Table C-10. Summary of BMD modeling results for increased relative kidney weight in P0 female S-D rats exposed to ETBE by daily gavage for a total of 18 weeks beginning 10 weeks 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M4)[b]	N/A	-283.41	1258	829	No model adequately fit the data.
Exponential (M3)	N/A	-290.99	1037	983	
Exponential (M5)	N/A[c]	-288.99	1037	983	
Hill	<0.0001	-276.90	error[d]	error[d]	
Power	<0.0001	-296.86	1648	1056	
Polynomial 3°	0.00528	-292.51	-9999	976	
Polynomial 2°	0.00236	-290.89	-9999	945	
Linear	1.92E-04	-285.88	40622	error[d]	

[a] Modeled variance case presented (BMDS Test 2 *p*-value = <0.0001, BMDS Test 3 *p*-value = <0.0001), no model was selected as a best-fitting model.

[b] For the Exponential (M4) model, the estimate of c was 0 (boundary). The models in this row reduced to the Exponential (M2) model.

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

[d] BMD or BMDL computation failed for this model.

Table C-11. Summary of BMD modeling results for absolute kidney weight in F1 male Sprague-Dawley rats exposed to ETBE by gavage in a 2-generation study (Gaoua, 2004b); BMR = 10% relative deviation from the mean.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)	6.30E-04	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 1000 mg/kg-d were -0.584, 0.717, 0.225, and -0.837, respectively.
[b] No available degrees of freedom to calculate a goodness of fit value.

Data from Gaoua (2004b)

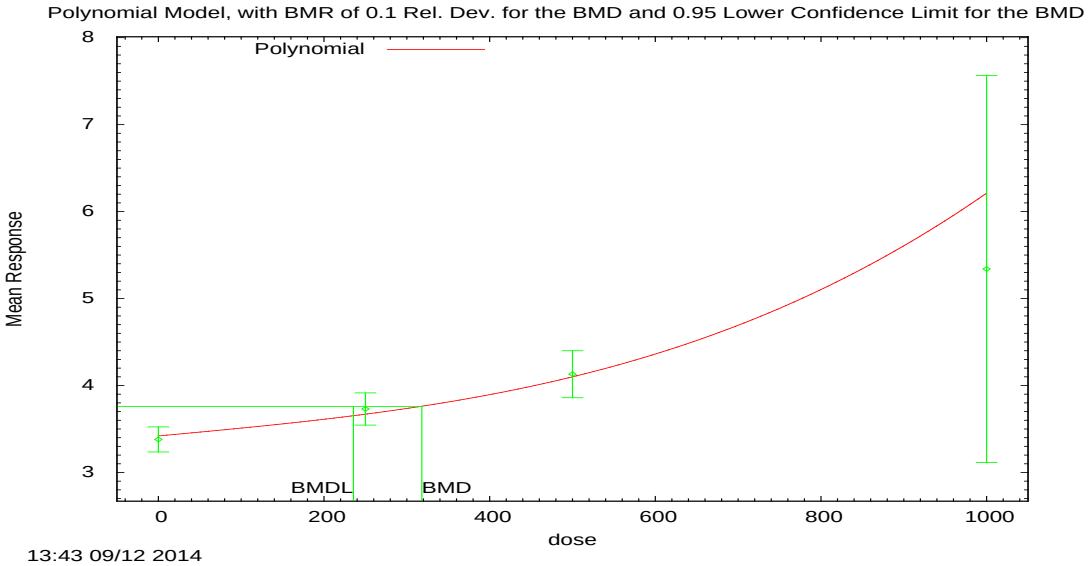

Figure C-8. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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
THE MODEL HAS PROBABLY NOT CONVERGED!!!

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
lalpha	-13.8779	2.02785
rho	9.40248	0
beta_0	3.41732	3.38
beta_1	0.000881597	0.00138667
beta_2	2.23248E-28	0
beta_3	0.00000000190507	0.000000000693333

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
1000	25	5.34	6.2	5.39	5.16	-0.837

Likelihoods of Interest

Model	Log(likelihood)	# Param's	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-12. Summary of BMD modeling results for relative kidney weight in F1 male Sprague-Dawley rats exposed to ETBE by gavage in a 2-generation study (Gaoua, 2004b); BMR = 10% relative deviation.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)	<0.0001	-298.20	249	194	No models provided an adequate fit and a valid BMDL estimate, therefore no model was selected.
Exponential (M3)	0.00994	-319.84	368	297	
Exponential (M4)	<0.0001	-287.10	239	196	
Exponential (M5)	N/A[b]	-315.83	382	306	
Hill	N/A[b]	-315.82	382	317	
Power	0.00326	-317.83	382	306	
Polynomial 3°	0.0592	-322.92	352	281	
Polynomial 2°	0.00360	-318.01	352	286	
Linear	<0.0001	-291.10	239	196	

[a] Modeled variance case presented (BMDS Test 2 *p*-value = <0.0001, BMDS Test 3 *p*-value = 0.0558), no model was selected as a best-fitting model.
[b] No available degrees of freedom to calculate a goodness of fit value.

Data from Gaoua (2004b)

Table C-13. Summary of BMD modeling results for absolute kidney weight in F1 female Sprague-Dawley rats exposed to ETBE by gavage in a 2-generation study (Gaoua, 2004b); BMR = 10% relative deviation.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	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	1016	679	
Exponential (M4)	0.121	-178.16	980	654	
Exponential (M5)	N/A[b]	-176.44	1019	613	

Hill	N/A[b]	-176.44	1019	611	
Power	0.145	-178.44	1019	666	
Polynomial 3°	0.184	-178.80	1001	684	
Polynomial 2°	0.159	-178.58	1002	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 1000 mg/kg-d were -0.05426, 0.8898, -1.173, and 0.3711, respectively.
[b] No available degrees of freedom to calculate a goodness of fit value.

Data from Gaoua (2004b)

