TOXICOLOGICAL REVIEW

OF

ETHYL TERTIARY BUTYL ETHER

(CAS No. 637-92-3)

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

July 2009

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LIST OF ABBREVIATIONS AND ACRONYMS

AC_{50}	concentration required to anesthetize half of study group
AIC	Akaike's Information Criterion
ALDH	aldehyde dehydrogenases
AUC	area under the curve
BMCL	benchmark concentration lower; 95% confidence limit
BMD	benchmark dose
BMDL	benchmark dose, lower 95% confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BrdU	5-Bromo-2'-deoxyuridine
CASE	computer automated structure evaluator
CASRN	Chemical Abstracts Service Registry Number
CHO	Chinese hamster ovary
CNS	central nervous system
CYP	cytochrome P450
DIPE	diisopropyl ether
ETBE	ethyl tertiary butyl ether
FOB	functional observation battery
GABA	γ-aminobutyric acid
GC-MC	gas chromatography-mass spectrometry
GD	gestational day
G/ETBE	gasoline/ETBE vapor condensate
HBA	2-hydroxybutyrate
HEC	human equivalent concentration
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
IRIS	Integrated Risk Information System
i.p.	intraperitoneally
K_m	Michaelis constant
K_{ow}	octanol:water partition coefficient
K_p	permeability coefficient
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LI	Labeling index
LOAEL	lowest-observed-adverse-effect level
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MN	micronucleus
MPD	2-methyl-1,2-propane diol
MTBE	methyl tertiary butyl ether
MULTICASE	multiple computer automated structure evaluation
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRC	National Research Council
NTP	National Toxicology Program
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic

PEX	polyethylene
PND	postnatal day
PFC	plaque-forming cell
POD	point of departure
RfC	reference concentration
RfD	reference dose
RfV	reference values
SAR	structure-activity relationships
SCE	sister chromatid exchange
SRBC	sheep red blood cell
SD	standard deviation
TAA	t-amyl alcohol
TAME	tertiary amyl methyl ether
TBA	tertiary butanol
UF	uncertainty Factors
U.S. EPA	U.S. Environmental Protection Agency
$\mathbf{V_{max}}$	Maximum substrate turnover velocity
WHO	World Health Organization

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to ethyl tertiary butyl ether (ETBE). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of ETBE.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 202-566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of ethyl tertiary butyl ether (ETBE). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for ETBE has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation*

Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998b), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through January 2009.

2. CHEMICAL AND PHYSICAL INFORMATION

In 1990, as part of comprehensive amendments to the Clean Air Act, automobile emissions were regulated in an effort to reduce CO and O_3 pollution. The 1990 amendments to the Clean Air Act mandated that, in areas with excessive levels of CO and O_3 air pollution, automotive gasoline must contain additives that improve automobile exhaust quality. The most common fuel oxygenates in 1999 were methyl tertiary butyl ether (MTBE), ethanol, ETBE, tertiary amyl methyl ether (TAME), diisopropyl ether (DIPE), and tertiary butanol (TBA), with MTBE accounting for 85% of the oxygenates used in the United States, or roughly 15 billion L/year (Blue Ribbon Panel on Oxygenates in Gasoline, 1999). Exact production numbers for ETBE are not available; however, it should be mentioned that in the United States, ETBE usage ranked far behind MTBE and ethanol. It should also be noted that the reduction in the use of MTBE makes the use of other oxygenates more likely for two reasons: (1) to replace the volume that MTBE previously contributed to gasoline (up to 15%), and (2) to meet air pollution reduction goals previously addressed by MTBE. While the production of oxygenates has continued in the United States after 2006 (U.S. DOE 2007a), the production of reformulated gasoline with ethers (e.g., MTBE or ETBE) as a fuel additive stopped in 2006. The amount of gasoline with ether as a fuel additive was effectively eliminated in 2006, going from a net production of 2–4 million barrels per month in 2005 to 0 barrels by the end of 2006 (and the import of reformulated gasoline with ether as a fuel additive dropped from several hundred thousand barrels per day in 2005 to 0 barrels also by the end of 2006) (U.S. DOE, 2007b). ETBE has been proposed as an oxygenate substitute for the use of MTBE in gasoline and was used more widely than MTBE in some European counties by the late 1990s; however, the use of ETBE has been relatively low in the United States (CFDC, 2001). Although ethanol, rather than ETBE, has replaced MTBE in gasoline in the United States, ETBE remains an alternative oxygenate for gasoline (CFDC, 2007).

ETBE is a colorless liquid with a characteristic strong odor that has been described as being reminiscent of ether, gasoline, or varnish, or as being sweet; its taste has been characterized as highly objectionable (Vetrano, 1993, unpublished report). An additional effect of adding ETBE to gasoline is that it may increase the emission of acetaldehyde and 1,3-butadiene into the atmosphere (Schuetzle, 1994). The chemical structure of ETBE is presented in Figure 2-1, and its main physicochemical properties are given in Table 2-1.

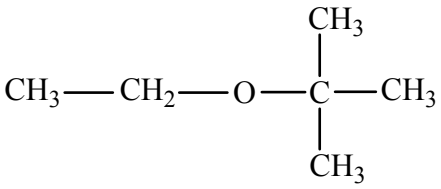


Figure 2-1. Chemical structure of ETBE.

Table 2-1. Physicochemical properties of ETBE

Characteristic		Reference
CASRN	637-92-3	Drogos and Diaz, 2002
Chemical formula	$C_6H_{14}O$	
Molecular weight	102.18	
Systematic name	2-ethoxy-2-methylpropane 2-methyl-2-ethoxypropane	National Library of Medicine/Special Information Services[a]
Synonyms	ethyl tert-butyl ether ethyl tert-butyl oxide methyl-2-ethoxypropane tert-butyl ethyl ether ETBE	
Melting point	-94°C	Drogos and Diaz, 2002
Boiling point	67–73°C	
Vapor pressure	130–152 mm Hg @ 25°C	
Density	0.73–0.74 g/cm^3 @ 25°C	
Water solubility	7,650–26,000 mg/L	
Oil/water partition coefficient (log k_{ow}) @ 25°C	1.48 1.74	Montgomery, 1994 Drogos and Diaz, 2002
Henry's law constant	2.7×10^{-3} atm-m^3/mol @ 25°C	Drogos and Diaz, 2002
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)	
Odor detection threshold (in water)	0.049 ppm (49 µg/L)	
Odor detection threshold (in water)	0.005 ppm (5 µg/L)	Durand and Dietrich, 2007
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 weight in mg/mmol ÷ 1,000 L/m^3

[a]Available online at http://chem.sis.nlm.nih.gov/chemidplus/jsp/common/ChemFull.jsp

The use of ETBE as a gasoline additive, at an amount of up to 17% by weight, indicates that this chemical could be produced in very large amounts depending on how widespread the use of ETBE becomes within the gasoline supply chain. Environmental concern surrounding

fuel oxygenates has arisen not only in connection with automotive emissions, but also with the potential of inhalation and/or dermal exposure while refueling motor vehicles. An additional concern associated with fuel additives is derived from the relatively high aqueous solubility of these additives and the fact that they have been shown to easily reach groundwater following leakage or spills (U.S. EPA, 1998b), with the potential of subsequent oral (drinking water) or dermal (bathing or showering) exposure.

The concentration of oxygenates, particularly ETBE and MTBE, in groundwater and surface water is likely to exceed the concentrations of other components of gasoline. This potential for groundwater contamination at higher concentrations by these oxygenates is due to the fact that when compared to other components of gasoline (e.g., benzene, toluene, ethylbenzene, and total xylenes), ETBE and MTBE have greater solubilities in water, are less likely to adhere to soil particles, and are more likely to resist biodegradation (Deeb et al., 2001; Fayolle et al., 2001; U.S. EPA, 1992). Therefore, ETBE and other oxygenates are likely to travel farther and faster in groundwater than other gasoline constituents. Although data specifically on ETBE associated with leaking underground fuel storage tanks are not available, the issue of water contamination with oxygenates in general has been raised due to the more than 400,000 reported and confirmed releases from underground fuel storage tanks in the United States since 1988 (Rothenstein, 2004), more than half of which potentially contained MTBE (U.S. EPA, 1998b). Data from over 7,200 monitoring wells on 868 leaking underground fuel storage tanks in the Los Angeles, California area collected by Shih et al. (2004) detected oxygenates roughly in proportion to their usage (e.g, MTBE at 82.7% and ETBE at 8.9% of the leaking sites). Apart from potential health concerns, the presence of MTBE in drinking water is associated with an unpleasant odor and taste that is unacceptable for many people even at relatively low concentrations (U.S. EPA, 1998b), and ETBE has odor and taste thresholds that allow ETBE to be detected at even lower concentrations in water or air (i.e., the detection thresholds of ETBE, 5–47 μg/L in water and 0.13 mg/m^3 in air, are 2.5–25 times lower than similar values for MTBE) (Durand and Dietrich, 2007; Vetrano, 1993).

Plastic plumbing pipe, particularly silane cross-linked polyethylene (PEX), represents an additional potential source of ETBE in drinking water (Durand and Dietrich, 2007). Durand and Dietrich (2007) measured ETBE leaching from a PEX pipe using a utility quick test designed for evaluating taste, odor, migration, and leaching of materials in water distribution systems. ETBE was observed leaching into tap water with and without the addition of free chlorine or monochloramine using solid phase microextration/gas chromatography-mass spectrometry (GC-MS). Aqueous concentrations of ETBE in the leachate ranged from 23 to >140 μg/L and decreased with increased flushing. A team of 10 panelists were recruited and trained for several weeks in flavor profile analysis in a research protocol approved according to the standards of the Virginia Tech Institutional Review Board for human subjects. Panelists were able to smell ETBE at a concentration of 5 μg/L (Durand and Dietrich, 2007).

3. TOXICOKINETICS

3.1. ABSORPTION

Most of the available 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 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 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, TBA, were determined in exhaled air samples. Blood was drawn before exposure, approximately every 10 minutes during, and for 1 hour following 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, TBA, and acetone concentrations were determined in blood and urine. The blood profiles of 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 appeared to reflect not only ETBE exposure, but also the physical activity, and were highly variable. Nihlén et al. (1998a) calculated the ETBE doses to the volunteers to be 0.58, 2.9, and 5.8 mmol for the 5, 25, and 50 ppm exposure levels, respectively. The concentrations of ETBE in blood rose sharply during the first 30 minute 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 50, 25, and 5 ppm, respectively. By 6 hours, they had fallen to very low levels (<1 μM) even after 50 ppm 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 were 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 excretion was calculated as 45–50% of the respiratory uptake, and since the net respiratory uptake and excretion 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 and 40 ppm of ETBE (actual exposure concentrations were 4.5 and 40.6 ppm,

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, TBA, were determined in blood, and the same two substances, plus additional metabolites of TBA, were assessed in urine. The authors estimated the received doses to be 1,092 µmol following exposure to 40 ppm ETBE, and 121 µmol following 4 ppm exposure, respectively. 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). Amberg et al. (2000) also exposed F344 NH rats, 5/sex/dose concurrent with the volunteers in the same exposure chamber. Blood was taken from the tail vein 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 40 and 4 ppm 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 rats or humans were identified. Dekant et al. (2001a) published a review article that presented an overview of their studies of the toxicokinetics of ETBE, MTBE, and TAME in both humans and rats following inhalation exposure at 4 and 40 ppm, 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. A synopsis of their findings is presented in Table 3-1. The data may provide some insight relative to uptake of ETBE following ingestion. The authors assumed 100% absorption of MTBE and TAME following ingestion.

Table 3-1. Doses received by humans and F344 rats following inhalation exposure to and oral ingestion of fuel oxygenates

	Dose received (µmol)	Percent of dose excreted	Dose received (µmol)	Percent of dose excreted
Inhalation	**4 ppm exposure level**		**40 ppm exposure level**	
ETBE				
Human	121	41	1,092	43
Rat	2.3	50	21	53
MTBE				
Human	161	35	1,387	69
Rat	3.8	42	33	39
TAME				
Human	102	53	1,033	58
Rat	1.9	40	20	42
Ingestion	**5 mg dose**		**15 mg dose**	
MTBE				
Human	57	46	170	49
TAME				
Human	49	9	147	14

Source: Dekant et al. (2001a).

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 (see Section 3.2 for details) 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), which are almost identical (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 2-1; Drogos and Diaz, 2002]), then values for ETBE and TAME should be similar. Data in Table 3-1 support the latter hypothesis, but there are limited data for the evaluation of either hypothesis. Note that, 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, since 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:

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

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 (2002), and converting cm/second values to cm/hour, yields a K_p value for ETBE of 0.0020–0.016 cm/hour and, for comparison, 0.0012–0.012 cm/hour for MTBE. These calculations predict that the dermal absorption rate of ETBE in humans would be between 1.3 and 1.7 times as high as 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 above 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 highlight experimental variables such as the importance of temperature as well as 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 to 38% for MTBE, which, as the authors point out, parallels the lower blood:air partition coefficient for ETBE (11.7) compared to MTBE (17.7). The AUC for the concentration-time curve was linearly related to ETBE exposure level, suggesting linear kinetics up to 50 ppm. Although comparison of log k_{ow} values with MTBE suggest that dermal absorption rates for ETBE would be higher than MTBE, no data are available on dermal or oral absorption of ETBE.

3.2. DISTRIBUTION

In vivo data on the tissue distribution of ETBE are not available. However, Nihlén et al. (1995) conducted a series of in vitro experiments, using blood samples from 10 human donors (5 males, 5 females), to assess the partitioning of ETBE, MTBE, TAME, and TBA between air and blood. Kaneko et al. (2000) performed a similar series of in vitro studies to determine partition coefficients of ETBE, MTBE, TAME, TBA, and t-amyl alcohol (TAA) using tissues from five male Wistar rats. 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, 17.7 and 14.2 for MTBE, 17.9 and 15.5 for TAME, and 462 and 531 for TBA for humans and rats respectively. Note the similarity between the values for humans and rats and that the blood:air partition coefficient for ETBE, MTBE, and TAME are much lower than for TBA. Nihlén et al. (1995) also estimated oil:water partition (log k_{ow}) coefficients and obtained values of -0.56 for TBA, 0.90 for MTBE, 1.36 for ETBE, and 1.45 for TAME. These values have a similar ranking, but are not identical, to those listed in a report by Drogos and Diaz (2002) (namely, 0.35 for TBA, 0.94–1.30 for MTBE, 1.48–1.74 for ETBE, and 1.55 for TAME). Nihlén et al. (1995) also used

these coefficients, and air:oil partition coefficients, to calculate blood:tissue partition coefficients. These values are listed in Table 3-2.

Table 3-2. Blood:tissue partition coefficients for gasoline ether additives and TBA

Partition coefficient	TBA	MTBE	ETBE	TAME
Blood:brain	1.05	1.40	2.34	2.58
Blood:muscle	1.06	1.18	1.78	1.93
Blood:fat	0.646	4.98	11.6	13.3
Blood:lung	1.02	0.783	0.835	0.837
Blood:kidney	1.06	1.04	1.42	1.51
Blood:liver	1.05	1.04	1.44	1.54

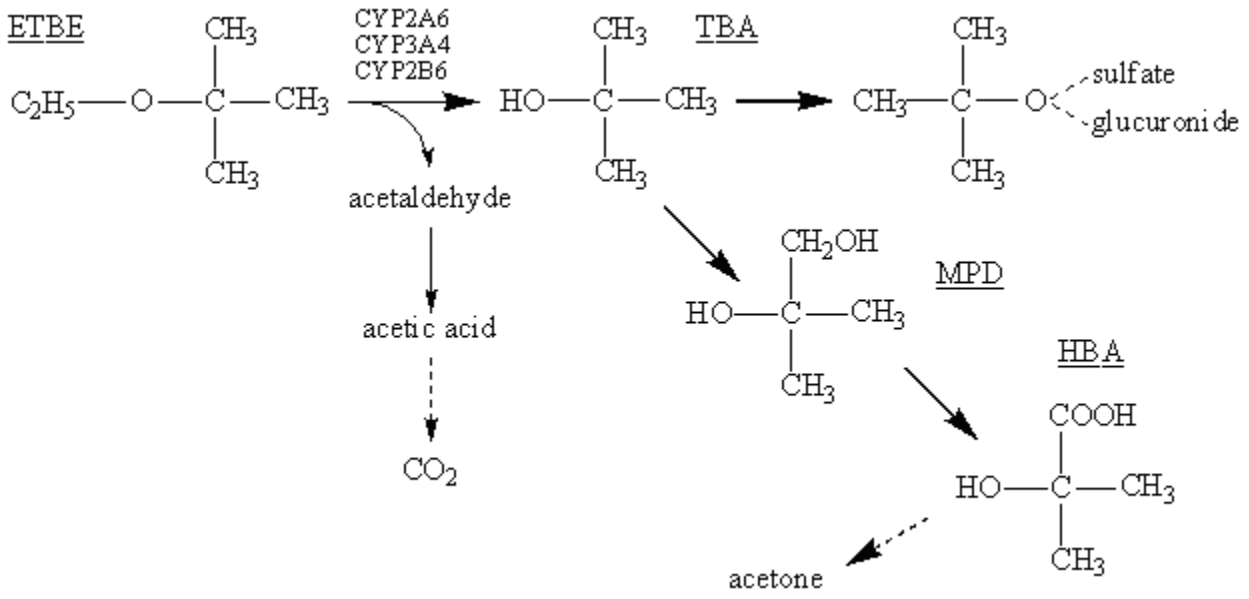
Source: Nihlén et al. (1995).