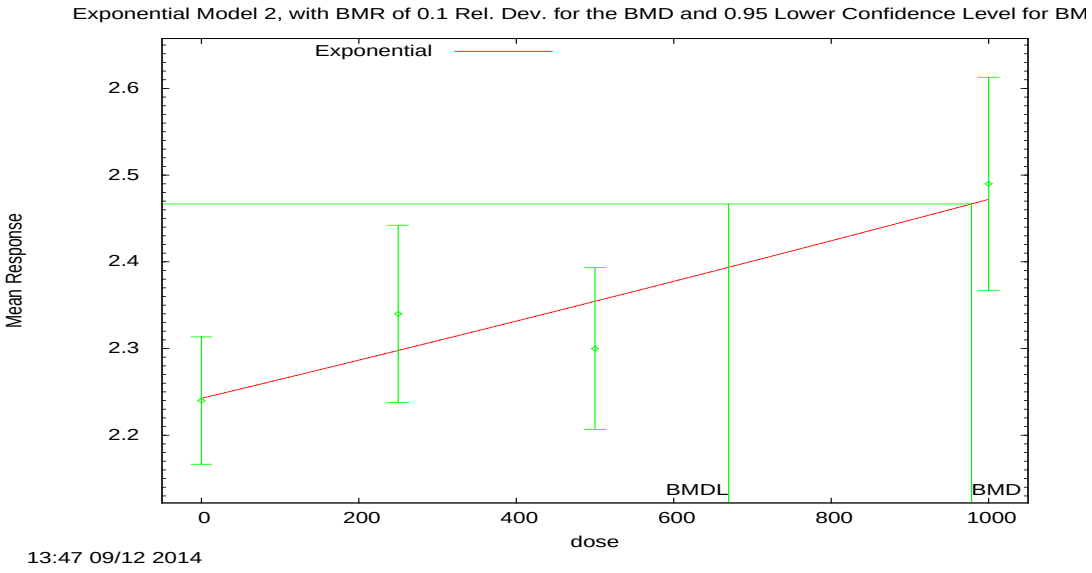

Figure C-9. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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
lnalpha	-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
1000	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-14. Summary of BMD modeling results for relative kidney weight in F1 female Sprague-Dawley rats exposed to ETBE by gavage in a 2-generation study (Gaoua, 2004b); BMR = 10% relative deviation.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.102	-412.25	1064	702	No models provided an adequate fit and a valid BMDL estimate, therefore no model was selected.
Exponential (M4) Exponential (M5)[c]	0.0333	-410.28	1067	489	
Hill	0.0335	-410.30	1069	466	
Power	1.02E-04	-398.44	6.5E+06	error[d]	
Polynomial 3°	0.0333	-410.29	1057	687	
Polynomial 2°[e] Linear	0.103	-412.26	1063	686	

[a] Modeled variance case presented (BMDS Test 2 *p*-value = 0.00542, BMDS Test 3 *p*-value = 0.061), no model was selected as a best-fitting model.
[b] For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] BMD or BMDL computation failed for this model.
[e] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from Gaoua (2004b)

Table C-15. 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			

Exponential (M2) Exponential (M3)[b]	0.668	-41.247	648	479	The Hill model was selected on the basis of lowest BMDL. (BMDLs were greater than 3-fold 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 1000 mg/kg-d were -0.202, 0.399, -0.232, and 0.0344, respectively.
[b] For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

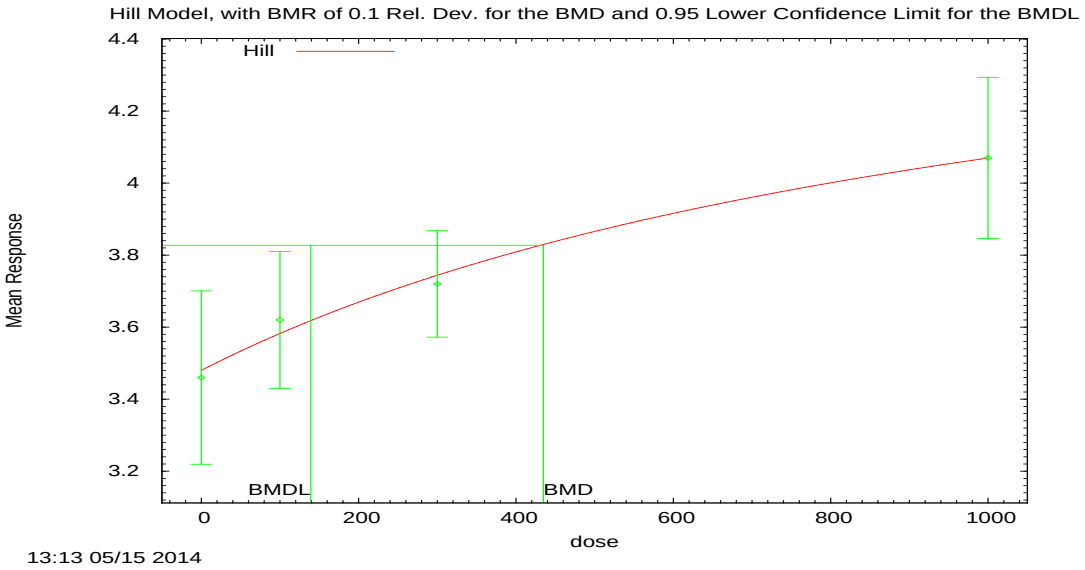

Figure C-10. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 434.715
BMDL at the 95% confidence level = 139.178

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.223598	0.2327
rho	n/a	0
intercept	3.47949	3.46
v	1.24601	0.61
n	1	0.27452
k	1122	1610

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
1000	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-16. BMD modeling results for increased relative 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.0530	-460.12	471	401	The Hill model is selected as the only adequately-fitting model.
Exponential (M4) Exponential (M5)[c]	0.0956	-461.22	256	150	
Hill	**0.108**	**-461.41**	**243**	**129**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0720	-460.73	439	367	

[a] Constant variance case presented (BMDS Test 2 *p*-value = 0.271), selected model in bold; scaled residuals for selected model for doses 0, 100, 300, and 1000 mg/kg-d were -0.602, 1.25, -0.78, and 0.133, respectively.
[b] For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

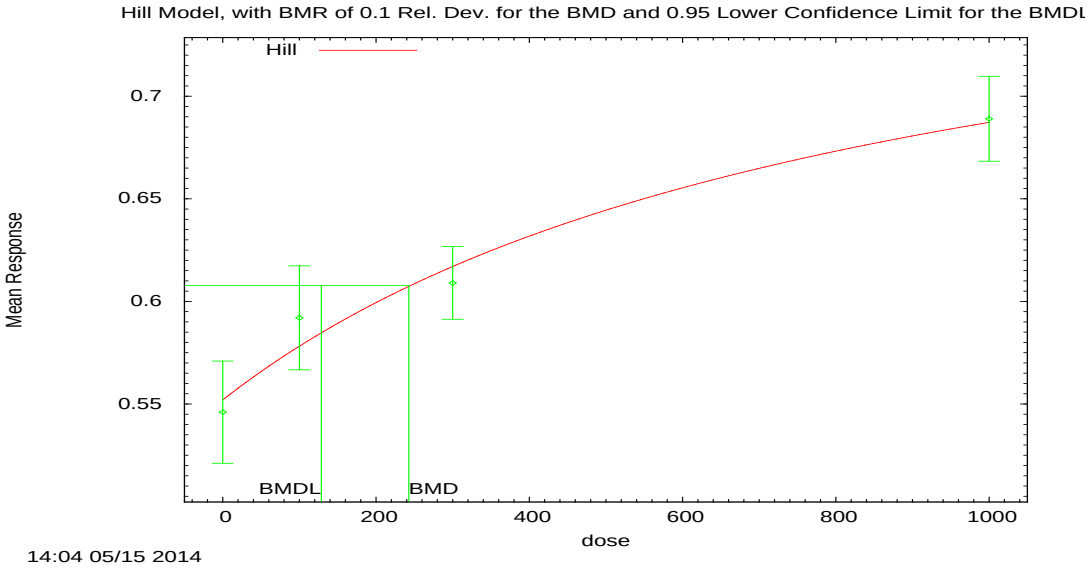

Figure C-11. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 242.739
BMDL at the 95% confidence level = 128.617

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.0027678	0.0028115
rho	n/a	0
intercept	0.552461	0.546
v	0.251763	0.143
n	1	0.204461
k	863.449	1625.63

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	24	0.546	0.552	0.059	0.0526	-0.602
100	24	0.592	0.579	0.06	0.0526	1.25
300	24	0.609	0.617	0.042	0.0526	-0.78
1000	24	0.689	0.688	0.049	0.0526	0.133

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	235.996644	5	-461.993287
A2	237.954442	8	-459.908884
A3	235.996644	5	-461.993287
fitted	234.705776	4	-461.411551
R	202.992245	2	-401.98449

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	69.9244	6	<0.0001

Test 2	3.9156	3	0.2707
Test 3	3.9156	3	0.2707
Test 4	2.58174	1	0.1081

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2

Table C-17. 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)	0.483	-199.73	1135	781	Polynomial 2° is selected on the basis of most parsimonious model with lowest AIC.
Exponential (M3)	0.441	-198.60	1089	826	
Exponential (M4)	0.217	-197.67	1144	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	1092	823	
Polynomial 3°[d] Polynomial 2°	**0.743**	**-200.60**	**1094**	**905**	
Linear	0.467	-199.67	1144	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 1000 mg/kg-d 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). The models in this row reduced to the Polynomial 2° model.

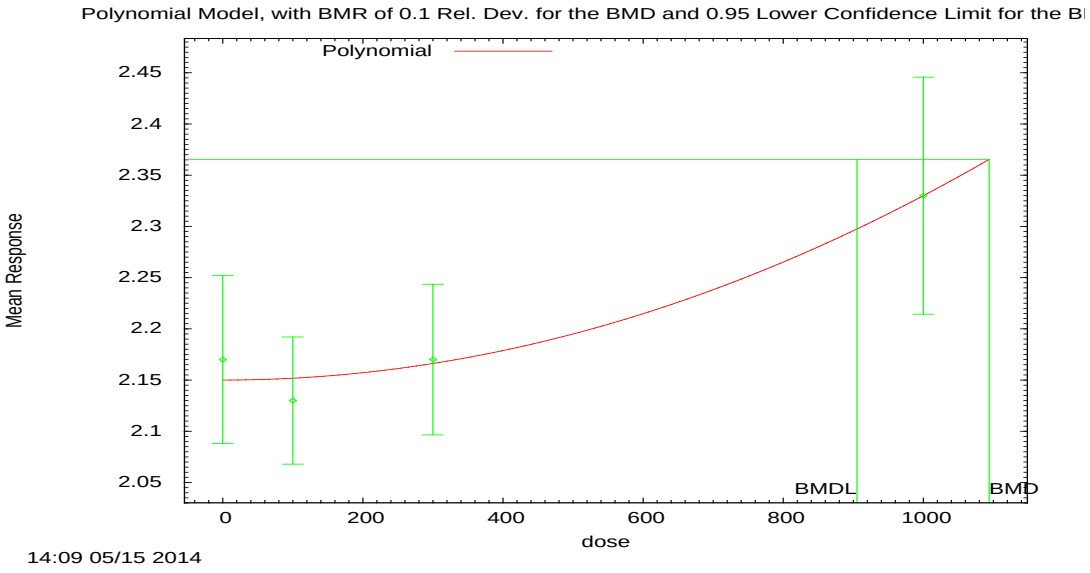

Figure C-12. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 1093.86
BMDL at the 95% confidence level = 905.267

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.0323691	0.0337309
rho	n/a	0
beta_0	2.1504	2.15624
beta_1	7.16226E-28	0
beta_2	0.000000179719	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
1000	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

Table C-18. Summary of BMD modeling results for increased relative 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-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)	0.367	-471.62	2953	1482	Polynomial 2° is selected on the basis of lowest AIC.
Exponential (M3)	0.208	-470.04	1573	1026	
Exponential (M4)	0.156	-469.61	3056	1506	
Exponential (M5)	N/A[b]	-468.07	error[c]	0	
Hill	N/A[b]	-468.07	error[c]	error[c]	
Power	0.208	-470.04	1592	1028	
Polynomial 3°	0.207	-470.03	1511	1172	
Polynomial 2°	**0.450**	**-472.03**	**1751**	**1254**	
Linear	0.366	-471.61	3055	1506	

[a] Constant variance case presented (BMDS Test 2 *p*-value = 0.665), selected model in bold; scaled residuals for selected model for doses 0, 100, 300, and 1000 mg/kg-d were 0.849, -0.925, 0.0742, and 0.00257, respectively.
[b] No available degrees of freedom to calculate a goodness of fit value.
[c] BMD or BMDL computation failed for this model.

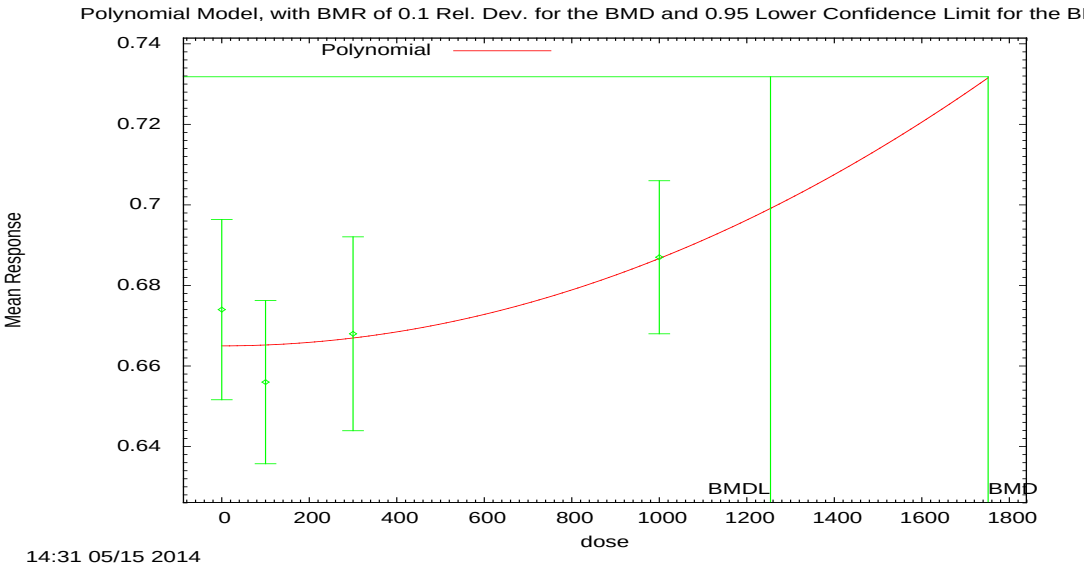

Figure C-13. Plot of mean response by dose, with fitted curve for selected model; dose shown in mg/kg-d.