Nihlén et al. (1998a) exposed eight healthy male volunteers (average age: 29 years) to 5, 25, and 50 ppm ETBE by inhalation for 2 hours. The volunteers performed light physical exercise during exposure. Profiles of ETBE, TBA, and acetone were established for blood throughout exposure and for up to 22 hours thereafter. The same laboratory conducted studies with MTBE by using the same experimental protocol. Net uptake of MTBE was 38% of the dose (compared to 26% net uptake for ETBE) and net exhalation was 28% of the net uptake for MTBE (compared to 31% net exhalation for ETBE) (Nihlén et al., 1998c). 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 to 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 twofold greater blood:fat partition coefficient (11.6 and 4.98 for ETBE and MTBE respectively).

3.3. 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 3-1. On the basis of structures of the metabolites elucidated, ETBE is initially metabolized by cytochrome P450 (CYP) enzymes via oxidative deethylation by introducing a hydroxy group into the ethyl or methyl moieties of the molecule (Bernauer et al., 1998). The resulting hemiacetal is unstable and decomposes spontaneously into TBA and acetaldehyde. In human liver microsome preparations, this step is catalyzed mainly by CYP2A6, with some contribution from CYP3A4 and CYP2B6, and possible contribution of CYP2E1 (Le Gal et al., 2001; Hong et al., 1999a). Using data from rat hepatic microsome preparations, Turini et al. (1998) suggest 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). TBA 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), and acetone. It should be noted that these metabolites have been identified in humans and rats for both ETBE and MTBE. 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.



Source: Adapted from Dekant et al., 2001a.

Figure 3-1. Proposed Metabolism of ETBE.

Zhang et al. (1997) used computer models to predict the metabolites of ETBE and their toxic effects. The metabolism model correctly predicted cleavage into TBA and acetaldehyde and that TBA would undergo glucuronidation and sulfation. However, for the further metabolism of TBA, 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.

3.3.1. Metabolism in Humans

3.3.1.1. *Metabolism of ETBE in Humans In Vivo*

Nihlén et al. (1998a) exposed eight healthy male volunteers (average age: 29 years) to 0, 5, 25, and 50 ppm ETBE by inhalation for 2 hours. Profiles of ETBE, TBA, and acetone were established for blood throughout exposure and for up to 22 hours thereafter. The blood profiles of parent compound and metabolites were similar at all three exposure levels, and reflected exposure concentrations, as judged by linear increases in concentration-time AUC values

calculated (but only reported graphically) by the authors. 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 50, 25, and 5 ppm, respectively. By 6 hours, ETBE concentrations had fallen to low levels even after exposures to 50 ppm. The blood concentration of TBA continued to rise for the full 2-hour exposure period, with peak values of about 13 and 7 μM at 50 and 25 ppm, 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 (TBA levels could not be determined following 5 ppm 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 (lower doses and controls) for about 1½ hours after exposure. 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 4 and 40 ppm of ETBE, respectively (actual exposure concentrations were 4.5 and 40.6 ppm, 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. Levels of parent ETBE and its primary metabolite, TBA, were determined in blood and urine. In urine, two further metabolites of TBA, MPD and HBA, were also assayed.

At an exposure level of 40 ppm, the peak concentration of TBA in blood was 13.9 ± 2.2 and 1.8 ± 0.2 μM at 4 ppm. At the and low high exposure concentrations, TBA disappeared from blood with half-lives of 9.8 ± 1.4, and 8.2 ± 2.2 hours. The time courses of metabolite appearance in urine after 40 and 4 ppm were similar, but relative urinary levels of metabolites after 4 ppm differed from those after 40 ppm. Using parent ETBE as the reference, molar ratios for total urinary excretion were 1:25:107:580 (ETBE:TBA:MPD:HBA) after 40 ppm and 1:17:45:435 after 4 ppm. 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.

3.3.1.2. *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. (1997a) coexpressed human CYP2A6 or CYP2E1 with human CYP reductase in insect SF9 cells. In this system, in the presence of 1 mM ETBE, TBA 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 (to TBA) by 10 human CYP isozymes: 2A6 > 3A4 ≈ 2B6 ≈ 3A4/5 » 2C9 > 2E1 ≈ 2C19 » 1A2 ≈ 2D6 ≈ 1A2. They characterized the correlation with CYP2A6 as high, with 3A4, 3A5, and 2B6 as good, and with 2C9, 2E1, and 2C19 as poor, and the remaining three CYP activities showed no correlation with ETBE metabolism. They also reported direct enzyme activities toward ETBE as the substrate (in pmol TBA formed per minute and pmol CYP): 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 TBA. 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 conclude that CYP2A6 is the major enzyme responsible for the oxidative metabolism of MTBE, ETBE, and TAME in human livers. Furthermore, they conclude 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, TAA from TAME, and TBA from ETBE and MTBE. The human microsomes showed higher catalytic activity towards MTBE and TAME at 0.5 mM, compared to 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, 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.

3.3.2. Metabolism in Animals

3.3.2.1. *Metabolism of ETBE in Animals In Vivo*

Bernauer et al. (1998) studied the metabolism and excretion of [^{13}C]-ETBE, MTBE, and TBA in rats. F344 rats, 2/sex, were exposed via inhalation to 2,000 ppm ETBE or MTBE for 6 hours, or three male F344 rats received 250 mg/kg TBA by gavage. Urine was collected for 48 hours. The metabolic profiles for ETBE and MTBE were essentially identical, with excretion of MPD > HBA > TBA-sulfate > TBA-glucuronide. Oral administration of TBA produced a similar metabolite profile, with HBA > TBA-sulfate > MPD » TBA-glucuronide ≈ TBA. TBA 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 (see Section 3.1). 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 TBA were much lower than in humans, 5.3 ± 1.2 and 21.7 ± 4.9 μM at 40 ppm and 1.0 ± 0.7 and 5.7 ± 0.8 μM at 4 ppm, respectively. Similar to humans, rats excreted mostly HBA in urine, followed by MPD and TBA. The molar ratios for total urinary excretion of TBA:MPD:HBA were 1:2.3:15 after exposure to 40 ppm and 1:1.5:11 after 4 ppm. 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. (2001b) focused on aspects of metabolism of MTBE and ETBE in humans and rats. They reported that, at a high exposure level (2,000 ppm), rats predominantly excreted the glucuronide of TBA in urine, which, at low levels (4 or 40 ppm) had been barely detectable. They concluded

that, at high exposure levels, the normally rapid metabolism of TBA 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 appears 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, TBA, HBA, or acetone, occur naturally in normal mammalian physiology, providing a highly variable background that needs to be corrected for in metabolic experiments.

3.3.2.2. *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-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. (1997b) 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 (i.p.) 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, 4.1/3.8; 2E1 induced, 4.7/1.6; 3A induced, 4.4/1.4; and 1A induced, not determined/0.9. 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 TBA to MPD, HBA, and even acetone, have not been fully characterized. However, it is clear that TBA is not subject to metabolism by alcohol dehydrogenases (Dekant et al., 2001a).

3.4. ELIMINATION

3.4.1. Elimination in Humans

Nihlén et al. (1998a) exposed eight healthy male volunteers (average age: 29 years) to 5, 25, and 50 ppm ETBE by inhalation for 2 hours. ETBE, TBA, and acetone were measured in urine for up to 22 hours after exposure. The blood profiles of parent compound and metabolites were similar at all three exposure levels, and reflected exposure concentrations. The authors estimated the ETBE amount to the volunteers to be 0.58, 2.9, and 5.8 mmol for the 5, 25, and 50 ppm 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 excretion was calculated at 45–50% of the inhaled ETBE (respiratory uptake) and net respiratory excretion was 31% (of the net respiratory uptake), of which TBA 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 retained after 5, 25, and 50 ppm 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 TBA 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 TBA 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 indicate 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 upon 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 4 and 40 ppm of ETBE, respectively (actual exposure concentrations were 4.5 and 40.6 ppm, 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 TBA were determined in blood and urine. Two further metabolites of TBA, HBA and MPD, were also determined in urine.

At 40 ppm, the peak concentration of ETBE in blood was 12.1 ± 4.0 μM, while that for TBA was 13.9 ± 2.2 μM. The corresponding values at 4 ppm 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. TBA 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 TBA 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 TBA (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 40 and 4 ppm, respectively, were similar, but relative urinary levels of HBA after 4 ppm were higher, while those for MPD were lower, as compared to 40 ppm. 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 40 and 4 ppm, respectively, while TBA 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 3.3.1, Amberg et al. (2000) calculated that 43 ± 12% of the 40 ppm dose and 50 ± 20% of the 4 ppm dose had been excreted in urine by 72 hours. Respiratory elimination was not monitored.

3.4.2. 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 TBA. Parent ETBE was not identified in rat urine. The half-life for TBA in rat urine was 4.6 ± 1.4 hours at 40 ppm, but could not be calculated at 4 ppm. 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 40 and 4 ppm 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 2,000 ppm ETBE or MTBE for 6 hours, or three male F344 rats received 250 mg/kg TBA 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 > TBA-sulfate > TBA-glucuronide. Oral administration of TBA produced a similar metabolite profile, with relative amounts of HBA > TBA-sulfate > MPD » TBA-glucuronide ≈ TBA.

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 (1995a, unpublished report) 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 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 Beskett (1995a, b, unpublished reports) for "Total" column in Table 3-3 or for the specific tissues in Table 3-4. 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 5,000 ppm 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 3-3. Elimination of [^{14}C]-ETBE-derived radioactivity from rats and mice within 96 hours following a single 6-hour inhalation exposure

Exposure level (ppm)	Volatile organics[a]	Exhaled CO_2[a]	Urine[a]	Feces[a]	Total[b]
F344 Rat[c]					
500	37	1	60	2	9.92
750	36	1	62	2	17.5
1,000	42	1	56	2	22.1
1,750	58	2	38	3	56.9
2,500	52	2	45	2	56.2
5,000[d]	63 (51)	2 (1)	34 (44)	1 (3)	97.5 (116)
CD-1 Mouse[e]					
500	10	1	74	16	6.38
750	28	2	60	10	7.94
1,000	29	2	64	6	12.8
1,750	42	2	46	10	13.7
2,500	42	2	47	10	22.7
5,000[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 (1995a, unpublished report); [d]values in parentheses: Borghoff (1996, unpublished report); [e]Sun and Beskitt (1995b, unpublished report)

Table 3-4. Radioactivity in blood and kidney of rats and blood and liver of mice, following 6 hours of [^{14}C]-ETBE inhalation exposure

Exposure level (ppm)	F344 Rat[a,]		CD-1 Mouse[a,]	
	Blood[b]	Kidney[c]	Blood[b]	Liver[c]
500	0.037	0.074	0.154	0.208
750	0.062	0.094	0.340	0.348
1,000	0.080	0.116	0.336	0.540
1,750	0.124	0.152	0.481	0.724
2,500	0.156	0.185	0.474	0.628
5,000	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 (1995a, 1995b, unpublished reports).

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 1,000 ppm; 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 500 ppm to 2% at 5,000 ppm, while fecal elimination remained rather 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, unpublished report), is given in Table 3-3. The authors concluded that the metabolic pathways leading to urinary excretion of ETBE degradation products became saturated at an exposure concentration of approximately 1,750 ppm.

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 5,000 ppm, $^{14}CO_2$ 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 3-4 (again combined with the mouse data from the parallel study). The major finding was that radioactivity levels increased up to 2,500 ppm, but leveled off in kidney and fell considerably in blood at 5,000 ppm. To the authors, these data were indicative of saturation of the absorption pathway at around 2,500 ppm. However, it is noteworthy that total elimination of ETBE-derived radioactivity increased steadily from 500 to 5,000 ppm (Table 3-3). The authors reported no deaths following 6 hours of ETBE exposure. The findings of Sun and Beskitt (1995a, unpublished report) at 5,000 ppm 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 at a later time point.

In a parallel study with an identical experimental protocol, Sun and Beskitt (1995b, unpublished report) exposed CD-1 mice (3/sex/dose) to 500, 750, 1,000, 1,750, 2,500, and 5,000 ppm [^{14}C]-ETBE. The only difference from the rat study (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 3-3 and 3-4, 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 1,000 ppm (Table 3-3), and excreted about 5 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 3-3 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 5,000 ppm showed no further increase over the

values at 2,500, indicating that the absorptive and metabolic capacities of mice had become saturated. Judging from the data in Table 3-4, saturation of blood and liver had occurred already at 1,750 ppm. The authors reported no deaths following 6 hours of ETBE exposure. It may be noted here that Sun and Beskitt (1995a, b, unpublished reports) did not state any estimates for absorbed dose. The data in Table 3-3, 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.

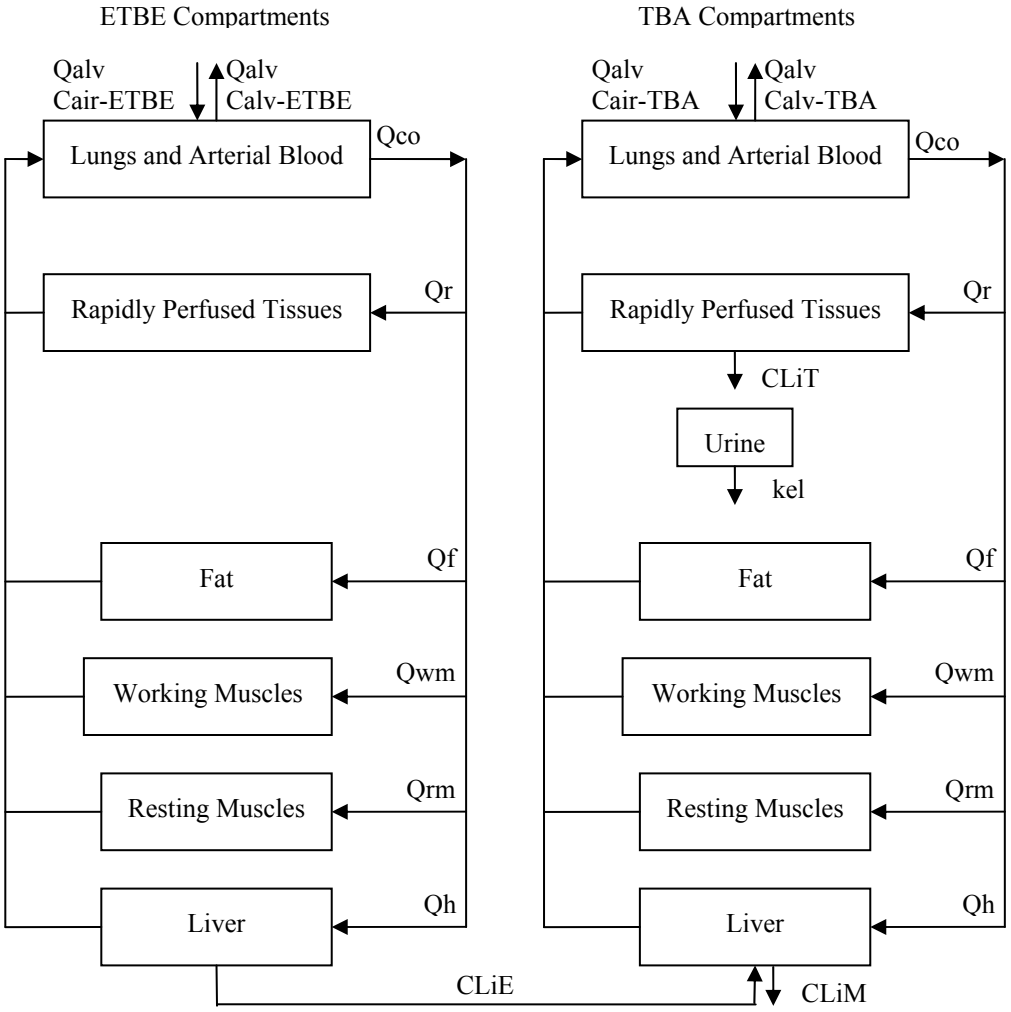
Borghoff (1996, unpublished report) conducted studies to establish experimental conditions for future bioassays of ETBE, based on the two studies previously conducted by Sun and Beskitt (1995a, b, unpublished reports). The experimental protocol and materials were identical to the ones used by Sun and Beskitt (1995a, b, 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 of 5,000 ppm. 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 3-3; 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 TBA 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. TBA 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. TBA 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, TBA, HBA, and MPD in urine were assayed. During 24 hours of collection, rats eliminated about 7 times as much TBA 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.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

A physiologically based toxicokinetic (PBTK) model of ETBE for humans has been developed (Nihlén and Johanson, 1999), but models are not available for any other species. Although physiologically based pharmacokinetic (PBPK) models for a structurally related substance, MTBE, exist that may allow for interspecies extrapolation of dosimetry between rodents and humans (Borghoff et al., 1996), and for predictions of internal dosimetry in humans exposed to MTBE in potable water (Rao and Ginsberg, 1997), no models are available for ETBE. In the Borghoff et al. (1996) model, the MTBE metabolic parameters (V_{max} and K_m) were estimated from gas uptake data by retrofitting only the parent compound module. Rao and Ginsberg (1997) updated the model by fitting these parameters simultaneously with both modules for MTBE and its metabolite, TBA, against concentrations of both compounds in blood. The utility of this MTBE model in risk assessment of MTBE was evaluated by performing cross-species extrapolations of internal dosimetry of the parent compound and TBA, and relating the animal acute toxicity data to predictions of internal doses in human brain after simulated bathing and showering in MTBE-contaminated water (Rao and Ginsberg, 1997).