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 = 1751.45
BMDL at the 95% confidence level = 1254.17

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.00253026	0.00259675
rho	n/a	0
beta_0	0.665286	0.668151
beta_1	2.84343E-27	0
beta_2	0.0000000216877	0

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	24	0.674	0.665	0.053	0.0503	0.849
100	24	0.656	0.666	0.048	0.0503	-0.925
300	24	0.668	0.667	0.057	0.0503	0.0742
1000	24	0.687	0.687	0.045	0.0503	0.00257

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	239.810603	5	-469.621206
A2	240.598408	8	-465.196816
A3	239.810603	5	-469.621206
fitted	239.01285	3	-472.0257
R	237.463901	2	-470.927802

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	6.26901	6	0.3937
Test 2	1.57561	3	0.6649
Test 3	1.57561	3	0.6649
Test 4	1.59551	2	0.4503

Inhalation Exposure Endpoints

Table C-19. Summary of BMD modeling results for slight urothelial hyperplasia of the renal pelvis in male F344 rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5d/wk, for 104 wks (JPEC, 2010b)BMR = 10% extra risk.

Model[a]	Goodness of fit		BMC_{10Pct} (mg/m³)	$BMCL_{10Pct}$ (mg/m³)	Basis for model selection
	p-value	AIC			
Gamma	**0.874**	**164.37**	**2734**	**1498**	**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	4329	3522	
LogLogistic	0.814	164.40	3010	1831	
Probit	0.202	165.59	4059	3365	
LogProbit	0.633	164.57	3050	1896	
Weibull	0.758	164.44	2623	1478	
Multistage 3°	0.565	164.69	2386	1412	
Multistage 2°	0.565	164.69	2386	1422	
Quantal-Linear	0.269	165.16	1541	1227	

[a] Selected model in bold; scaled residuals for selected model for doses 0, 2089, 6268, and 20893 mg/m³ were 0.036, -0.107, 0.104, and -0.040, respectively. Exposure concentrations were converted from 0, 500, 1500, and 5000 ppm to mg/m³ using the calculation mg/m³ = (102.17, molecular weight of ETBE) × ppm ÷ 24.45.

Data from JPEC2010b

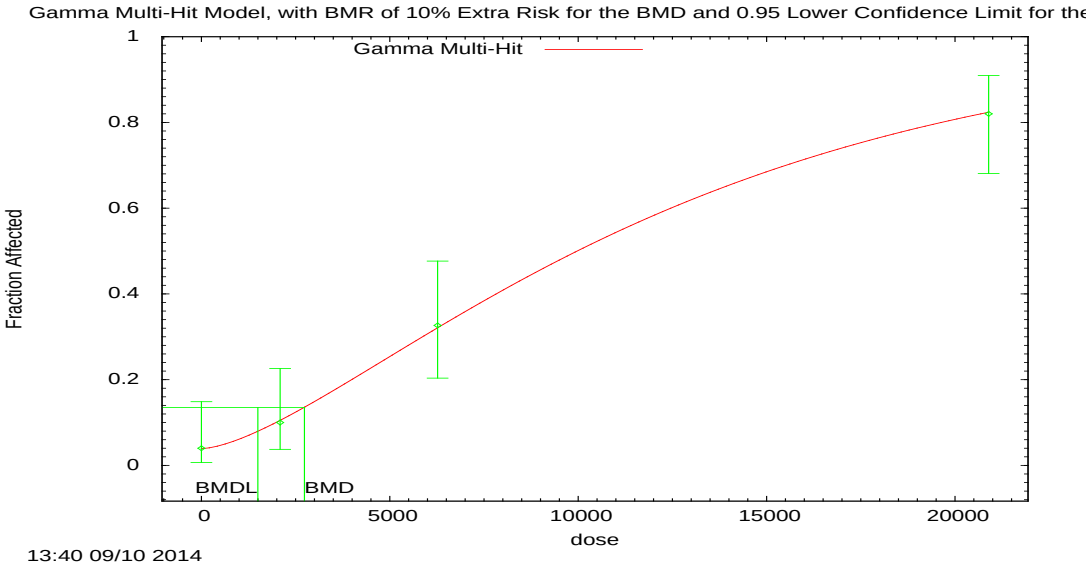

Figure C-14. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in mg/m^3.

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 cummulative Gamma distribution function
Power parameter is restricted as power >=1

Benchmark Dose Computation.
BMR = 10% Extra risk
BMD = 2734.41
BMDL at the 95% confidence level = 1497.7

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
2089	0.1046	5.231	5	50	-0.107
6268	0.3196	15.659	16	49	0.104
20893	0.8222	41.109	41	50	-0.04

Chi^2 = 0.03 d.f = 1 P-value = 0.8737

Table C-20. 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 wks JPEC (2008b); 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]	0.0115	-47.349	5505	3234	The Hill model was selected on the basis of lowest AIC.
Exponential (M4)[c]	0.416	-54.646	327	39.2	
Exponential (M5)[d]	0.416	-54.646	327	39.2	
Hill	**0.507**	**-55.041**	**218**	**16.2**	
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	0.0121	-47.465	5401	3086	

[a] Constant variance case presented (BMDS Test 2 *p*-value = 0.662), selected model in bold; scaled residuals for selected model for doses 0, 150, 500, 1500, and 5000 ppm were -0.0403, 0.29, -0.727, 0.792, and -0.315, respectively.

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

[c] The Exponential (M4) model may appear equivalent to the Exponential (M5) model, however differences exist in digits not displayed in the table.

[d] The Exponential (M5) model may appear equivalent to the Exponential (M4) model, however differences exist in digits not displayed in the table.

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

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

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

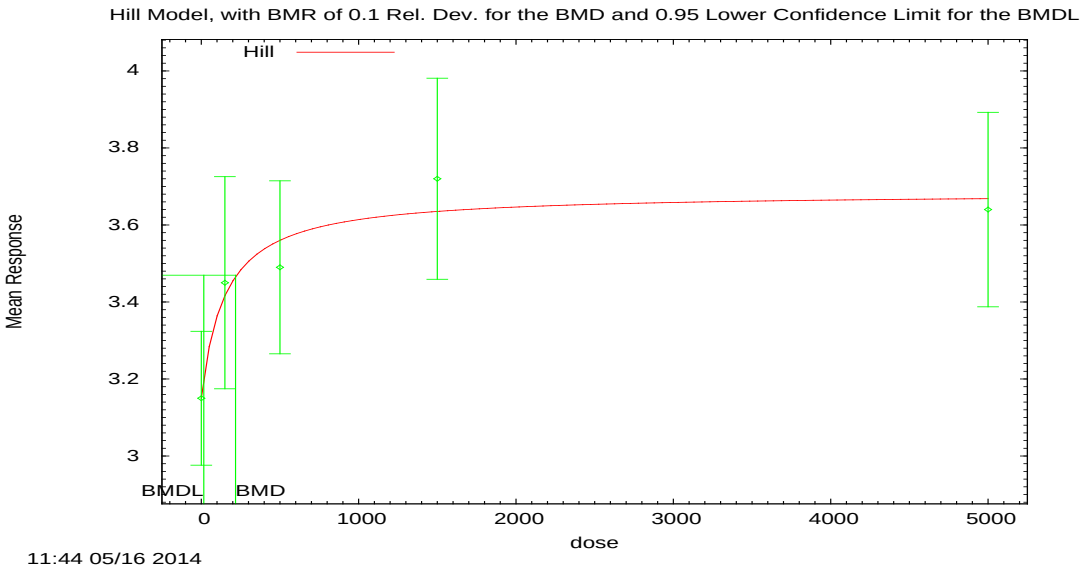

Figure C-15. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

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 = 217.735
BMDL at the 95% confidence level = 16.1532

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.104264	0.112741
rho	n/a	0
intercept	3.15411	3.15
v	0.533715	0.57
n	1	0.287502
k	150.7	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	3.15	0.243	0.323	-0.0403
150	10	3.45	3.42	0.385	0.323	0.29
500	10	3.49	3.56	0.314	0.323	-0.727

1500	10	3.72	3.64	0.365	0.323	0.792
5000	10	3.64	3.67	0.353	0.323	-0.315

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	32.20061	6	-52.401221
A2	33.401145	10	-46.80229
A3	32.20061	6	-52.401221
fitted	31.520704	4	-55.041408
R	24.155193	2	-44.310386

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	18.4919	8	0.01783
Test 2	2.40107	4	0.6624
Test 3	2.40107	4	0.6624
Test 4	1.35981	2	0.5067

Table C-21. Summary of BMD modeling results for increased relative kidney weight in male S-D rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5 d/wk for 13 wks 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]	0.00625	-225.68	2954	2226	The Hill model was selected on the basis of lowest AIC.
Exponential (M4) Exponential (M5)[c]	0.152	-232.27	623	256	
Hill	**0.175**	**-232.55**	**470**	**133**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.00771	-226.13	2792	2051	

[a] Constant variance case presented (BMDS Test 2 *p*-value = 0.321), selected model in bold; scaled residuals for selected model for doses 0, 150, 500, 1500, and 5000 ppm were -0.599, 1.37, -1.04, 0.241, and 0.0322, respectively.
[b] For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

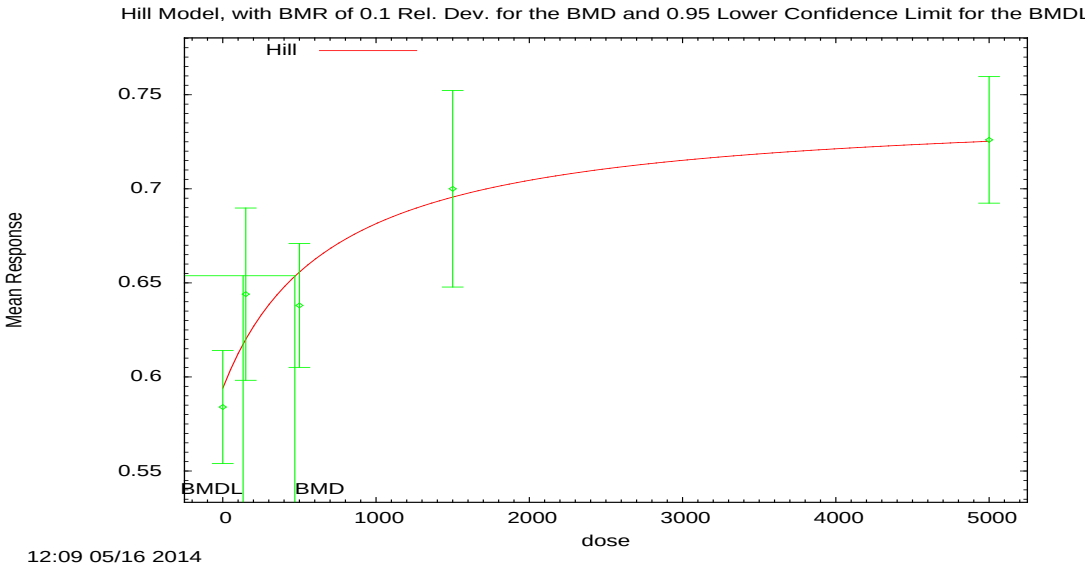

Figure C-16. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

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 = 470.166
BMDL at the 95% confidence level = 132.528

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.00299441	0.0031028
rho	n/a	0
intercept	0.594365	0.584
v	0.149823	0.142

n	1	0.147616
k	714.991	2225.81

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	10	0.584	0.594	0.042	0.0547	-0.599
150	10	0.644	0.62	0.064	0.0547	1.37
500	10	0.638	0.656	0.046	0.0547	-1.04
1500	10	0.7	0.696	0.073	0.0547	0.241
5000	10	0.726	0.725	0.047	0.0547	0.0322

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	122.020272	6	-232.040543
A2	124.363765	10	-228.727531
A3	122.020272	6	-232.040543
fitted	120.275236	4	-232.550472
R	106.075094	2	-208.150188

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	36.5773	8	<0.0001
Test 2	4.68699	4	0.3209
Test 3	4.68699	4	0.3209
Test 4	3.49007	2	0.1746

Table C-22. 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 wks 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	6790	4046	The Linear model is selected based on lowest AIC; however, the
Exponential (M4)	0.731	-133.76	error[c]	0	

Exponential (M5)	0.760	-132.29	error[c]	0	BMD is higher than the maximum dose.
Hill	0.760	-132.29	error[c]	error[c]	
Power[d] **Polynomial 3°[e]** **Polynomial 2°[f]** **Linear**	**0.806**	**-135.40**	**6840**	**3978**	

[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, 1500, and 5000 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). The models in this row 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. The models in this row reduced to the Linear model.
[e] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