The PBTK model of Nihlén and Johanson (1999) addresses human inhalation exposure only and describes the pharmacokinetics of ETBE and its main metabolite, TBA, in lungs, liver, fat, rapidly perfused tissues, and resting and working muscles (Figure 3-2). The authors assumed that ETBE is metabolized in the liver by a first-order process, and that TBA (the metabolite) is excreted in the urine also by a first order elimination process. This perfusion-limited model differs in several ways from conventional PBPK models that usually follow an anatomically representative, typical description (e.g., Andersen, 1991), as introduced by Ramsey and Andersen (1984). Thus, in the Nihlén and Johanson (1999) PBTK model, tissue volumes and blood flows were calculated from individual data on body weight and height, and, moreover, the blood flows are expressed as functions of physical activity (oxygen uptake above rest, VOD, in L/minute, linked in turn by an empirical function with workload, W, in watts), unlike the conventional PBPK models in which tissue volumes, blood flows, and Michaelis-Menten kinetic constants can be scaled with allometric adjustments solely to body weight. Compartments for slowly perfused tissues and gastrointestinal tract are not included in this PBTK model, and the liver perfusion is described as a single blood flow, Qh (in L/minute), without splitting into arterial and portal circulations. Free fat mass (in kg) and lean body volume (in L) are expressed in terms of total body water (in L), which in turn is linked, by an empirical function, with body height (in m) and body weight (in kg). Such a model structure precludes allometric scaling of variables.



Symbols of parameters and variables
Qalv - alveolar ventilation (L/min)
Cair-ETBE - concentration of ETBE in ambient air (microM)
Calv-ETBE - concentration of ETBE in alveolar air (microM)
CairTBA - concentration of TBA in ambient air (microM)
CalvTBA - concentration of TBA in alveolar air (microM)
CLiT - clearance of TBA to urine (L/min)
kel - first order excretion rate constant of TBA (1/min)
Qco - Cardiac output (L/min)
Qr - Blood flow to rapidly perfused compartment (L/min)
Qf - Blood flow to fat compartment (L/min)
Qwm - Blood flow to working muscle compartment (L/min)
Qrm - Blood flow to resting muscle compartment (L/min)
Qh - Blood flow to liver compartment (L/min)
CLiE - Intrinsic hepatic clearance of ETBE (L/min)
CLiM - Intrinsic hepatic clearance of TBA (L/min)

Source: Modified from Nihlén and Johansen (1999).

Figure 3-2. Structure of the PBTK model for ETBE and TBA.

While two partition coefficients for ETBE—water:air and blood:air—were measured in vitro (Nihlén et al., 1995), the tissue:blood partitioning was calculated for each tissue based on its water and lipid contents. The metabolism and excretion clearances and elimination rate constant were also estimated individually by fitting the model to the experimental data from eight volunteers, exposed to 5, 25, and 50 ppm of ETBE in air (Nihlén et al., 1998a). The same individual data were used in the PBTK model validation.

Although some limited pharmacokinetic data from rodents exposed to ETBE are available in the literature (Dekant et al., 2001a, b; Borghoff, 1996, unpublished report), the human PBTK model from Nihlén and Johanson (1999) cannot be used for rodents. The structural simplifications of this human PBTK model, single route of exposure, and the same limited data sets used to calibrate and validate the model, limit its potential for application in human health risk assessment. Therefore, at this time, sufficient information is not available to allow interspecies extrapolation of ETBE dosimetry between rodents and humans, or to apply the existing PBPK models for MTBE to the case of ETBE.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS - EPIDEMIOLY, CASE REPORTS, CLINICAL CONTROLS

No epidemiologic studies in humans or case reports of accidental exposure to ETBE have been reported.

4.1.1. Studies in Humans

Nihlén et al. (1998b) exposed eight healthy male volunteers (range 21–41 years, mean body weight 82 kg) to ETBE vapor for 2 hours during light exercise on a bicycle ergometer. The study was carried out 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 by the volunteers. The ETBE vapor was generated at four nominal levels (0, 5, 25, and 50 ppm) in a 20 m^3 exposure chamber with a controlled climate (average temperature 19°C, 43% humidity, 16 air exchanges per hour). Each subject was exposed at least once to each concentration, with a 2-week interval between exposures. Measurements of ocular, nasal, and pulmonary physiological function were conducted prior to exposures, during the exposures, and afterward. In addition, the subjects rated symptoms of irritation, discomfort, and central nervous system (CNS) effects in a questionnaire. Significantly, dose-related ratings of solvent smell (recorded on a 100-mm visual analog scale graded from "not at all" to "almost unbearable"; $p = 0.001$, repeated measures analysis of variance) occurred as volunteers entered the chamber containing ETBE vapor. However, the ratings declined slowly with time during exposure and after the exposure period had been completed. Significantly elevated ratings of discomfort in the throat and airways were reported during and after exposure to 50 ppm ETBE in comparison to exposure to clean air, but ratings at lower concentrations, although somewhat higher than control values, did not differ significantly from control responses. Questions on discomfort in the eyes, fatigue, nausea, dizziness, and intoxication had the highest average ratings at the 50 ppm exposure level. However, no exposure concentration vs. effect correlation was seen, and none of the ratings differed significantly from the clean air ratings. No significant acute effects of ETBE were seen regarding eye redness, measured or reported tear-film breakup time, or conjunctival epithelial damage. Increases in eye-blinking frequency of 10–14 blinks per minute by as much as 50% ($p = 0.01$) were reported. Increased nasal swelling ($p = 0.001$, compared with pre-exposure values) was indicated by a 6–15% decrease in nasal volume using acoustic rhinometry. Analysis of nasal lavage fluid for total cells and markers of inflammation (albumin, lysozyme, eosinophilic cationic protein, myeloperoxidase, interleukin 8) showed some sporadic changes, but these were not related to exposure levels ($p > 0.05$). Slightly impaired pulmonary function (vital capacity -3.2, -3.4%, and forced vital capacity -3.6, -4.4% at 25 and 50 ppm, respectively) was observed compared with values measured 35–50 minute after exposures. Single breath

carbon monoxide diffusing capacity was reduced with borderline significance after exposure to 25 ppm ETBE, but not to 50 ppm. Some individuals reported a "bad taste in their mouth" after exposure to 25 and 50 ppm. Thus, healthy subjects exposed for 2 hours to 25 or 50 ppm ETBE experienced irritation in throat and airways, nasal swelling, a bad taste in the mouth, and slightly impaired lung function. However, the low number of experimental subjects reduced the statistical power of this study. Amberg et al. (2000) and Bernauer et al. (1998) also conducted studies in humans, presented in detail in Chapter 3, but these studies focused on metabolism of ETBE in humans, not on health effects.

Vetrano (1993, unpublished report) evaluated odor and taste thresholds for ETBE (99.0–99.5% purity) and MTBE (99.9% purity). Using six or seven subjects (six women and sometimes a single man), the average calculated detection and recognition threshold values for aerosolized ETBE were determined. Test agent samples (0.6 μL) were vaporized in hydrocarbon-free air in a Tedlar® bag and subsequently diluted; airborne concentrations were confirmed by gas chromatographic analysis. Each airborne sample was presented to each test panel member using a dynamic triangle olfactometer operated according to American Society for Testing and Materials Standard Practice E 679. Each concentration was presented 3 times for evaluation. Variability of panel responses was tabulated in the report, but was not summarized. Both the detection threshold, defined as the minimum airborne concentration at which 50% of the test subjects could differentiate between a test sample and odor free air, and the recognition threshold, defined as the minimum concentration at which 50% of subjects recognized or identified the odorant, were determined. For ETBE, detection and recognition thresholds were 0.013 and 0.024 ppm, respectively, and for MTBE they were 0.053 and 0.08 ppm, respectively. Thus, ETBE was detected at approximately a fourfold lower concentration as an airborne olfactory stimulus than MTBE. In other experiments, odor threshold values for various concentrations of MTBE and ETBE in water were measured. The average odor detection and recognition limits for ETBE in water were determined to be 0.049 and 0.106 ppm, respectively. Average detection and recognition limits for MTBE in water were determined to be 0.095 and 0.193 ppm, respectively; therefore, ETBE was detected at approximately twice as low of a concentration as MTBE in water. Substances with odor thresholds of less than 1 ppm are generally categorized as highly odorous. Finally, taste detection thresholds for ETBE and MTBE in water were measured. The average taste detection threshold values for ETBE and MTBE in drinking water were reported to be 0.047 and 0.134 ppm, respectively. Thus, ETBE was detected at approximately a threefold lower concentration than MTBE. The taste of both oxygenates was described as highly objectionable.

In an investigation conducted on behalf of the American Petroleum Institute, TRC Environmental Corp. (1993) repeated work for MTBE and examined the effects of various oxygenate additions on the odor of gasoline blends using methods identical to those described by Vetrano (1993, unpublished report). Odor detection threshold and odor recognition values for

97% pure MTBE in air were determined to be 0.053 and 0.125 ppm, similar to previously reported values (Vetrano, 1993, unpublished report). Odor detection threshold and odor recognition values for MTBE in water were 0.045 and 0.055 ppm, respectively, considerably less than earlier reported values. The taste threshold for 97% MTBE in water was found to be 0.039 ppm, 3 times lower than the value reported in Vetrano (1993, unpublished report). The taste of MTBE was described as bitter, nauseating, like rubbing alcohol, etc. In addition to studies with ETBE and MTBE alone, the oxygenates MTBE, ETBE, and TAME were added to one or more of three gasoline blends to evaluate their effects on gasoline odor detection and recognition thresholds. When the summer blend of gasoline was mixed with 15% ETBE (99% purity), the average odor detection and recognition threshold values were 0.064 ppm and 0.139 ppm, respectively, a reduction of 89% compared with the odor threshold for the summer blend of gasoline alone.

Durand and Dietrich (2007) evaluated odor threshold and intensity of ETBE standards as part of a study measuring ETBE leaching from a PEX pipe using a utility quick test designed to assess taste, odor, migration, and leaching of materials in water distribution systems. ETBE was observed leaching into tap water with and without the addition of free chlorine or monochloramine using solid phase microextration/GS-MS. Aqueous concentrations of ETBE in the leachate ranged from 23>140 μg/L and decreased with increased flushing. A team of 10 panelists were recruited and trained for several weeks in flavor profile analysis in a research protocol approved according to the standards of the Virginia Tech Institutional Review Board for human subjects. Four to six panel members were present for all tests. ETBE for quantification was purchased from Chem Service, Inc. (purity not reported). Panelists were asked to identify and describe the odor of known concentrations (5–50 μg/L) of ETBE in experimental tap water alone or with 2 mg/L Cl_2 or 4 mg/L NH_2Cl as Cl_2. Panelists reported a chemical or solvent odor and a burning sensation during the flavor profile analysis of ETBE samples that was experienced by most panelists in the absence or presence of chlorine disinfectants. The ability of panel members to detect ETBE was reduced in the presence of chlorinous odor from free chlorine, but not monochloramine. Panelists were able to smell ETBE at a concentration of 5 μg/L, the lowest concentration tested (Durand and Dietrich, 2007).

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Subchronic Studies—Oral

No subchronic studies of oral exposure to ETBE were found in the literature.

4.2.2. Chronic Studies—Oral

As part of series of carcinogenicity studies, Maltoni et al. (1999) carried out chronic exposure studies of petroleum components and additives, including ETBE (BT959). The authors

indicated that the publication was preliminary in nature; however, no further explanation was given to this characterization despite the fact that as of 2008, this was still the only publication of these data. Male and female CRC/RF Sprague-Dawley rats, 60/sex/group, were dosed by gavage with ETBE (purity >94%) at dose levels of 0, 250, and 1,000 mg/kg for 104 weeks. Impurities in the test solution included ethyl alcohol (2.88%), TBA (1.59%), MTBE (1%), 2-ethoxy-butane (0.12%), olefin C8 (0.11%), ter-butyl-isopropyl ether (0.09%), and methyl alcohol (0.01%). ETBE was administered in olive oil 4 days/week (Monday, Tuesday, Thursday, and Friday). The study authors state that administration of a dose of 1,000 mg/kg for 5 or more days/week would not have been tolerated, suggesting that the maximum tolerated dose may have been exceeded under standard dosing regimes. Rats were 8 weeks of age at study initiation; they were weighed and examined for gross lesions weekly for the first 13 weeks, then biweekly for the remainder of the bioassay. No major effects of dosing on food and water intake or body weights were observed.

Starting at week 40, a dose-related increase in mortality for both male and female rats was reported. The authors present two survival curves (Figures 7 and 8 from Maltoni et al., 1999) and state that "there was a dose-correlated increase of [sic] the mortality rate in males starting from the 40th week until the end of the experiment … and in females from the 40th to the 88th week of the biophase." Other than this statement, the authors did not identify a lowest-observed-adverse-effect level (LOAEL) for mortality, did not indicate if the increased mortality was statistically significant, and only presented the data graphically. Attempts to digitize the data from the survival curves support the increased mortality of high-dose females relative to control animals from weeks 56 to 88 by approximately 8–20% with increased survival relative to control females from weeks 104 to 136 by approximately 4–7%. Mortality of the low-dose females was similar to the controls (± 2–3%) with the exception that at 88 weeks of age, mortality was approximately 11% higher than controls and at 120 weeks of age, mortality was approximately 5% lower than controls. Mortality in the high dose males was approximately 8–30% higher than controls from weeks 56 to 120, whereas the low-dose males exhibited higher mortality by approximately 7–17% from weeks 56 to 88 and were similar to controls at other time points. Using the digitized numbers from the survival curves in Maltoni et al. (1999), ETBE-exposure is associated with increased mortality relative to controls in animals of both sexes 56 to 88 weeks of age and increased survival relative to controls in females ≥104 weeks of age. At 56 weeks of age, 250 mg/kg is the LOAEL in males for a 7% increase in mortality and 1,000 mg/kg is the LOAEL in females for an 8% increase in mortality. At 104 weeks in age, 1,000 mg/kg is the LOAEL in males for a 13% increase in mortality and ETBE-exposure in females is associated with decreased mortality. The treated-related effects on mortality are relative to considerable mortality in control animals. Less than approximately 30% of control rats of either sex remained alive by 104 weeks of age. No explanation was provided for the low survival rate displayed among control animals in the study. Historical control data on mortality

were not provided by the authors. Average mortality data observed in gavage studies in female Sprague-Dawley rats are available from the National Toxicology Program (NTP, 2005) and are provided here for purposes of comparison. Survival of female rats in the NTP studies treated with corn-oil gavage averaged 42% by 104 weeks (range 28–47%), suggesting that the animals from the Maltoni et al. (1999) study with olive oil gavage were at the low end of survival based on the limited NTP data set. Rats in the Maltoni et al. (1999) study were allowed to live out their natural lives or until week 137, when the study was terminated. Upon death, rats were necropsied and tissue/organs were taken for microscopic examination.

The incidence and multiplicity of tumors were determined in all treatment groups (Table 4-1). The data were presented according to dose group, tumor site, and histiotype. In addition, the data were tabulated as incidence and number of benign tumors/treatment group and incidence and number of malignant tumors/treatment group. Some grouped totals included only tumors (e.g., total malignant tumors of the uterus), whereas others included precancers and tumors (e.g., defined by the authors as pathologies of oncological interest of the mouth epithelium and forestomach included acanthomas, dysplasias, and carcinomas). Total benign and malignant tumor incidences at both doses were comparable with the control group in both sexes. The total number of malignant tumors per 100 animals was significantly greater in female rats dosed with 250 mg/kg-day ETBE (55 per 100) than the total number of malignant tumors per 100 animals in female control rats (25 per 100; $p < 0.05$).

Table 4-1. Tumor incidences resulting from 2-year gavage exposure of Sprague-Dawley rats to ETBE

Tumor formation	Administered dose (mg/kg-day)					
	0	250	1,000	0	250	1,000
	Males			Females		
Benign (total)	40/60	40/60	32/60	50/60	53/60	49/60
Malignant (total)	11/60	14/60	14/60	9/60	21/60	19/60
Mouth epithelium (total)	6/60	14/60	15/60a	14/60	16/60	18/60
acanthomas	1/60	0/60	2/60	1/60	2/60	3/60
squamous cell dysplasias	5/60	14/60	11/60	11/60	11/60	12/60
squamous cell dysplasias with in situ carcinoma	0/60	0/60	1/60	2/60	1/60	2/60
squamous cell carcinomas	0/60	0/60	1/60	0/60	2/60	1/60
Forestomach (total)	13/60	24/60	13/60	12/60	10/60	11/60
acanthomas	5/60	7/60	4/60	5/60	3/60	6/60
squamous cell dysplasias	8/60	14/60	9/60	7/60	4/60	5/60
squamous cell carcinomas	0/60	3/60	0/60	0/60	3/60	0/60
Uterus (total malignant)				2/60	10/60[a,b]	2/60
carcinomas				1/60	2/60	0/60
sarcomas				1/60	8/60[c]	2/60
Hemolymphoreticular (total)	3/60	8/60	6/60	3/60	6/60	5/60
lymphoblastic lymphoma	0/60	1/60	0/60	0/60	1/60	0/60
lymphocytic lymphoma	0/60	0/60	0/60	0/60	1/60	0/60
lymphoimmunoblastic lymphoma	2/60	4/60	5/60	1/60	3/60	2/60
histiocytic sarcoma	1/60	1/60	1/60	2/60	0/60	2/60
myeloid leukaemia	0/60	2/60	0/60	0/60	1/60	0/60

[a]Statistically significant ($p < 0.05$), as calculated by the authors.
[b]Not statistically significant by $\chi 2$ test (the same test used by the authors on the grouped data) when the vaginal schwannomas are removed from the "uterus".
[c]Identified as including four malignant schwannomas of the uterus-vagina

Source: Maltoni et al. (1999).

Two significantly increased tumor types or combined tumors and precancers were reported. In each case, only the total tumors or combined tumors and precancers were significantly increased and there was no significant increase in individual histiotypes for tumors or precancers. In the first example, the incidence of total pathologies of oncological interest, which includes tumors and precancers (i.e., acanthomas, squamous cell dysplasia, squamous cell dysplasia borderline with carcinoma in situ, and squamous cell carcinoma of oral cavity, tongue, and lips) was significantly increased in male rats of the 1,000 mg/kg group (25 vs. 10% in the male control group; $p < 0.05$). In the second example, total malignant tumors of the uterus (carcinomas and sarcomas) were noted in 250 mg/kg females (16.7% incidence, $p < 0.05$ that

appears to also include vaginal tumors), but uterine malignancies were diagnosed in only 3.3% of females in the 1,000 mg/kg group and in 3.3% of females in the control group.

Two examples of nonstatistically increased tumors were also reported. There was a nonsignificant increase in the incidence of total pathologies of oncological interest in the forestomach for males in the 250 mg/kg-day group (24/60) but not females (10/60) or animals of either sex at the high dose (13/60 male and 11/60 female), which is similar to controls (13/60 males and 12/60 females). In addition, hemolymphoreticular neoplasias (lymphomas, sarcomas, and leukemias) and, in particular, lymphoimmunoblastic lymphomas (8.3% incidence in the 1,000 mg/kg males), were increased overall in the male (3/60, 8/60, and 6/60 at 0, 250, and 1,000 mg/kg-day respectively) and female (3/60, 6/60, and 5/60) treatment groups, but none of the increases noted were statistically significant. The authors attributed the lack of adequate dose-response to the relatively high mortality in the treatment groups. However, a survival analyses was not presented, nor were data provided on tumor incidence for individual animals by week or month that would allow for a time-to-tumor analysis. A summary of total tumor and precancer incidences is given in Table 4-1.

The number of acanthomas of the mouth epithelium in both males (2/60) and females (3/60) of the 1,000 mg/kg group was about twice that in corresponding control rats (1/60). Squamous cell carcinoma of the forestomach occurred in 5% of both male and female rats in the 250 mg/kg group (not statistically significant), but no squamous cell carcinomas of the forestomach were found in either sex of the control group or in the 1,000 mg/kg treatment group.

The authors identified three limitations of the study, including the use of only two dose groups and a single animal species and increased mortality of the animals exposed to ETBE. However, the authors concluded that in spite of the study limitations, the study results show a statistically significant increased incidence of total pathologies of oncological interest of the mouth epithelium in males and total malignant tumors of the uterus in females and total malignant tumors (which were only increased in females at the low dose when tabulated as the number of tumors per 100 animals), and a nonsignificant increase in total pathologies of oncological interest of the forestomach in males and of hemolymphoreticular neoplasias.

4.2.3. Subchronic Studies—Inhalation

4.2.3.1. *Subchronic Inhalation Studies—Rats*

In subchronic inhalation experiments, ETBE was administered as a vapor at target chamber concentrations of 0 (filtered air control), 500, 2,000, and 4,000 ppm (mean analytical concentrations: 0, 501, 2,090, and 3,910 ppm) to groups of Sprague-Dawley rats. The rats (10/sex/group and 9 weeks of age at initial exposure) were exposed for 6 hours/day, 5 days/week, for 4 weeks (published as White et al., 1995; IIT Research Institute, 1991, unpublished report). The rats were observed daily, and salivation and redness around the nose/mouth/face were occasionally reported for test animals during exposures. A functional

observation battery (FOB) was administered 1 week prior to the exposures and about 60 minutes after 1, 5, or 20 exposures to evaluate neuromuscular function and sensory perception (described in detail in Section 4.4.1). Ataxia and sedation, which are overt signs of CNS depression, were seen following exposure termination in the 4,000 ppm group, but ETBE-exposed rats appeared normal within 15 minutes of the end of exposure. Mean body temperature was reduced 2.00–2.14% in 4,000 ppm males after the fifth exposure, and a trend for increased hind limb splay in both sexes of the high concentration group occurred. These effects were described by the authors as being associated with transient CNS depression. No other indications of CNS depression or neurotoxicity were detected. No premature mortality occurred, and no statistically significant effect of treatments on weekly body weights was observed. Necropsy was performed 18 hours after the final exposure to ETBE, at which time blood for serum chemistry and hematology was taken, and the following tissues were weighed and prepared for histological examination: brain, adrenal glands, gonads, heart, kidneys, liver, lungs, and spleen. Approximately 31 additional tissues were also collected and prepared for histological comparison between the high-dose group (4,000 ppm) and controls. At termination, no significant effects of ETBE exposure were seen in serum chemistry (liver function enzymes [creatine kinase, alanine aminotransferase, asparatate aminotransferase, and alkaline phosphatase], electrolytes [sodium, potassium, and chloride], glucose, triglycerides, cholesterol, creatinine, blood urea nitrogen, total serum protein, and albumin) or on hematology evaluations (red cell count, hemoglobin, mean corpuscular volume [MCV], total and differential leukocyte counts, and platelet count). The only exception was a significant increase in white blood cell count (leukocytes) in females exposed to 2,000 and 4,000 ppm ETBE. This finding was noted to be of questionable toxicological significance because it was not accompanied by changes in histopathology. In rats exposed to 4,000 ppm, absolute and relative liver weights in males at termination were increased 16.8 and 16.1%, respectively; absolute and relative liver weights in females were increased 9.5 and 12.5%, respectively. Relative liver weights were also increased 10% in female rats exposed to 2,000 ppm ETBE. In addition, absolute kidney and adrenal weights were increased 12.8 and 13.7%, respectively, in male rats exposed to 4,000 ppm of ETBE. No observations attributed to ETBE exposures were recorded at necropsy or upon histological examination of any tissues from the high-dose animals, including gonads, adrenal glands, kidneys, and liver.

Subchronic 13-week ETBE inhalation studies using both rats and mice were conducted by the Chemical Industry Institute of Toxicology (Medinsky et al., 2006 [erratum]; Medinsky et al., 1999; Bond et al., 1996a, b, unpublished reports). Male and female F344 rats (6.5 weeks old) and male and female CD-1 mice (7.5 weeks old; described in Section 4.2.3.2 below) were exposed in whole-body chambers to 0 (control), 500, 1,750, or 5,000 ppm ETBE (97.5% pure) for 6 hours/day, 5 days/week, for 13 weeks. For each exposure level group of F344 rats, the total number of 48 rats/sex was subdivided into a series of subgroups: a basic core subgroup

(11 rats/sex), a neurotoxicology subgroup (12 rats/sex), an interim clinical pathology (chemistry and hematology) subgroup (10 rats/sex), and a cell replication subgroup (15 rats/sex).

Special attention was given to the assessment of effects in rat kidneys and mouse livers, including serum enzyme assays, on the basis of effects previously noted in chronic oncogenicity studies with MTBE (reviewed in Ahmed, 2001; Cal EPA, 1999; Mennear, 1997). In addition, studies of cell turnover and induced cell proliferation were evaluated in kidneys from male and female rats and livers from male and female mice (five animals/sex/group), using 5-bromo-2'-deoxyuridine (BrdU) labeling after 1, 4, and 13 weeks of exposure. A wide range of clinical chemistry and hematological parameters was evaluated after 6 and 13 weeks in rats, but only after 13 weeks in mice. At termination, a broad suite of tissues and organs was examined in control and high-concentration animals; potential target organs (lungs, liver, kidneys) and gross lesions were examined in all groups. Mallory-Heidenhain staining for possible accumulation of hyaline droplets in renal tubules, and immunohistochemical staining for renal tubular $alpha_{2u}$-globulin were conducted on thin sections of rat kidneys. In addition, testicular seminiferous tubules of male rats from all treatment groups were analyzed for degenerative changes. Significant findings in rats that are related to ETBE exposure are summarized in Table 4-2 (see Table 4-6 in Section 4.2.3.2 for the summary of significant findings in mice).

Table 4-2. Summary of significant results from the 13-week subchronic ETBE inhalation study in F344 rats

Endpoint	Measurement frequency	Observations
Basic core subgroup[a]		
Mortality	Twice daily	No exposure-related mortality
Growth rate	Weekly	Females: increased, 5,000 ppm only
Clinical signs	Weekly	Males: ataxia postexposure, 5,000 ppm
Organ weights	Study termination	Males: increased kidney, liver weights at 1,750, 5,000 ppm; increased adrenal weights at 5,000 ppm Females: increased adrenal, liver weights at 5,000 ppm; increased kidney weights at 1,750, 5,000 ppm; increased heart weights at 500, 5,000 ppm
Gross pathology	Study termination	Not exposure-related
Histopathology	Study termination	Males: renal effects at 500, 1,750, 5,000 ppm; increased percentage of seminiferous tubules with degeneration of spermatocytes at 1,750, 5,000 ppm Females: bone marrow congestion at 1,750, 5,000 ppm
Clinical pathology subgroup[a]		
Hematology	Interim (6 wks)	Males: increased platelets and decreased mean corpuscular hemoglobin concentration (MCHC) and increased MCV at 5,000 ppm Females: decreased white blood cells and increased[b] MCV at 5,000 ppm
	Study termination	Males: decreased MCHC at 1,750, 5,000 ppm; increased platelets at 5,000 ppm Females: increased MCV at 1,750, 5,000 ppm
Clinical chemistry	Interim (6 wks)	Males: increased creatinine, total protein and albumin; decreased chloride and sodium at 5,000 ppm Females: decreased bilirubin and phosphorus at 5,000 ppm
	Study termination	Males: decreased chloride at 1,750, 5,000 ppm; increased total protein 5,000 ppm Females: decreased bilirubin at 500 ppm
Cell replication subgroup[c]		
Renal labeling index (LI)	Wks 1, 4, and 13	Males: increased LI wks 1, 4, 13 at 5,000 ppm; 13 wks at 500, 1,750 ppm Females: increased LI wk 1 at 500, 1,750, 5,000 ppm; wk 4 at 5,000 ppm; no effect at 13 wks
Neurotoxicology subgroup[b,d]		
FOB, neuropathology	18 hrs after exposure days 1, 6, 10, 20, 42, 65	No significant findings

[a]10 animals/sex/exposure group
[b]MCV is listed as decreased in table 1 from Medinsky et al. (1999), but increased in the text. Note: Table 1 from Medinsky et al. (1999) contain errors that can be resolved by reading text and referring to Bond et al. (1996a, unpublished report).
[c]15 animals/sex/exposure group
[d]12 animals/sex/exposure group

Sources: Medinsky et al. (2006, 1999); Dorman et al. (1997); Bond et al. (1996a, unpublished report).

Mean analytical concentrations for the ETBE exposures were 0, 505 ± 13, 1,748 ± 59, and 4,971 ± 155 ppm for rats. Transient ataxia (lasting <1 hour) was sometimes observed in male rats exposed to 5,000 ppm shortly following daily exposure, and decreases (~25%) in body weight gain were observed during the first week of exposure in male and female rats exposed to 1,750 and 5,000 ppm ETBE. At termination, body weights of female rats in the 5,000 ppm group were significantly higher than controls, but body weights of other groups, both male and female, did not differ significantly from those of controls. Significant increases in absolute mean kidney and liver weights occurred in male rats exposed to 1,750 and 5,000 ppm ETBE compared to controls (9.8 and 19.3% increases for kidneys; 14.2 and 32.4% increases for liver), and mean adrenal weights were significantly increased 34.3% in male rats at 5,000 ppm. Significantly increased absolute weights of kidney (21.3%), adrenal (17.8%), and liver (25.8%) were also noted in female rats exposed to 5,000 ppm ETBE, and increased kidney weight (12.2%) was noted in female rats exposed to 1,750 ppm ETBE. Increased heart weight was found in female rats exposed to 500 ppm (10.1%) and 5,000 ppm (12.3%) ETBE, but not 1,750 ppm. No significant findings were noted in the histopathology of adrenal glands or livers of high-dose animals.

Slight but statistically significant increases in various clinical chemistry parameters (Table 4-2) were seen, but these effects were reported to be of uncertain toxicological significance. At both the interim and 13-week time points, 3–6% decreases in levels of serum chloride and 9–11% increases in total protein were observed in male rats exposed to 5,000 ppm ETBE. No consistent changes in clinical chemistry parameters compared to controls were seen in female rats exposed to ETBE. The changes seen in peripheral hematology parameters (Table 4-2) were not considered to be clinically significant by the authors. There was a 1% increase[1] in the MCV in female rats exposed to 5,000 ppm ETBE (58.33 ± 0.31 compared to 57.62 ± 0.40 in controls at 6 weeks) at the interim sampling point and to females exposed to both 1,750 (57.97 ± 0.49) and 5,000 ppm (58.11 ± 0.60 compared to 57.26 ± 0.73 in controls) at the 13-week time point. There was a 4% decrease in mean corpuscular hemoglobin concentration (MCHC) in male rats exposed to 5,000 ppm (35.13 ± 0.38 compared to 36.46 ± 1.09 in controls) ETBE at the interim sampling point and a 2.5% decrease in males exposed to both 1,750 (35.00 ± 0.30) and 5,000 ppm (35.01 ± 0.65 compared to 35.91 ± 0.67 in controls) at the 13-week time point.

Kidneys and testes of males and femoral bone marrow of females were the only tissues with significant histological findings in ETBE-exposed rats. Renal effects occurred in male rats exposed to ETBE and consisted of a higher incidence and mean number of regenerative foci and increased hyaline droplet severity in all three ETBE exposure groups (Tables 4-3 and 4-4).