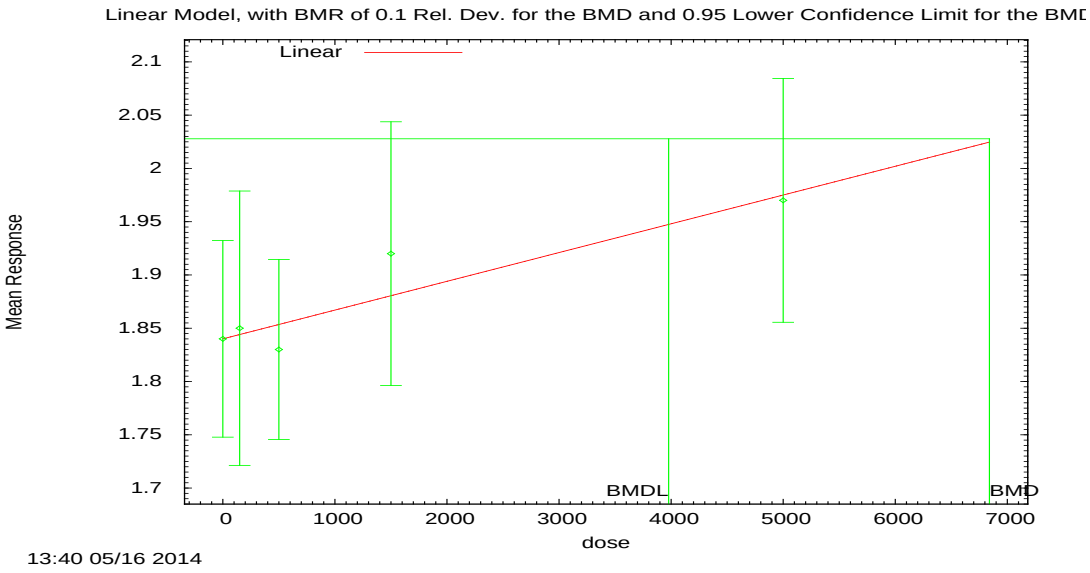

Figure C-17. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

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 = 6840.02

BMDL at the 95% confidence level = 3978.09

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	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
1500	10	1.92	1.88	0.173	0.147	0.774
5000	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-23. Summary of BMD modeling results for increased relative kidney weight in female S-D rats exposed to ETBE by whole-body inhalation for 6 hr/d, 5 d/wk for 13 wks 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]	0.147	-248.04	3288	2482	The Hill model was selected on the basis of lowest BMDL.
Exponential (M4) Exponential (M5)[c]	0.240	-248.55	1471	557	
Hill	**0.264**	**-248.74**	**1330**	**316**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.162	-248.26	3167	2334	

[a] Constant variance case presented (BMDS Test 2 *p*-value = 0.388), selected model in bold; scaled residuals for selected model for doses 0, 150, 500, 1500, and 5000 ppm were -0.874, 1.29, -0.235, -0.308, and 0.125, respectively.

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

[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.

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

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

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

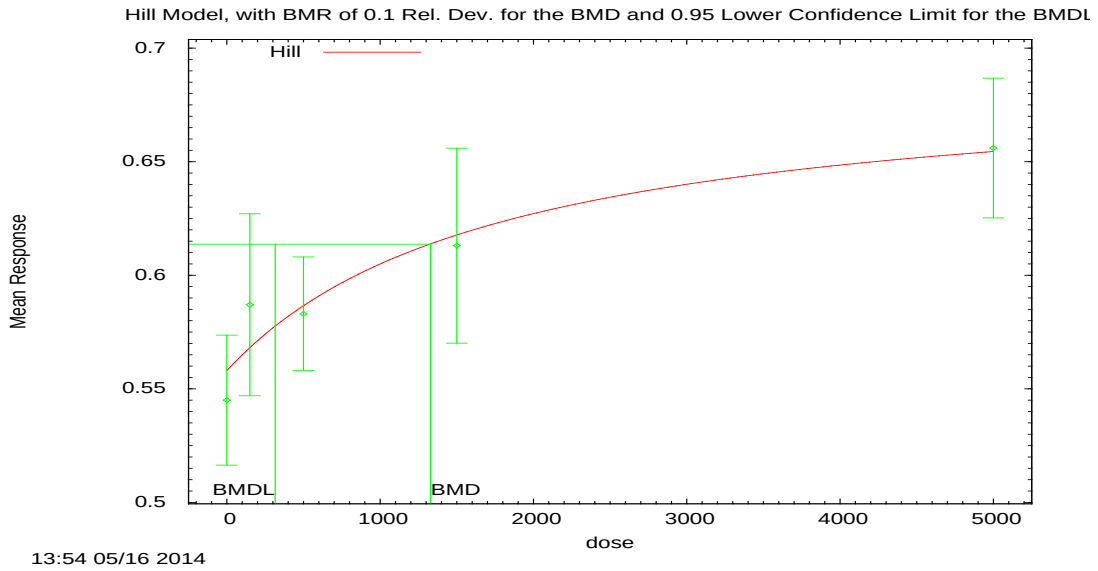

Figure C-18. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

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 = 1329.5
BMDL at the 95% confidence level = 315.543

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.00216632	0.002282
rho	n/a	0
intercept	0.557859	0.545
v	0.130692	0.111
n	1	0.226907
k	1785.17	1916.67

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	10	0.545	0.558	0.04	0.0465	-0.874
150	10	0.587	0.568	0.056	0.0465	1.29
500	10	0.583	0.586	0.035	0.0465	-0.235

1500	10	0.613	0.618	0.06	0.0465	-0.308
5000	10	0.656	0.654	0.043	0.0465	0.125

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	129.701589	6	-247.403177
A2	131.770538	10	-243.541076
A3	129.701589	6	-247.403177
fitted	128.368125	4	-248.73625
R	117.090968	2	-230.181936

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	29.3591	8	0.0002742
Test 2	4.1379	4	0.3877
Test 3	4.1379	4	0.3877
Test 4	2.66693	2	0.2636

Table C-24. 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 wks (Medinsky et al., 1999; Bond et al., 1996); 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	3107	2439	The Hill model was selected on the basis of lowest BMDL.
Exponential (M4) Exponential (M5)[c]	0.199	-129.71	1798	808	
Hill	**0.224**	**-129.89**	**1667**	**603**	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.208	-130.22	2980	2288	

[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, 1750, and 5000 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). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d] For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

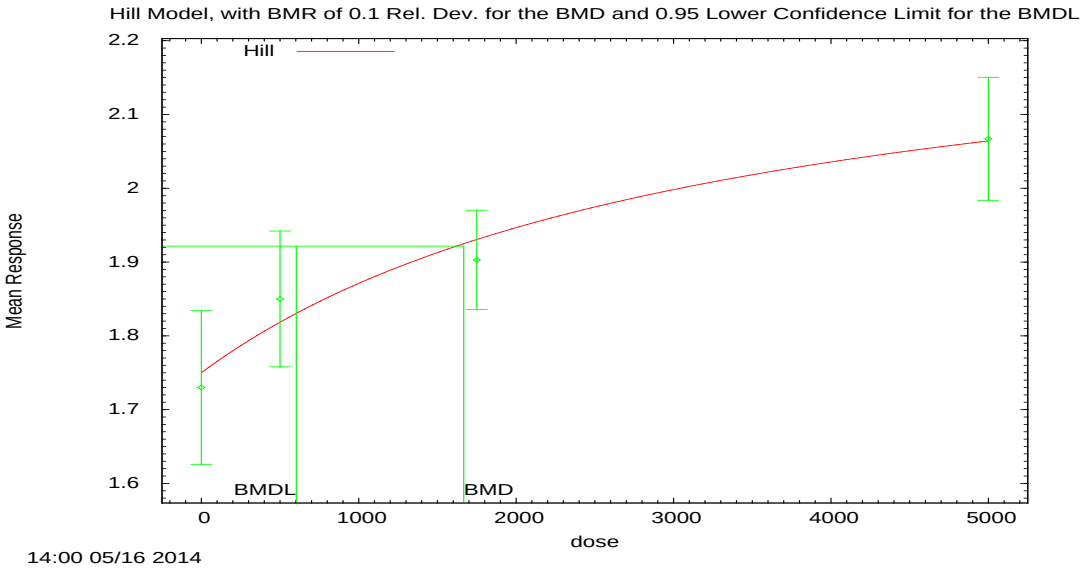

Figure C-19. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

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 = 1666.92
BMDL at the 95% confidence level = 603.113