[1] Note: Table 1 from Medinsky et al. (1999) contains discrepancies that are not consistent with the text (e.g., MCV is listed as decreased in female rats at 6 weeks in the table and increased in the text). The text is consistent with the unpublished study results and data tables from the unpublished report (Bond et al., 1996a, unpublished report).

Regenerative foci are associated with repair of damaged tubules. Therefore, the presence of regenerative foci represents indirect evidence of necrosis, as some cells of the proximal tubule would have had to have died and the remaining cells undergone regenerative proliferation for regenerative foci to be observed. The increased incidence and mean number of regenerative foci was exposure- and time-related, but the appearance of hyaline droplets in kidney sections stained with Mallory-Heidenhain stain was only exposure-related. No regenerative foci were observed in any treatment group after 1 week of exposure, or in control or 500 ppm treatment groups after 4 weeks of exposure. The mean number of regenerative foci was 2 and 4 in the 1,750 and 5,000 ppm dose groups, respectively, after 4 weeks of exposure. After 13 weeks of exposure there was a dose-response relationship in the mean number of regenerative foci observed with 2, 11, 17, and 34 foci for 0, 500, 1,750, and 5,000 ppm ETBE respectively. Although Medinsky et al. (1999) presented hyaline droplet severity (expressed as a mean grade scored with 1 for minimal, 2 for less than 10%, 3 for 10–25%, 4 for 25–50%, and 5 for greater than 50% of the cortex involved) as concentration-dependent (Table 4-4), there were no statistics presented. Alpha$_{2u}$-globulin immunoreactivity was observed in the hyaline droplets of the renal proximal tubular epithelium of male rats from all exposure groups. Medinsky et al. (1999) stated that accumulation of alpha$_{2u}$-globulin-containing droplets was directly related to treatment-dependent increases in renal effects and the cell proliferation or labeling index (LI). In male rats exposed to ETBE, after 1 or 4 weeks of exposure, a greater than twofold increase in the LI occurred at the high dose only (Table 4-5). After 13 weeks of exposure, there was a greater than twofold increase in the LI for all doses of ETBE relative to control, but there were no differences in LI between ETBE doses. The authors characterized the differences in LI as time- and ETBE-concentration-dependent (Medinsky et al., 2006; Medinsky et al., 1999[2]; Bond et al., 1996a, unpublished report). In female rats exposed to ETBE, after 1 week of exposure, a statistically significant, but less than twofold increase in the LI occurred at the 500, 1,750, and 5,000 ppm levels and after exposure to 5,000 ppm for 4 weeks (Medinsky et al., 2006; Medinsky et al., 1999; Bond et al., 1996a, unpublished report). No effect on the renal LI in female rats was seen at any exposure concentration after 13 weeks of exposure to ETBE. The higher LI in control rats at weeks 1 and 4 was characterized as growth-related, and the lower LI at the end of the study was considered typical of a mature kidney. Nephropathy was not observed in female rats exposed to ETBE, consistent with the absence of alpha$_{2u}$-globulin immunoreactivity in F344 females. The increased cell replication, hyaline droplet accumulation, and presence of alpha$_{2u}$-globulin immunoreactivity in males led the authors to conclude that the observed renal effects were due to an alpha$_{2u}$-globulin mode of action and, therefore, were not relevant for humans exposed to ETBE through inhalation. An evaluation of human relevance of the alpha$_{2u}$-

[2] Note: Tables 1 and 3 from Medinsky et al. (1999) contain errors (e.g., data from males and females are reversed) and the reversed data from males and females were resolved in a published erratum (Medinsky et al., 2006).

globulin accumulation as discussed in the Risk Assessment Forum Technical Panel Report (U.S. EPA, 1991b) is discussed in Section 4.5.3.

Table 4-3. Incidence of lesions in kidney, seminiferous tubules, and bone marrow of F344 rats from 13-week subchronic ETBE inhalation study

Sex	Tissue	Exposure group (ppm)			
		Control	500	1,750	5,000
Male	***Kidney***				
	Unaffected	7/11 (64%)	1/11 (9%)	0/11 (0%)	0/11 (0%)
	Kidneys with regenerative foci	4/11 (36%)	10/11[a] (91%)	11/11[a] (100%)	11/11[a] (100%)
	Testes				
	Unaffected	0/11 (0%)	0/11 (0%)	0/11 (0%)	1/10 (10%)
	Testes with degenerated spermatocytes	11/11 (100%)	11/11 (100%)	11/11 (100%)	10/11 (91%)
	Seminiferous tubules with some degenerated spermatocytes[b]	2.1%	2.4%	7.8%[a]	12.7%[a]
	Testes with sloughed epithelium	7/11 (64%)	3/11 (27%)	3/11 (27%)	7/11 (64%)
	Seminiferous tubules with lumenal debris[b]	2.1%	0.7%	2.8%	1.0%
Female	***Kidney***				
	Unaffected	10/10 (100%)	11/11 (100%)	11/11 (100%)	11/11 (100%)
	Femoral bone marrow				
	Unaffected	5/10 (50%)	8/11 (73%)	4/11 (36%)	0/11 (0%)
	Congestion	0/10 (0%)	0/11 (0%)	5/11[a] (45%)	11/11[a] (100%)
	Necrosis	5/10 (50%)	3/11 (27%)	3/11 (27%)	2/11 (18%)

[a]Significantly different to control ($p < 0.05$).
[b]The incidence of tubules with spermatocyte degeneration or lumenal debris was quantified by counting the number of affected tubules out of 100 total tubules in a cross-section of testes from each rat.

Sources: Medinsky et al. (1999); Bond et al. (1996a, unpublished report).

Table 4-4. Mean number of regenerative foci and hyaline droplet severity in kidneys from male F344 rats exposed to ETBE in a 13-week subchronic inhalation study

Exposure Group	Regenerative foci[a]			Hyaline droplet severity grade[b]		
	1 wk	4 wks	13 wks	1 wk	4 wks	13 wks
Control	0	0	2	1.2	1.8	1.8
500 ppm ETBE	0	0	11	3.4	2.6	3.0
1,750 ppm ETBE	0	2	17	4.0	3.4	3.2
5,000 ppm ETBE	0	4	34	4.6	3.8	3.8

[a]Mean number of regenerative foci per two kidney sections/rat.
[b]Data expressed as mean grade using the following Mallory Heidenhain scoring scale: 1 for minimal, 2 for <10% of the cortex involved, 3 for 10–25% of the cortex involved, 4 for 25–50% of the cortex involved, and 5 for >50% of the cortex involved.

Sources: Medinsky et al. (1999); Bond et al. (1996a, unpublished report).

Table 4-5. Cell division or LI in proximal tubule cells from male and female F344 rats exposed to ETBE in a 13-week subchronic inhalation study

Exposure Group	LI (%) in males			LI (%) in females		
	1 wk	4 wks	13 wks	1 wk	4 wks	13 wks
Control	3.52	3.27	0.91	2.65	1.38	0.59
500 ppm ETBE	4.90	4.04	2.16[b]	4.25[a]	1.42	1.02
1,750 ppm ETBE	4.34	2.80	3.40[c]	4.97[a]	1.59	0.97
5,000 ppm ETBE	7.12[b]	8.98[c]	2.47[c]	3.96[a]	1.81[a]	0.87

[a]Difference from control at $p < 0.05$.
[b]$p < 0.01$
[c]$p < 0.001$

Sources: Medinsky et al. (2006, 1999); Bond et al. (1996a, unpublished report).

A treatment-related increase in the incidence of congestion in the bone marrow of female rats was observed (Table 4-3), but, in spite of the congestion, the hematopoietic cell population of bone marrow appeared to be unaffected. No additional description of the observation was provided by the authors. The presence of bone marrow congestion in the absence of hematopoietic changes was considered insignificant and of no clinical relevance by the authors.

Degenerated spermatocytes were found in the testes of rats from all treatment groups, including controls. Male rats exposed to ETBE displayed a dose-related increase in the mean percentage of testicular seminiferous tubules with spermatocytes that displayed signs of degeneration (Table 4-3). Significant increases occurred in rats exposed to 1,750 and 5,000 ppm, with seminiferous tubules containing degenerated spermatocytes observed at 7.8 and 12.7%, respectively, relative to 2.1% in controls. The occurrence of debris in the lumen of seminiferous

tubules was not affected by ETBE treatment. A no-observed-adverse-effect level (NOAEL) of 500 ppm was suggested by the authors on the basis of the dose-response data for the percent of seminiferous tubules with degenerated spermatocytes.

4.2.3.2. *Subchronic Inhalation Studies—Mice*

Subchronic 13-week ETBE inhalation studies using both rats and mice were conducted by the Chemical Industry Institute of Toxicology (Medinsky et al., 2006 [erratum]; Medinsky et al., 1999; Bond et al., 1996a, b, unpublished reports). Male and female F344 rats (6.5 weeks old, described above in Section 4.2.3.1) and male and female CD-1 mice (7.5 weeks old) were exposed in whole-body chambers to 0 (control), 500, 1,750, or 5,000 ppm ETBE (97.5% pure) for 6 hours/day, 5 days/week, for 13 weeks. Each exposure group of 40 mice/sex was subdivided into a basic core and clinical chemistry subgroup (15 mice/sex), a hematology subgroup (10 mice/sex), and a cell replication subgroup (15 mice/sex).

Mean analytical concentrations for the ETBE exposures to CD-1 mice were 0, 501 ± 14, 1,754 ± 50, and 4,962 ± 140 ppm (Medinsky et al., 1999; Bond et al., 1996b, unpublished report). In male and female mice, transient ataxia was sometimes observed shortly after daily exposure in 5,000 ppm groups. No ETBE-related alterations in body weights were observed in male or female mice. However, for both male and female mice, significant increases in absolute liver weights occurred in the 1,750 and 5,000 ppm groups (13 and 18% for males; 19 and 33% for females) compared with controls. Other organ weights were comparable to control values. A dose-related increase in the LI was seen in the livers of male mice exposed to 1,750 and 5,000 ppm after 1 and 4 weeks of exposure, but the LI had returned to control values at 13 weeks. In female mice, a similar dose-related increase was seen after exposure to ETBE for 1 week and 13 weeks to 1,750 ppm and 5,000 ppm; however, effects on liver LI were not significant at 4 weeks. The increases in the LI were thought to be consistent with a mitogenic response of the liver to ETBE.

A statistically significant increase in serum total protein (15%) was observed in male mice exposed to 5,000 ppm ETBE. Statistically significant increases in serum albumin levels (6%) and total protein (12%) were noted in female mice exposed to 5,000 ppm ETBE as well. The only significant histopathological lesion in exposed mice was centrilobular hypertrophy in livers of both male (8/10) and female (9/14) mice exposed to 5,000 ppm ETBE for 13 weeks. The incidences of centrilobular hypertrophy in males (2/15) and females (1/15) exposed to 1,750 ppm, or in males (0/15) and females (2/15) exposed to 500 ppm ETBE, were not statistically significant. Minimal hepatocellular necrosis was occasionally reported in all groups, including controls, and was not exposure-related. A synopsis of results is provided in Table 4-6. Bond et al. (1996b, unpublished report) suggested a NOAEL of 500 ppm in mice.

4.2.4. Chronic Studies—Inhalation

No chronic studies by the inhalation route were found in the literature.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Berger and Horner (2003) assessed in vivo effects of gasoline oxygenates on gamete quality. Female Simonson rats (a Sprague-Dawley-derived strain), 3–4 rats per test agent, were treated with 0.3% ETBE, MTBE, TAME, or MPD, a metabolite of MTBE and ETBE, in drinking water for 2 weeks. Oocytes from the rats were then recovered after injection of donors with pregnant mare serum gonadotropin and human chorionic gonadotropin to stimulate ovulation. Sperm obtained from untreated males was used to fertilize the oocytes in vitro. The percentages of oocytes fertilized and number of sperm heads and attached sperm per oocyte were counted using a fluorescent microscope after 20 hours of incubation at 37°C with 5×10^3 sperm in 120 μL medium. The fertilization efficiency of oocytes from treated females was compared with that of oocytes from untreated control rats (n = 6). There were no effects of treatment on the percentage of females ovulating or the number of oocytes per ovulating female. The authors noted that oocytes from females treated with MPD tended to be more fragile during removal of the zona pellucida (45% of oocytes were intact compared to 57% for controls). No significant differences in the mean percentage of oocytes fertilized were observed (84, 82, 80, and 78%, respectively) for control, ETBE-, MTBE-, and MPD-treated rats. A significant reduction to 65% fertilization efficacy occurred in oocytes from TAME-exposed females. The mean number of penetrated sperm per oocyte for each of the treatment groups was comparable with the mean value for oocytes from untreated control rats. The data indicate that ETBE and MTBE did not affect female gamete quality.

The potential effects of ETBE on reproduction, the male and female reproductive systems in general and a specific examination of the potential effects of ETBE on spermatogenesis were assessed in F344 and Sprague-Dawley rats (CIT, 2003, unpublished report). The report states that it was conducted under GLP. Five groups of male F344 rats (12/dose group) were administered daily oral (gavage) doses of vehicle alone (corn oil, 4 mL/kg-day), or ETBE at 50, 250, 500, or 1000 mg/kg-day in corn oil for 12 weeks. Five groups of male (12/dose group) Sprague-Dawley rats were administered daily oral (gavage) doses of vehicle alone (corn oil, 4 mL/kg-day), or ETBE at 50, 250, 500, or 1000 mg/kg-day in corn oil for 12 weeks (10 pre-mating and 2 weeks during mating). Five groups of female (24/dose group) Sprague-Dawley rats were administered daily gavage doses of vehicle alone (corn oil, 4 mL/kg-day), or ETBE at 50, 250, 500, or 1,000 mg/kg-day in corn oil during a 2-week premating period, a 2 week mating period, and pregnancy through gestation day (GD) 19 or until the end of lactation (postnatal day [PND] 21). In-life clinical signs and mortality were checked daily and body weight and food consumption were monitored at scheduled intervals for both rat strains. The mating index, pre-coital time, and fertility index were calculated and the males were euthanized at the end of the mating period. Male rats of each strain were subjected to a macroscopic examination. The testes

Table 4-6. Summary of significant results from the 13-week subchronic ETBE inhalation study in CD-1 mice

Endpoint	Measurement frequency	Observations
Basic core subgroup [a]		
Mortality	Twice daily	No exposure-related mortality
Growth rate	Weekly	No changes
Clinical signs	Weekly	Males and females: ataxia postexposure, 5,000 ppm
Organ weights	Study termination	Males and females: increased liver weights at 1,750, 5,000 ppm
Gross pathology	Study termination	Not exposure-related
Histopathology	Study termination	Males and females: centrilobular hypertrophy at 5,000 ppm (53% males, 60% females, 0% controls)
Clinical pathology subgroup [b]		
Hematology	Interim (6 wks)	No changes
	Study termination	Males: increased hemoglobin and hematocrit at 1,750 ppm; females: no effect
Clinical chemistry	Interim (6 wks)	No changes
	Study termination	Males: increased total protein at 5,000 ppm; decreased chloride at 500, 1,750 ppm Females: increased total protein and albumin at 5,000 ppm; increased blood urea nitrogen at 1,750 ppm
Cell replication subgroup [c]		
Hepatic LI	Wks 1, 4, and 13	Males: increased LI, wks 1, 4 at 1,750, 5,000 ppm Females: increased LI, wks 1, 13 at 1,750, 5,000 ppm

[a]15 animals/sex/exposure group.
[b]10 animals/sex/exposure group.
[c]15 animals/sex/exposure group.

Sources: Medinsky et al. (1999); Bond et al. (1996b, unpublished report).

and epididymides were weighed separately and sperm were sampled from one testis and one epididymis for evaluation of spermatozoa count and viability. One testis and one epididymis from F344 and Sprague-Dawley males of the control and high dose (1,000 mg/kg-day) groups were fixed in Bouin's fluid and examined histopathologically. Half of the females were euthanized on GD 20 and subjected to gross pathological examination. The fetuses were removed by hysterectomy, and the following parameters were recorded: weight of gravid uterus, number of corpora lutea, implantation sites, early and late resorptions, and dead and live fetuses. The fetuses were weighed, sexed, and submitted to external examination. The remaining females were allowed to deliver normally and gestational duration was calculated. The litters were observed for clinical signs daily during the lactational period and body weight and sex ratio were recorded.

No changes in body weight were observed in males of either strain at any dose of ETBE. Increased food consumption was noted in F344 males from week 4 to week 12 of 10–18% and in Sprague-Dawley males in weeks 4 and 5 of 8–12%, food consumption was not different for either strain when calculated over the entire treatment period. Minor changes in body weight gain were noted at all doses during the pre-mating period; these changes were not dose-related and were therefore considered unrelated to treatment by the authors. No difference in body weight or weight gain was noted at 50 or 250 mg/kg-day ETBE in dams during pregnancy. A nonsignificant decrease (6%, $p > 0.05$) in body weight gain was recorded for pregnant females exposed to 500 mg/kg-day ETBE and an 11% ($p < 0.05$) decrease in body weight gain was noted in females exposed to 1,000 mg/kg-day. No changes were noted in body weight or weight gain in females at any dose during the lactation period. No changes in food consumption were observed at any dose for females during any period.

Excessive salivation (ptyalism) was noted in male rats of both strains at the higher doses as follows (F344 males at 50 [1/12], 250 [1/12], 500 [1/12], and 1,000 mg/kg-day [6/12]; Sprague-Dawley males at 250 [1/12], 500 [1/12], and 1,000 mg/kg-day [10/12]). Ptyalism was also observed in Sprague-Dawley female rats at the higher doses, and data were collected separately by reproductive time-period as follows (pre-mating at 1,000 mg/kg-day only [3/12]; pregnancy at 500 [1/12] and 1,000 mg/kg-day [8/12]; and lactation at 500 [1/12] and 1,000 mg/kg-day [6/12]). The study authors stated that ptyalism was not observed every treatment day, but specific data on frequency of symptoms were not provided. The study concludes that there were no effects of ETBE treatment at doses up to 1,000 mg/kg-day on gonadal function, mating behavior, fertility, embryo-fetal development, or parturition and, therefore, reports a no-observed-effect level (NOEL) of 1,000 mg/kg-day for parental and fetal toxicity in F344 rats and Sprague-Dawley rats. The authors also report a NOEL of 500 mg/kg-day for maternal toxicity based on the lower body weight gain in Sprague-Dawley dams observed at 1,000 mg/kg-day during pregnancy.

The potential effects of ETBE on pregnancy and embryo-fetal development were assessed in Sprague-Dawley rats (CIT, 2004a, unpublished report). As described below, because endpoints from the CIT (2004a, b, unpublished reports) studies were considered for benchmark dose (BMD) modeling and the derivation of an RfD, the study reports were externally peer-reviewed by EPA in November 2008. The study was conducted under EPA's testing guidelines (OPPTS 870.3700; U.S. EPA, 1998c). Female rats (24/dose group) that had been impregnated by unexposed males were administered daily gavage doses of vehicle alone (corn oil, 4 mL/kg-day), or ETBE at 250, 500, or 1,000 mg/kg-day in corn oil on GDs 5–19. Scheduled hysterectomy and necropsy were performed on GD 20. In-life clinical signs and mortality were checked daily. On GD 20, the dams were euthanized and subjected to gross pathological examination. The fetuses were removed by hysterectomy, and the following parameters were recorded: weight of gravid uterus, number of corpora lutea, implantation sites, early and late resorptions, and dead and live fetuses. The fetuses were weighed, sexed, and submitted to external examination. Half of the fetuses from each treatment group were fixed in Harrison's fluid and subjected to a detailed, serial-section examination of soft tissues, while the remainder underwent a detailed skeletal examination following staining of bone with alizarin red and cartilage with alcian blue.

Significantly lower maternal body weights (-11%, $p < 0.05$) and decreased net weight gains since the start of treatment (-17%, $p < 0.05$) occurred in the dams dosed with 1,000 mg/kg-day ETBE when compared with untreated controls, and the decreased weight change was not accompanied by decreased food consumption. No effects on embryo-fetal development were recorded at this dose level. No significant treatment-related effects on maternal weights or on embryo-fetal development were observed at ETBE dose levels of 500 or 250 mg/kg-day. The study authors reported a NOAEL for maternal ETBE toxicity of 500 mg/kg-day when administered via gavage in the rat, and a NOAEL of 1,000 mg/kg-day with regards to embryo-fetal development.

A two-generation reproductive toxicity study of ETBE was conducted in rats by CIT (2004b; unpublished report). As described below, because endpoints from the CIT (2004a, b, unpublished reports) studies were considered for BMD modeling and the derivation of an RfD, the study reports were externally peer-reviewed by EPA in November 2008. The study was conducted under EPA's testing guidelines (OPPTS 870.3800; U.S. EPA, 1998d). Sprague-Dawley rats (25/sex/dose group) were administered ETBE via gavage at dose levels of 0 (corn oil vehicle), 250, 500, or 1,000 mg/kg-day. Reproductive parameters evaluated included gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation, and weaning, as well as growth and development of offspring of treated rats. Dosing of all females in the F0 generation groups commenced 10 weeks before mating, continued during a 2-week mating period, throughout gestation, and until the end of lactation (PND 21) for a total of 18 weeks; corresponding males were dosed for an identical length of time. Direct gavage

treatment of the progeny of the F0 generation (F1 pups) began at weaning and continued under the same experimental conditions described for the F0 generation. Progeny of the F1 generation (F2 pups) were treated from weaning until sexual maturity. For both F1 and F2 generations, on PND 4, litters were culled to eight pups per litter (four males, four females). On PND 22, 25 F1 rats/sex from each dose group were selected for subsequent mating. Time and acquisition of sexual milestones for each animal were noted, and neurobehavioral and reflex development tests were conducted at designated intervals. Testicular and epididymal sperm parameters were evaluated for F0 and F1 males. The estrous cycle of females was monitored 3 weeks prior to mating and during the mating period. Histological examinations were conducted for gross lesions, reproductive organs, adrenal glands, and pituitary glands from all parental rats; organs with gross abnormalities were also examined.

For all generations, ptyalism was observed in a dose-related trend in most treated males and some females. The incidence of ptyalism resolved within 1 hour of dosing in all females and most males (all but one male of the F0 generation, most males of the F1 generation, and all of the males of the F2 generation). There were no apparent effects of ETBE on mating, fertility, gestation, fecundity, or delivery, and no significant effects were observed on the progeny from birth until weaning in any of the treatment groups. Sperm parameters were not affected in any of the male treatment groups.

In the F0 generation at 1,000 mg/kg-day, males showed significantly lower body weight gains in the last quarter of the treatment period (-22%, $p < 0.01$ compared to controls over days 85–113; Table 4-7). The decreased weight gain was not different from controls when analyzed over the entire treatment period (days 1–113), and was not accompanied by a decrease in food intake. F0 females at this dose consumed 10% more food during the lactation period (PND 1–21, $p < 0.001$). Absolute and relative liver weights in 1,000 mg/kg-day males were increased by 17 and 24% ($p < 0.01$), respectively, apparently related to the slight to moderate centrilobular hypertrophy in liver tissue of high-dose parental males (3/3) and not seen in the one control male subjected to histopathological examination. Only the livers of those four males were examined out of all of the rats from the F0 generation. Absolute and relative kidney weights were significantly increased in 1,000 mg/kg-day males by 21 and 28% ($p < 0.01$), respectively, and correlated with the appearance of acidophilic globules in renal tissue from 5/6 males examined. Only the kidneys of those six high-dose males were examined out of all of the rats from the F0 generation. In addition, tubular basophilia (4/6), peritubular fibrosis (3/6), and proteinaceous casts (1/6) were observed in the male rat's kidneys at the high dose. In male rats in the 500 mg/kg-day group, significantly lower body weight gain was noted at the end of the treatment period (-29%, $p <0.001$) and absolute and relative kidney weights were increased by 15 and 18% ($p < 0.01$), respectively. In the 250 mg/kg-day F0 generation males, absolute (+11%, $p < 0.05$) and relative (+11%, $p < 0.01$) kidney weights were increased, but no such effects were found in

females. In the 250 mg/kg-day group, transient ptyalism was observed in a few males and females, but no other abnormal effects were noted.

Table 4-7. Effects of oral ETBE treatment on parental Sprague-Dawley rats in a two-generation reproduction and fertility study

Dose	Mean body weight gain (percent change as percent of control)				Mean liver weight (percent of control)			Centrilobular hypertrophy (incidence)[a]	Mean kidney weight (percent of control)			Renal acidophilic globules	
	Days[b] 1–113	*p*-Value	Days[c] 85–113	*p*-Value	Absolute	Relative	*p*-Value		Absolute	Relative	*p*-Value	Incidence[a]	Severity
F0 Generation													
1,000 mg/kg-day													
Males	-5	>0.05	-22	<0.01	+17	+24	<0.01	3/3	+21	+28	<0.01	5/6	slight-moderate
Females	0	>0.05	NA		+6	+4	>0.05		+5	+3	>0.05		
500 mg/kg-day													
Males	-3	>0.05	-29	<0.001	+2	+6	>0.05		+15	+18	<0.01		
Females	+2	>0.05	NA		+4	+8	>0.05		+2	+5	>0.05		
250 mg/kg-day													
Males	0	>0.05	-3	>0.05	+2	+3	>0.05		+11	+11	<0.01		
Females	+1	>0.05	NA		+1	+10	>0.05		+1	+9	>0.05		
F1 Generation													
1,000 mg/kg-day													
Males	+1	>0.05	-13	>0.05	+27	+25	<0.01	2/2	+58	+58	<0.01	3/4	slight-marked
Females	0	>0.05	NA		+10	+9	<0.05		+11	+10	<0.01		
500 mg/kg-day													
Males	+4	>0.05	-7	>0.05	+14	+11	<0.05		+22	+19	<0.01	1/1	marked
Females	-2	>0.05	NA		+3	+6	>0.05		+3	+6	>0.05		

Table 4-7. Effects of oral ETBE treatment on parental Sprague-Dawley rats in a two-generation reproduction and fertility study

Dose	Mean body weight gain (percent change as percent of control)				Mean liver weight (percent of control)			Centrilobular hypertrophy (incidence)[a]	Mean kidney weight (percent of control)			Renal acidophilic globules	
	Days[b] 1–113	*p*-Value	Days[c] 85–113	*p*-Value	Absolute	Relative	*p*-Value		Absolute	Relative	*p*-Value	Incidence[a]	Severity
250 mg/kg-day													
Males	0	>0.05	-2	>0.05	0	0	>0.05		+10	+11	<0.05, <0.01		
Females	-1	>0.05	NA		+1	+3	>0.05		+4	+6	>0.05		
F2 Generation													
All doses	No effects noted at any dose level; rats killed prior to mating												

[a]Number observed/number examined.
[b]Body weight of F1 generation females was only tracked for 105 days (64 days of premating, 20 days of pregnancy, and 21 days of lactation).
[c]NA – Not applicable; the corresponding period (days 85–113) in females includes a portion of pregnancy (day 14 to 20) and all of lactation.

Source: CIT (2004b, unpublished report).

Body weight, body weight gain, and food consumption were not affected by exposure in either sex of the F1 parental dose groups. Transient ptyalism was observed in most males and in a majority of females. Increases in absolute (+27%, $p < 0.01$) and relative (+25%, $p < 0.01$) liver weights occurred in males dosed with 1,000 mg/kg-day, with accompanying centrilobular hypertrophy in males (2/2) examined. Only the livers of those two high-dose males were examined out of all of the rats from the F1 generation. Kidney weights, both absolute (+58%, $p < 0.01$) and relative (+58%, $p < 0.01$), were significantly increased in high-dose males, and slight to moderate acidophilic globules were seen in the kidney tissue of high-dose males (3/4) examined microscopically as a result of observed macroscopic lesions. Histology was also performed on kidneys from one control male and one mid-dose (500 mg/kg-day) male due to the presence of macroscopic lesions in these animals. Only the kidneys of those six males were examined out of all of the rats from the F1 generation. Tubular basophilia was observed in the control male, the mid-dose male, and two of the four high-dose males. Peritubular fibrosis was also observed in the control male, the mid-dose male, and two of the four high-dose males. The one mid-dose male examined also had "sloughed degenerated/necrotic cells" in the tubular lumen. Similar but less striking increases in absolute (+10%, $p < 0.05$) and relative (+9%, $p < 0.05$) liver and absolute (+11%, $p < 0.01$) and relative (+10%, $p < 0.01$) kidney weights appeared in high-dose females (Table 4-7), although no macroscopic effects were noted and no histology was performed. In the 1,000 mg/kg-day group, pup body weight gains were slightly, but not significantly lower during the first 4 days of lactation. Two pups born to 1,000 mg/kg-day F1 females exhibited gross external malformations (absence of tail with anal atresia also observed in one pup); however, the authors state that the incidence of these malformations was comparable to laboratory or external historical control data. In the 500 mg/kg-day F1 generation, absolute (+14%, $p < 0.05$) and relative (+11%, $p < 0.01$) liver and absolute (+22%, $p < 0.01$) and relative (+19%, $p < 0.01$) kidney weights were increased in parental males, but no such effects were found in parental females. In the 250 mg/kg-day F1 generation, relative (+11%, $p < 0.01$) kidney weights were increased in parental males, but no such effects were found in parental females. No other macroscopic or histological effects were noted in parental males and females or in their progeny. No other effects were observed in the F1 parental rats or their progeny at 250 mg/kg-day, but transient ptyalism was observed in a majority of males and some females.

In the F2 generation, transient ptyalism was seen in approximately half of the high-dose males and females and in a few rats of lower-dose groups. Significant effects on body weight, body weight gain, food consumption, or liver and kidney weights were not observed. There were no adverse macroscopic or histological findings.

In summary, significant decreases in body weight gain in male parental rats were recorded for the F0 generation dosed with 1,000 or 500 mg/kg-day ETBE. Absolute and relative kidney weights were increased in high- and mid-dose males, which was associated with the presence of acidophilic globules in the limited number of animals examined histopathologically.

Increases in absolute and relative liver weights, with concomitant centrilobular hypertrophy, were also recorded. Histological examination of the kidney or liver was only performed when lesions were observed macroscopically. Similar macroscopic effects in females were not observed, and therefore, no histology of the kidney or liver in females was performed. In the F1 generation, both males and females dosed with 1,000 mg/kg-day showed increases in absolute and relative liver and kidney weights, but the effects were far less pronounced than in males. Increases in absolute and relative kidney and liver weights were seen in males, but not in females, dosed with 500 mg/kg-day.

The study authors suggested a NOAEL of 250 mg/kg-day based on systemic toxicity (specifically on the increased incidence of reduced body weight gain at the end of the treatment period in F0 males and the increased liver and kidney weights in high dose F0 females and mid- and high-dose F0 and F1 males as described above). For fertility, gonadal function, reproductive performance, parturition, and lactation in the parental generations, and development of the offspring to weaning or sexual maturity, the authors suggested a NOAEL of 1,000 mg/kg-day (highest dose tested). The authors note that ptyalism is common following gavage of unpalatable chemicals, and, therefore, the transient ptyalism observed was not considered to represent an adverse effect of ETBE exposure; a lowest-observed-effect level of 250 mg/kg-day was noted. Although the increased relative kidney weights observed at 250 mg/kg-day in the F0 and F1 generation males and the increased absolute kidney weights in F0 generation males represent a LOAEL, these effects were not identified in the study summary. Data tables from CIT (2004b, unpublished report) report relative kidney weights in males treated at 250 mg/kg-day ETBE were significantly increased relative to controls as determined by Dunn's test at 1% for the F1 males and by Dunnett's test at 1% based on pooled variances. Data tables from CIT (2004b, unpublished report) also report increased absolute kidney weights as determined by Dunnett's test at 5% in F0 generation males treated at 250 mg/kg-day ETBE relative to controls.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute Studies

4.4.1.1. *Oral*

Roudabush (1966, unpublished report) reported the oral dose to kill half of the animals (LD_{50}) for ETBE to be >1,600 mg/kg in rats, but no experimental details were provided. An acute oral study with ETBE was performed in which four groups containing two male and two female albino rats were administered single doses of 500, 1,000, 2,500, or 5,000 mg/kg ETBE (no vehicle was employed) by gavage (MB Research Laboratories, Inc., 1988a, unpublished report). The rats were observed for mortality and toxicity 1, 2, and 4 hours post-dose and twice daily for 14 days. No deaths or abnormal physical signs were noted. Body weight gains were normal except for a single female (2,500 mg/kg) that lost weight during the second week of the

study. Necropsy results were normal. On the basis of data presented, the acute oral LD_{50} is >5,000 mg/kg. No other acute oral studies of ETBE were found.