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
α	0.0160221	0.0170425
rho	n/a	0
intercept	1.74684	1.73
v	0.521534	0.337
n	1	0.225826
k	3309.8	1856.13

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
1750	11	1.9	1.93	0.1	0.127	-0.635
5000	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

1
2
3

Table C-25. 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 wks (Medinsky et al., 1999; Bond et al., 1996); 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	2706	2275	The Exponential (M4) model was selected as the most parsimonious model of adequate fit.
Exponential (M4) Exponential (M5)[c]	**0.956**	**-191.20**	**1342**	**816**	
Hill	N/A[d]	-189.20	1325	741	
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	0.0928	-188.45	2552	2111	

[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, 1750, and 5000 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). The models in this row reduced to the Exponential (M2) model.
[c] For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row 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. The models in this row reduced to the Linear model.
[f] For the Polynomial 3° model, the b3 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g] For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from Medinsky et al. (1999)

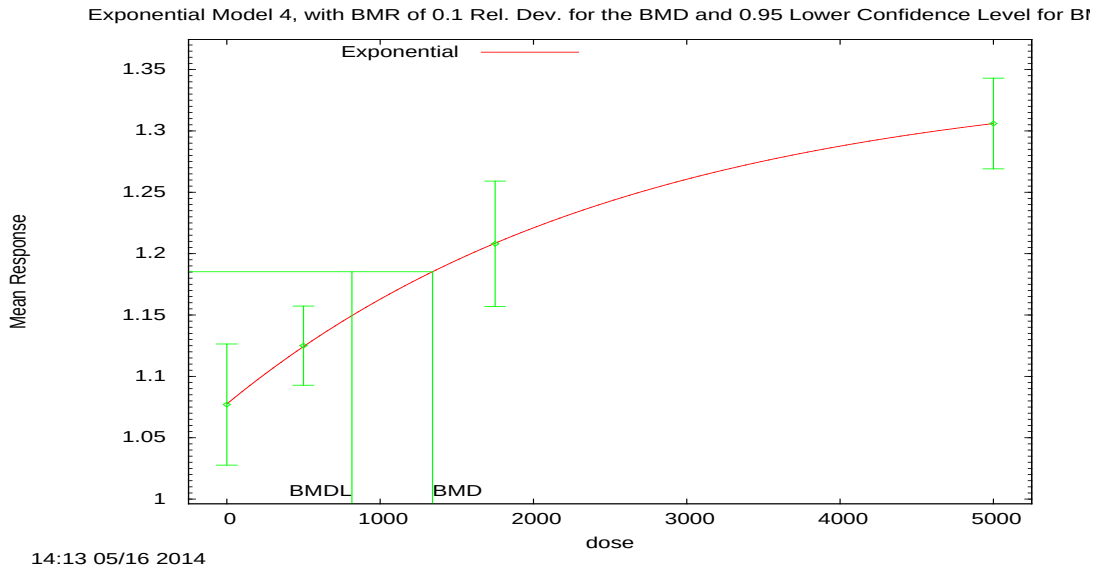

Figure C-20. Plot of mean response by dose, with fitted curve for selected model; dose shown in ppm.

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 = 1341.66
BMDL at the 95% confidence level = 815.742

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
lnα	-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
1750	11	1.208	1.208	0.076	0.05983	-0.02385

5000	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 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 three-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

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 dataset. The data were modeled using three different exposure metrics: administered concentration as ppm, administered concentration as mg/m^3, and an internal PBPK exposure concentration of ETBE metabolized.

Table C-26. Cancer endpoints selected for dose-response modeling for ETBE.

Species / Sex Endpoint	Doses and Effect Data				
Hepatocellular adenomas and carcinomas JPEC (2010b)	Exposure Concentration (ppm)	0	500	1500	5000
	Exposure Concentration (mg/m^3)	0	2089	6268	20,893
	PBPK Concentration (mg/hr)	0	1.145	2.7316	4.125
	Incidence / Total	0 / 50	2 / 50	1 / 49	10 / 50

C.1.2.1. Modeling Results

Below are tables summarizing the modeling results for the cancer endpoints modeled. For the multistage cancer models, the coefficients were restricted to be non-negative (beta's ≥ 0).

[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).

Table C-27. 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 wks; 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	2942	1735	Multistage 1° was selected on the basis of lowest AIC.
Two	0.264	0.000, 1.284, -1.000, and 0.137	83.093	2756	1718	
One	**0.490**	**0.000, 1.009, -1.144, and 0.309**	**81.208**	**2605**	**1703**	

[a] Selected model in bold.

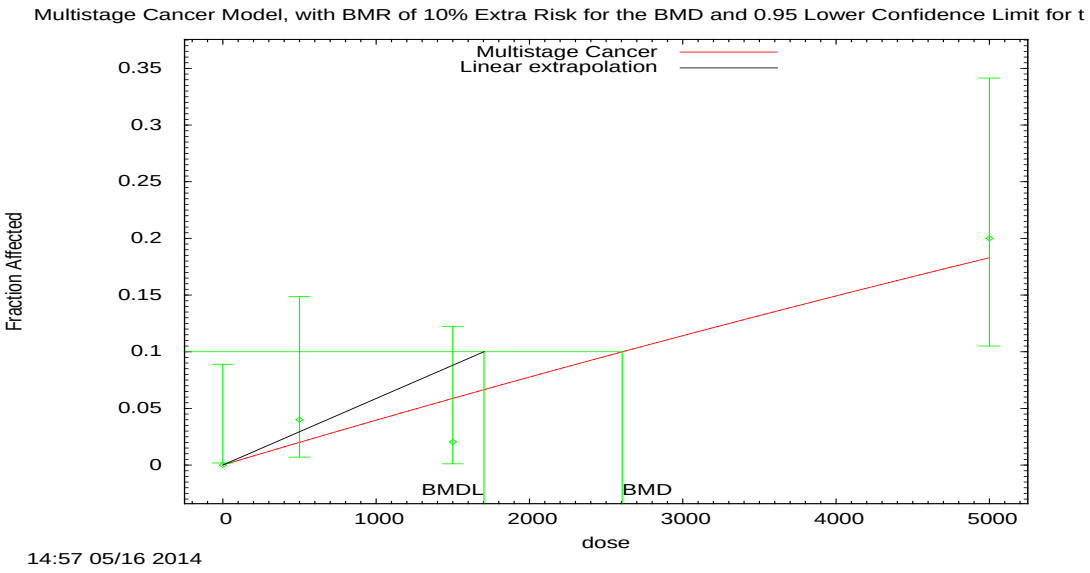

Figure C-21. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in ppm.