4.4.1.2. *Inhalation*

The comparative anesthetic activity of a series of commonly available aliphatic ethers with 2–10 carbon atoms (31 compounds), including ETBE, was determined in inhalation studies using white mice (Marsh and Leake, 1950). The test agents were placed in a 20 L Pyrex™ jar and volatilized. Four mice (18–24 g) were then placed in the jar and the experiment was run five times for a total of 20 animals. The concentration of test agent producing anesthesia within 30 minutes in 9–11 of the 20 mice, the AC_{50} (concentration in mmol/L required to anesthetize half of mice), was calculated using the jar volume and the amount of test agent volatilized. The median lethal concentration (LC_{50}), was determined in a corresponding fashion. The AC_{50} for ETBE was determined to be 0.7 mmol/L (17,112 ppm), while the LC_{50} was 1.2 mmol/L (29,334 ppm). The Therapeutic Index (LC_{50}/AC_{50}) was thus calculated to be 1.7, and the Certain Safety Factor ($LC_{1–5}/AC_{95–99}$) was determined to be 1.25. For comparison, the Therapeutic Index and Certain Safety Factor for diethyl ether are 3.0 and 1.8, respectively. The AC_{50} and LC_{50} values for MTBE were found to be 1.2 and 1.5 mmol/L, respectively, using identical experimental methods.

In an acute inhalation study (IIT Research Institute, 1989a, unpublished report), ETBE vapor was administered nose-only to a group of five CD rats/sex at a concentration of 5.88 mg/L (1,407 ppm). Since none of the rats died after a single 4-hour exposure, it was concluded that the LC_{50} is >5.88 mg/L (1,407 ppm).

4.4.2. Direct Administration Studies

4.4.2.1. *Dermal Administration*

ETBE was applied neat (without vehicle) at a dose of 2 g/kg to the shaved backs of five male and five female rabbits (IIT Research Institute, 1989b, unpublished report). The test article was left in contact with the skin under occlusion for 24 hours and then removed. The rabbits were observed during that period and for 14 days thereafter. Since no deaths occurred during the study, the dermal LD_{50} was estimated to be >2 g/kg. Dermal irritation, edema, erythema, and eschar formation were observed at the sites of application of all rabbits. No gross pathological lesions were observed in any of the rabbits at necropsy.

Similar results were found in a second acute toxicity study designed to determine the dermal LD_{50} performed by MB Research Laboratories, Inc. (1988b, unpublished report). A 2 g/kg dose of ETBE was applied neat to the shaved backs of five male and five female healthy New Zealand albino rabbits. The test article was kept in contact with the skin under occlusion for 24 hours. Rabbits were observed 1, 2, and 4 hours post-dosing, and then twice daily for 14 days. Nine of the 10 rabbits survived the dermal dose of 2 g/kg. Physical signs noted in

surviving rabbits included diarrhea, few feces, yellow nasal discharge, lacrimation, and soiling of the anogenital area. Five of the rabbits lost weight during the study, while body weight changes in four rabbits were normal. Dermal reactions at the site of application were well-defined to moderate on day 1, slight to severe on day 7, and absent to severe on day 14. Instances of poor hair regrowth, indicative of dermal injuries in depth, were noted on day 14. Necropsy of survivors revealed abnormalities in skin at the site of treatment and isolated instances of gastrointestinal and lung abnormalities. Yellow staining of the nose/mouth area and soiling of the anogenital area were also noted during necropsy. Based on the findings from this study, the dermal LD_{50} for ETBE in rabbits was estimated to be >2.0 g/kg.

Roudabush (1966, unpublished report) applied ETBE (dose not reported) to the shaved skin of guinea pigs under occlusion, which resulted in only slight skin irritation being observed. No information on dermal absorption was provided. In another dermal irritation study (MB Research Laboratories, Inc., 1988c, unpublished report), six New Zealand albino rabbits were dosed with 0.5 mL ETBE at two intact and two abraded sites. ETBE was kept in contact with the skin for 4 hours under occlusion, and then the wrappings were removed. Dermal reactions were scored at 4, 24, 48, and 72 hours after application of the test article, and also after 7 and 14 days. Although absent at 4 hours, erythema was well-defined at the site of application at 24 and 48 hours and slight to well-defined at 72 hours. By day 7, erythema was absent at some sites, but well-defined at other sites, and by day 14, no erythema was detected. The Primary Irritation Index was 3.08 out of a possible 8.0, and abraded sites were no more severely affected than intact sites.

A subchronic dermal study was conducted in Sprague-Dawley rats by UBTL Inc. (1994, unpublished report). Groups of 10 male and 10 female young adult rats were administered ETBE (no carrier) to clipped areas on their backs 5 days/week for 4 weeks (28 days) at 0 (control), 0.05, 0.25, and 1.0 mL/kg-day. The application site was occluded for at least 6 hours following dosing. Rats were observed twice daily for viability and once daily for signs of toxicity. Irritation was evaluated just prior to dosing and 24 hours after the fifth dose each week. At necropsy, blood samples were taken for clinical chemistry evaluation and selected tissues were collected for histopathological evaluation. ETBE caused slight to moderate dermal irritation in both males and females at 1.0 mL/kg-day; there were visible lesions and histopathological changes in the skin. Less irritation was observed in rats in the mid-dose group, and very slight dermal irritation was observed in the 0.05 mL/kg-day dose group. There was an increase in the incidence and severity of lymphoid hyperplasia of the axillary lymph nodes in males and females of the 0.25 and 1.0 mL/kg-day groups, findings considered to be secondary to the dermal irritation observed. Under the conditions of the study, the authors reported the dermal NOEL to be <0.05 mL/kg-day in male and female rats; the systemic NOEL for dermal exposure was reported to be 1.0 mL/kg-day for both male and female rats.

4.4.2.2. *Ocular Administration*

Eye irritation characteristics were evaluated using the Draize test (MB Research Laboratories, Inc., 1988d, unpublished report). ETBE, 0.1 mL, was placed in the conjunctival sac of one eye of each of nine healthy New Zealand rabbits. Three eyes were washed with lukewarm water for 1 minute at 20–30 seconds after dosing and the treated eyes of the remaining six animals remained unwashed. The eyes were examined and scored at intervals for corneal opacity, iritis, and conjunctival irritation. For unwashed eyes, slight corneal opacity that cleared by day 7 occurred in 1/6 eyes; iritis in 1/6 eyes that cleared by day 2, and conjunctival irritation in 6/6 eyes that cleared by day 7. In the washed eyes, corneal opacity was noted in 1/3 eyes; this opacity was first observed on day 1, and it had not cleared by day 14. However, no iritis was observed in the washed eyes, and conjunctival irritation that was observed in 1/3 eyes had cleared by day 7. Mean Draize scores were 12.2, 8.3, 4.0, 0, and 0 for unwashed eyes on days 1, 2, 3, 7, and 14, respectively, and 6.7, 5.3, 4.0, 3.3, and 3.3 for washed eyes at these same time points.

4.4.3. Neurological Studies

The ability of ETBE and other oxygenated fuel additives to inhibit the binding of a convulsant ligand ([^{3}H]t-butylbicycloorthobenzoate) to the γ-aminobutyric acid$_A$ ($GABA_A$) receptor was tested in vitro in Sprague-Dawley rat brain membrane preparations (Martin et al., 2002). Membrane preparations (100 µL) were incubated on ice for 60 minutes with 20 nM of [^{3}H]t-butylbicycloorthobenzoate and one of the six chemicals (MTBE, ETBE, TAME, TBA, TAA, or ethanol) to generate concentration-response curves for binding inhibition. Chemicals were tested over a concentration range from 10^{-4} to 1 M and incubations were performed in triplicate. Subsequent incubations were performed on ice for 90 minutes to generate saturation curves. Parallel incubations were included with picrotoxin to establish nonspecific binding. Concentration loss due to evaporation was monitored and was less than 5% for all agents tested. The $GABA_A$ receptor has distinct recognition sites for GABA, depressants, and convulsants (Mehta and Ticku, 1999). There was a general correlation of the potentiation of $GABA_A$ receptor responses with their ability to induce general anesthesia (Krasowski and Harrison, 1999). Martin et al. (2004, 2002) suggested that acute neurological symptoms associated with short-term exposure to oxygenates such as ETBE (e.g., headache, dizziness, and nausea) may reflect the participation of the $GABA_A$ receptor. The potency of the inhibition of convulsant ligand binding was in the rank order: TAA > TAME > ETBE > TBA > MTBE > ethanol. In a follow up study, the uptake of $^{36}Cl^-$ was measured in synaptoneurosomes (which included pre- and postsynaptic membranes) from adult Sprague-Dawley rat cerebral cortex (Martin et al., 2004). The oxygenates and oxygenate metabolites tested produced concentration-dependent enhancement of muscimol-stimulated uptake of $^{36}Cl^-$. The potency of enhancement was as follows MTBE = TAME > TAA = ETBE > TBA > ethanol. Concentrations that facilitated

muscimol-stimulated $^{36}Cl^-$ uptake ranged from 0.06 to 3 nM, which were described by the authors as being in the range of blood concentrations reported in experimental animals following exposures known to induce CNS effects and ataxia (Martin et al., 2004).

In subchronic inhalation experiments, ETBE was administered as a vapor at target chamber concentrations of 0, 500, 2,000, and 4,000 ppm to groups of Sprague-Dawley rats for 4 weeks as described in Section 4.2.5.1 (published as White et al., 1995; IIT Research Institute, 1991, unpublished report). A FOB (which included tail pinch, rotorod performance, body temperature, righting reflex, auditory response, hind limb extension, foot splay, grip strength, home-cage observation, hand-held observation, open-field observation, extensor thrust, catalepsy, visual placing, tactile placing, negative geotaxis, vision, eye-blink, and pupil response) was administered 1 week prior to the exposures and about 60 minutes after 1, 5, or 20 exposures (6 hours/day, 5 days/week) to evaluate neuromuscular function and sensory perception. Ataxia and sedation, which are overt signs of CNS depression, were seen following exposure termination in the 4,000 ppm group, but rats appeared normal 15 minutes after the end of exposure. Mean body temperature was reduced 2.00–2.14% in 4,000 ppm males after the fifth exposure, and a trend for increased hind limb splay in both sexes of the high concentration group occurred. These effects were described by the authors as being associated with transient CNS depression. No other indications of CNS depression or neurotoxicity were detected.

Dorman et al. (1997) performed an evaluation of possible ETBE neurotoxicity with male and female F344 rats from the 13-week inhalation study described in Section 4.2.3.1 (Medinsky et al., 1999). Rats from the neurotoxicology subgroup (12/sex/concentration), 8 weeks of age at study initiation, were exposed to 500, 1,750, or 5,000 ppm ETBE (>97% pure) for 6 hours/day and 5 days/week for a total of 65 exposures over a 13-week period. Details of the experimental design and nonneurological effects were provided earlier (Section 4.2.3.1). On exposure days, all rats were observed for mortality and overt clinical signs of toxicity prior to exposure and shortly following exposure, while on non-exposure days, the rats were observed once daily. The following observations of individual rats were conducted during daily cage-side examinations: body appearance, piloerection, fur appearance, facial crust, skin temperature and color, breathing pattern, salivation, and an evaluation of eyes, lacrimation, and mucous membranes. Effects on the respiratory, circulatory, autonomic (e.g., diarrhea, salivation), and central nervous systems (e.g., ataxia, head tilt, seizures) were evaluated, in addition to specific assessments of somatosensory activity (e.g., photophobia) and behavior patterns (e.g., circling, aggressiveness). Motor activity assessments and an FOB were also performed on all rats. These latter tests were conducted 4 days prior to initial ETBE exposure, and at least 18 hours after the end of days 1, 6, 10, 20, 42, and 65 of exposure, to detect possible persistent neurological effects. The FOB consisted of observations of spontaneous activity and behavior in an open field, assessment of visual approach response, auditory startle response, tail pinch response, surface righting reflex, visual placing response, forelimb and hindlimb grip strength, hind leg splay, and pupillary reflex.

Each animal was also evaluated for posture, tremors, spasms, convulsions, palpebral closure, handling reactivity, and muscle tone.

At termination, rats in the 5,000 ppm and control groups were anesthetized and then perfused with 1.5% glutaraldehyde/4% formaldehyde for preparation of nervous tissue. Brains were weighed and measured, and paraffin sections of Gasserian ganglia, dorsal spinal root ganglia, spinal root fibers, brain, spinal cord, eye, and optic nerve were stained with hematoxylin/eosin to provide a direct evaluation of possible structural neuropathy. Additionally, portions of sciatic, tibial, and sural nerves were embedded in glycol methacrylate, sectioned, and stained with Lee's methylene blue basic fuchsin for more extensive histological examination.

The only noteworthy clinical finding during daily observations was transient ataxia, typically observed in male rats immediately following a 5,000 ppm exposure, and lasting less than 1 hour. Such effects are typically seen with various ethers, including MTBE and diethyl ether. Ataxia was only noted during the first 5 weeks of the 13-week study. There were no significant effects on body appearance, and no significant effects on motor activity during the post-exposure test sessions were noted. No evidence of sensorimotor dysfunction (altered acoustic response, tail pinch response, approach response, or visual placing response) or neuromuscular dysfunction was observed in control or ETBE-exposed rats. No signs of ataxia, piloerection, excessive vocalization, muscle tremors or spasms, clonic or tonic seizures, increased salivation, abnormal respiration, or abnormal pupillary reflex were observed in ETBE-exposed rats. No gross or microscopic abnormalities were observed in the central, peripheral, or autonomic nervous systems of rats exposed to 5,000 ppm ETBE. No significant effects of exposures on brain weight or size were observed. The authors concluded that in spite of transient ataxia in rats following high-level exposure, there was no indication that ETBE is a neurotoxicant under the conditions tested.

Neurobehavioral endpoints were assessed as part of a two-generation gavage study conducted in Sprague-Dawley rats as described in Section 4.3 (CIT 2004b, unpublished report). The study was conducted under EPA's testing guidelines OPPTS 870.3800 (U.S. EPA, 1998d). Groups of Sprague-Dawley rats (25/sex/dose group) were administered ETBE at dose levels of 0, 250, 500, or 1,000 mg/kg-day by gavage in corn oil. Dosing of the F0 generation groups commenced 10 weeks before mating, continued during a 2-week mating period, and throughout the time period required for gestation and lactation for a total of 18 weeks. Treatment of the progeny of the F0 generation (F1 pups) began at weaning and continued under the same experimental conditions described for the F0 generation. The following neurobehavioral and reflex development tests were conducted in rats of the F1 generation: acoustic startle response and pupil constriction were assessed at 4 weeks of age, and spontaneous locomotor activity was evaluated when the animals were between 7 and 8 weeks of age. There was no effect of treatment on neurobehavioral parameters.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Genotoxicity

4.5.1.1. *In Vitro Bacterial Assays*

Zeiger et al. (1992) conducted Salmonella mutagenicity test using different *Salmonella typhimurium* strains for 311 chemicals, including ETBE, both in the absence and presence of metabolic activation (S9). Preincubation protocol was followed as described in Haworth et al. (1983). Chemicals known or suspected to be volatile, such as ETBE, were incubated in capped tubes. ETBE (99% purity) did not show any mutagenic activity both in the presence and absence of S9.

4.5.1.2. *In Vitro Mammalian Assays*

The potential clastogenicity of ETBE (>98% purity; containing 13 ppm AO22, an antioxidant stabilizer) was evaluated in the in vitro Chinese hamster ovary (CHO) chromosome aberration assay (Vergnes, 1995, unpublished report). Concentrations of ETBE ranging from 0.1 to 5.0 mg/mL culture medium, both in the presence and absence of rat liver S9 metabolic activation system, were tested on cultures of CHO K1-BH4 cells. No significant cell cycle delays were noted in preliminary experiments; consequently, mitotic cells from the highest four concentrations were harvested 10 hours after treatment and scored for chromosomal aberrations. Treatment of cultured CHO cells with ETBE did not result in statistically significant or concentration-related increases in the frequency of chromosome aberrations in the presence or absence of the S9 metabolic activation system. Treatment of positive control cells with Mitomycin C demonstrated significantly positive numbers of chromosomal aberrations. ETBE was, therefore, not considered clastogenic under the conditions of this assay. Although the study description notes that glass bottles were used because of the solvent properties of ETBE, the methods do not state that the volatility of ETBE was controlled for; therefore, exposure concentrations may have been significantly reduced by evaporation of ETBE. The effect of the antioxidant stabilizer, AO22, was not discussed by the authors, but this compound has the potential to decrease the sensitivity of the assay for compounds that work through oxidative stress (e.g., antioxidant inhibition of iodoacetic acid genotoxicity measured in CHO cells in vitro as described in Cemeli et al., 2006). CHO K1-BH4 cells were used to evaluate the mutagenicity of ETBE by using the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward mutation assay (Vergnes and Kubena, 1995a, unpublished report). The assay detects mutations in the HGPRT gene, which are scored after killing all nonmutated cells by addition of the purine analog, 6-thioguanine, to cell cultures. Duplicate cultures of CHO cells were treated with five concentrations of ETBE (>98% purity; 13 ppm AO22–see above for potential effect of AO22) ranging from 0.1 to 5.0 mg/mL ETBE, both in the presence and absence of S9 activation. No cytotoxicity was observed. No statistically significant, concentration-related increase in the

HGPRT mutation frequency was observed at any of the ETBE concentrations tested. Mutation frequencies in the positive control plates treated with 200 μg/mL ethylmethanesulfonate were highly significantly elevated compared with dimethyl sulfoxide vehicle-treated control cells. ETBE was thus considered nonmutagenic in the HGPRT forward mutation assay. However, as above, the methods do not state that the volatility of ETBE was controlled for and, therefore, exposure concentrations may have been significantly reduced by evaporation of ETBE.