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 = 2604.82
BMDL at the 95% confidence level = 1703.47
BMDU at the 95% confidence level = 4634.52
Taken together, (1703.47, 4634.52) 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)	0.0000404483	0.0000438711

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
1500	0.0589	2.885	1	49	-1.144
5000	0.1831	9.155	10	50	0.309

Chi^2 = 2.42 d.f = 3 P-value = 0.4898

Table C-28. 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 wks; modeled with doses as mg/m³ JPEC (2010b); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMD_{10Pct} (mg/m3)	$BMDL_{10Pct}$ (mg/m3)	Basis for model selection
	p-value	Scaled residuals	AIC			
Three	0.0991	-0.040, 1.382, -0.897, and 0.048	84.961	12300	7251	
Two	0.264	0.000, 1.284, -1.000, and 0.137	83.093	11514	7179	
One	**0.490**	**0.000, 1.009, -1.144, and 0.309**	**81.209**	**10884**	**7118**	

[a] Selected model in bold.

Data from JPEC (2010b)

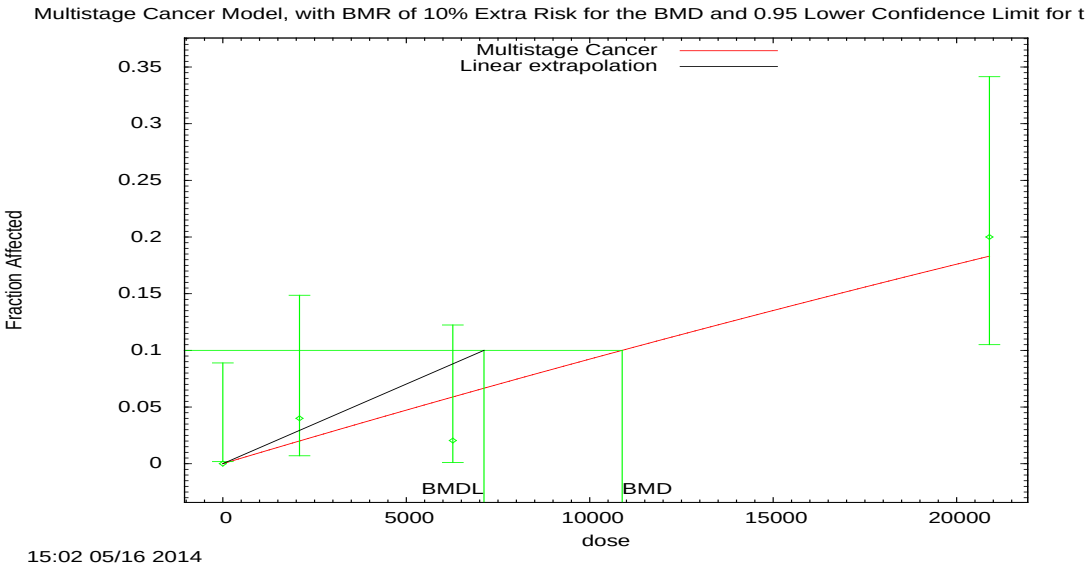

Figure C-22. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in mg/m³.

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 = 10884.4
BMDL at the 95% confidence level = 7118.08
BMDU at the 95% confidence level = 19366.3
Taken together, (7118.08, 19366.3) 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)	9.6799E-06	0.0000104989

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
2089	0.02	1.001	2	50	1.009
6268	0.0589	2.885	1	49	-1.144
20893	0.1831	9.155	10	50	0.309

Chi^2 = 2.42 d.f = 3 P-value = 0.4897

Table C-29. 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 wks; modeled with PBPK doses as ETBE metabolized, mg/hr (JPEC, 2010b); BMR = 10% extra risk.

Model[a]	Goodness of fit			BMC_{10Pct} (mg/hr)	$BMCL_{10Pct}$ (mg/hr)	Basis for model selection
	p-value	Scaled residuals	AIC			
Three	0.177	0.000, 1.033, -1.433, and 0.587	84.574	3.20	2.34	Multistage 1° was selected on the basis of lowest AIC
Two	0.144	0.000, 0.871, -1.574, and 0.798	85.271	3.09	2.19	
One	**0.184**	**0.000, 0.035, -1.713, and 1.378**	**84.446**	**3.03**	**1.98**	

[a] Selected model in bold.

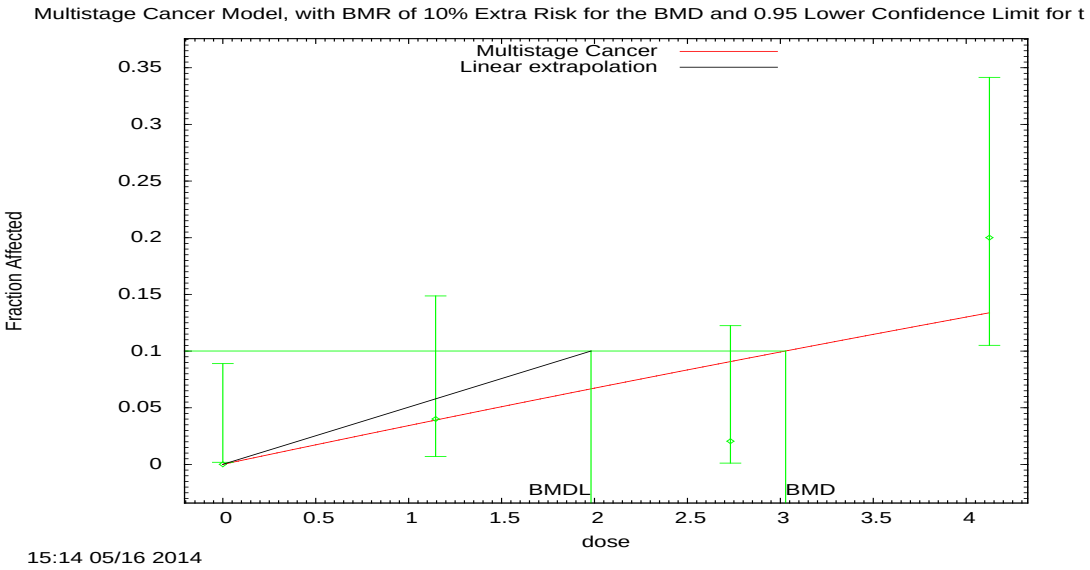

Figure C-23. Plot of incidence rate by dose, with fitted curve for selected model; dose shown in mg/hr.

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 = 3.02863
BMDL at the 95% confidence level = 1.98128
BMDU at the 95% confidence level = 5.02417
Taken together, (1.98128, 5.02417) 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)	0.0347882	0.0464377

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	p-value
Full model	-38.2989	4			
Fitted model	-41.2229	1	5.84813	3	0.1192
Reduced model	-48.0344	1	19.4711	3	0.0002184

AIC: = 84.4459

Goodness of Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled Resid
0	0	0	0	50	0
1.145	0.039	1.952	2	50	0.035
2.7316	0.0907	4.442	1	49	-1.713
4.125	0.1337	6.684	10	50	1.378

Chi^2 = 4.83 d.f = 3 P-value = 0.1844

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

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