4.5.1.3. *In Vivo Assays*

Vergnes and Kubena (1995b, unpublished report) conducted an in vivo micronucleus (MN) test in mice in response to ETBE exposure. Male and female CD-1 mice (five/sex/group) were exposed to ETBE by inhalation (>98% purity; 13 ppm AO22) at target concentrations of 0 (air-only control), 400, 2,000, and 5,000 ppm ETBE for 6 hours/day for 5 days. Following treatment, mice were killed and femurs were removed. Aspirated bone marrow cells were smeared on slides and treated with Giemsa stain. At least 2,000 polychromatic erythrocytes per mouse were scored in bone marrow smears. No statistically significant increases in the mean percentages of micronucleated polychromatic erythrocytes were observed in mice of either sex when exposed to ETBE. Mice of both sexes exposed to the positive control agent cyclophosphamide (15 mg/kg) showed significant increases in the percentage of polychromatic erythrocytes with micronuclei.

4.5.2. Studies with ETBE–Gasoline Mixtures

There are unpublished toxicology reports for inhalation exposure to gasoline/ETBE vapor condensate (G/ETBE). The interpretation of the results of these mixture studies for toxicity of individual components is confounded by co-exposure to other chemicals.

Huntingdon Life Sciences (2002, unpublished report) conducted a subchronic inhalation toxicity study in rats using G/ETBE that included a neurotoxicity assessment, a 4-week immunotoxicity assessment (White, 2002, unpublished report), and a 4-week in vivo-in vitro genotoxicity assessment (Gudi and Brown, 2002, unpublished report; Mason, 2002, unpublished report), each of which will be discussed in further detail below. The test material was prepared to simulate the composition of headspace vapor from an automotive fuel tank at near maximum in-use temperatures. The G/ETBE sample contained 16.3 area percent ETBE prepared from a baseline gasoline sample that contained 2.1 area percent benzene and other low boiling-point hydrocarbons (Daughtrey et al., 2004, abstract only). Conventional unleaded gasoline often contains MTBE at a low percentage, although MTBE was not reported as a contaminant by Daughtrey et al. (2004, abstract only).

Male and female Sprague-Dawley rats were exposed by inhalation to the G/ETBE at target concentrations of 0, 2,000, 10,000, and 20,000 mg/m^3 for 6 hours/day, 5 days/week, for 13 weeks (Huntington Life Sciences, 2002, unpublished report). Twenty rats/sex/concentration

were used for the high concentration and air control groups, while 10 rats/sex/concentration were included in the mid- and low-dose groups. Possible neurobehavioral effects were monitored using an FOB and motor activity tests that were performed prior to initiation of exposure, and after 4, 8, and 13 weeks of exposure. Ten rats/sex/group were killed at 13 weeks; the remaining rats in the high concentration and control groups were maintained without subsequent exposures for 4 weeks of untreated recovery. At the week 13 necropsy, 5 rats/sex/group were transcordally perfused and the nervous system tissue was prepared for neuropathological evaluation; tissues of all other rats were prepared for routine microscopic pathology examination. Mean analytical exposure concentrations of G/ETBE were 0, 2027 ± 193, 10,060 ± 691, and 19,930 ± 1,031 mg/m^3. Mean body weight gains were decreased in females exposed to 20,000 mg/m^3 during study weeks 4–12, but the differences abated during the recovery period; similar effects did not occur in males. There were no apparent exposure-related effects of G/ETBE on FOB or motor activity tests. Neuropathology evaluations were negative in all animals. Increased absolute and relative kidney weights occurred in males, but not females, exposed to 20,000 mg/m^3 (data not shown). This effect was reversible, or nearly so, during the 4-week recovery period. Microscopic findings attributed to G/ETBE exposure occurred only in the kidneys of male rats exposed to 20,000 mg/m^3, and consisted of eosinophilic hyaline granules within the cytoplasm of renal proximal tubular epithelial cells and evidence of tubular regeneration with corticomedullary intralumenal tubular casts. These changes were thought to be consistent with hyaline droplet nephropathy, and the authors suggested that the observations were potentially attributable to accumulation of alpha$_{2u}$-globulin within renal tubular cells. Whether or not the available data on G/ETBE nephrotoxicity are consistent with the alpha$_{2u}$-globulin accumulation mode of action as discussed in the Risk Assessment Forum Technical Panel Report (U.S. EPA, 1991b) has not been determined for the limited data in these unpublished reports. Unleaded gasoline is one of the model chemicals cited by that report to produce renal toxicity, and therefore, the exposure to this mixture would prevent attribution of the observed renal effects to ETBE. The concentration level of 10,000 mg/m^3 was considered by the study authors to be the NOAEL for this study.

White (2002, unpublished report) described the immunotoxicity portion of the Huntington Life Sciences (2002, unpublished report) study of G/ETBE. The immunotoxicity study used the same exposure concentrations (i.e., target concentrations of 0, 2,000, 10,000, and 20,000 mg/m^3 G/ETBE) and dosing regimen (i.e., 6 hours/day, 5 days/week) as the larger study, with the exception that only female rats (10/dose group) were exposed to the test agents and for a duration of only 4 weeks. Mean analytical exposure concentrations were 0, 2040 ± 161, 9978 ± 632, and 19,710 ± 1,044 mg/m^3. Rats were sensitized by intravenous injection of 2×10^8 sheep red blood cells (SRBCs) 4 days prior to the end of the G/ETBE exposure. As a positive control for immunotoxicity, 50 mg/kg cyclophosphamide was given i.p. on the last 4 days of the study. At study termination, 1 day after the last exposure, body weight was recorded, animals were

killed, and the thymus and spleen were removed and weighed. The spleen was placed in Earle's balanced salt solution for later determination of total splenocytes, and use in the plaque-forming cell (PFC) assay to measure the SRBC-specific antibody response. G/ETBE had no effect on the terminal body weight, the absolute or relative weights of the spleen and thymus, or the total number of splenocytes/animal. Exposure to G/ETBE at 10,000 and 20,000 mg/m^3 did result in a suppression of the PFC response. When evaluated as activity per 10^6 splenocytes, reductions of 76 and 72% were observed in the PFC for the 10,000 and 20,000 mg/m^3 groups, respectively. When evaluated as total activity per spleen, reductions of 74 and 70% were observed in the PFC for the 10,000 and 20,000 mg/m^3 groups, respectively. The cyclophosphamide positive control was effective, reducing spleen and thymus weight, total splenocytes, and the PFC response. Although not stated by the authors, the findings of this study support consideration of 2,000 mg/m^3 of G/ETBE as a NOAEL for elicitation of immunotoxicity in the rat.

The genotoxicity data from the Huntington Life Sciences (2002, unpublished report) study of G/ETBE were reported separately by Mason (2002, unpublished report) (MN study) and Guidi and Brown (2002, unpublished report) (sister chromatid exchange [SCE] study). As in the main study, Sprague-Dawley rats (5/sex/group) were exposed to target concentrations of 0, 2,000, 10,000, and 20,000 mg/m^3 of G/ETBE 6 hours/day, 5 days/week. However, as in the immunotoxicity study, the exposure duration was only for 4 weeks. Positive control groups (5 rats/sex/group) were given cyclophosphamide i.p. (5 mg/kg for the SCE study and 40 mg/kg for the MN study) 24 hours prior to study termination. At termination, blood from the abdominal aorta was collected and lymphocytes were cultured for each SCE analysis. Approximately 21 hours after initiation of cultures, lymphocytes were exposed to 5 μg/mL BrdU, and colcemide was added at 68 hours of culture. The cells were harvested at 72 hours and processed for evaluation of SCE. No significant effects were seen in tests for induction of SCE by G/ETBE. For the micronucleus analysis, femurs were removed from rats at termination; smears of femoral bone marrow were placed on slides and stained using a modified Feulgen method. At least 2,000 immature erythrocytes were examined from each animal; the proportion of immature erythrocytes containing satellite micronuclei was tabulated for each rat. There was an increase in the number of micronucleated immature erythrocytes in female rats at the 10,000 mg/m^3 G/ETBE dose (3.8) relative to control (0.8), although no significance was found at the 20,000 mg/m^3 G/ETBE dose (2.0) or at any dose in the male rats. Trend analysis detected a significant responsiveness in the frequency of MN as a function of G/ETBE exposure, but only if all doses tested were included in the evaluation. For both the 10,000 and 20,000 mg/m^3 groups, the mean values for the frequency of micronucleated immature erythrocytes were greater than the historical negative control range. The author concluded that G/ETBE may cause an increase in the frequency of micronuclei in immature erythrocytes; however, the authors also concluded that data from this study do not support bone marrow cell toxicity, in part, because of the lack of significant effect at low and high doses. The utility of genotoxicity results from a mixture study

is limited as noted above. The presence of 2.1% benzene and other possibly genotoxic agents in gasoline condensate (Daughtrey et al., 2004, abstract only) confound a determination of whether ETBE is acting alone, in concert with some other constituent, or not at all, to cause increases in MN. However, the positive response at 10,000 mg/m^3 should be noted and suggests the need for confirmation in a study of ETBE alone.

4.5.3. Structure-Activity Relationship Evaluations

The structure-activity relationships (SAR) for both MTBE and ETBE were evaluated using two computer-based systems (computer automated structure evaluator or CASE and TOPKAT). These software programs were developed to relate structural fragments of test molecules to structural determinants previously recognized as being associated with carcinogenicity in rodents, mutagenicity in the *S. typhimurium* (Ames) test, induction of SCEs and/or chromosomal aberrations in cultured mammalian cells, or other structural alerts for genotoxicity (Zeiger et al., 1996; Rosenkranz and Klopman, 1991). Structural alerts by an expert chemist were used to test the programs. Using the structures of 100 chemicals, the three methods predicted mutagenicity at 71–76% concordance with the *S. typhimurium* results. The results of the software analysis of the various endpoints indicated that, except for a marginal induction of SCEs in cultured mammalian cells by ETBE, the two ethers were not predicted to be either genotoxicants or carcinogens.

Zhang et al. (1997) used CASE and multiple computer automated structure evaluation (MULTICASE) computer models to predict the toxicity of ETBE, MTBE, TAME, DIPE, and metabolites of ETBE. One of the predicted metabolites, ethylene oxide, was predicted to be a rodent carcinogen, and one was predicted to have marginal carcinogenicity (N-hydroxy-N-acetylglycine). All parent ethers were predicted to be negative for rodent carcinogenicity, salmonella mutagenicity, sensory irritation, eye irritation, contact sensitization, and developmental toxicity. The principal metabolite, TBA, was also predicted to be negative in all areas of toxicity, and no predictions for toxic actions of acetaldehyde were provided because the molecule was too small for the model. Results observed for MTBE, which in contrast to model predictions, appears to be a multi-site and multi-species carcinogen in animals (reviewed by Cal EPA 1999; Mennear, 1997) suggest that the CASE, MULTICASE, or TOPKAT programs used by (Zhang et al., 1997; Zeiger et al., 1996; Rosenkranz and Klopman, 1991) may be of limited value in predicting potential carcinogenicity associated with exposure to ETBE.

4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

Liver and kidney toxicity are the primary noncancer health effects associated with exposure to ETBE based on limited available animal data. Increased liver and kidney weights were observed following oral exposure in both the parent and offspring generations and both sexes of rats in a two-generation reproductive toxicity study (CIT 2004b, unpublished report).

Increased kidney weight was also observed following subchronic inhalation exposure to ETBE in male and female F344 rats (Medinsky et al., 1999) and male Sprague-Dawley rats (White et al., 1995). The increased kidney weight was associated with histopathological changes in male rats (including regenerative foci, which are histological formations that demonstrate response to cellular necrosis) in males and sustained increase of greater than twofold in cellular proliferation (Medinsky et al., 1999). The increased kidney weight was not associated with histopathological changes in female rats, although an increased cellular proliferative response of less than twofold was observed at early time points (after 1 and 4, but not 13 weeks of exposure) (Medinsky et al., 1999). Increased liver weight was also observed following subchronic inhalation exposure to ETBE in mice and rats of both sexes (Medinsky et al., 1999; White et al., 1995). No changes in histopathology or serum levels of hepatic enzymes were observed in rats. Dose-related increases in hepatic proliferation were observed in male and female mice at some time periods during a 13-week exposure, and not observed at others (Medinsky et al., 1999). The results of subchronic and chronic studies are discussed below.

4.6.1. Oral

There is limited information concerning noncancer effects of ETBE following oral dosing and almost all of the studies were designed to investigate reproductive or developmental toxicity. There is a single chronic oral ETBE exposure carcinogenicity study that did not report any noncancer effects with the exception of mortality (i.e., Maltoni et al., 1999), a 2-week oral exposure study of potential ETBE effects on oocyte quality (Berger and Horner, 2003), and a series of unpublished oral exposure studies on prenatal developmental toxicity and two-generation reproduction and fertility effects (CIT, 2004a, b, 2003, unpublished reports). ETBE treatment had no apparent effects on oocyte quality in the only published reproductive toxicology study. Oral exposure to ETBE had no effect on sperm parameters, mating, fertility, gestation, fecundity, or delivery in the CIT (2004a, b, 2003, unpublished reports) studies.

Data from the two-generation reproductive toxicity study (CIT 2004b, unpublished report) suggest effects of ETBE on the liver and kidneys. Absolute and relative liver weights were increased in F0-generation male rats exposed to 1,000 mg/kg-day ETBE, F1 generation animals of both sexes exposed to 1,000 mg/kg-day ETBE, and F1 generation males exposed to 500 mg/kg-day ETBE. However, histology was only performed if abnormal morphology was detected at necropsy, and, therefore, there are limited histological data to support hepatotoxicity. Slight to moderate centrilobular hypertrophy was observed in all of the high-dose animals examined (five total males [3/3 F0 and 2/2 F1]) and was not seen in the single control F0 generation male examined. Only the livers from these six males were examined histologically out of all of the rats in all three generations. Absolute and relative kidney weights were also significantly increased in F0 generation males and F1 generation males and females. Kidney weight was increased at lower doses (i.e., at 250 mg/kg compared to 1,000 mg/kg) and to a

greater extent (e.g., for F1 generation rats exposed to 1,000 mg/kg-day kidney weight was up by 58% for both absolute and relative weight in males and up by 11 and 10% for absolute and relative weight in females) in males compared to females. Acidophilic globules were observed in 5/6 F0 and 3/4 F1 generation males at the high dose. In addition, tubular basophilia (4/6), peritubular fibrosis (3/6), and proteinaceous casts (1/6) were observed in the F0 males at the high dose (1,000 mg/kg-day). Histology was also performed on kidneys from six F1 males (four high-dose, one mid-dose [500 mg/kg-day], and one control) due to the presence of macroscopic lesions in these animals. Tubular basophilia was observed in the control male, the mid-dose male, and two of the four high-dose males. Peritubular fibrosis was also observed in the control male, the mid-dose male, and two of the four high-dose males. The one mid-dose male examined also had sloughed degenerated/necrotic cells in the tubular lumen. Only the kidneys from these 12 males were examined histologically out of all of the rats in all three generations. It is difficult to determine conclusions with this limited and select histology. No data are available to determine the presence or absence of acidophilic globules in females or if the presence of acidophilic globules was dose-responsive in males because histology was only performed if abnormal morphology was detected at necropsy.

For all generations, transient ptyalism (excessive salivation) was observed in a dose-related trend in most treated males and some females. The ptyalism was associated with dosing and was resolved within 1 hour of gavage for all females and the majority of males. Although reduced weight gain was observed in some of the parental animals as part of the developmental toxicity and reproductive toxicity studies over short-term exposure, there was no effect of ETBE exposure on weight gain over longer treatment periods. For example, pregnant Sprague-Dawley rats treated with ETBE for 2 weeks (i.e., GDs 5–19) at 1,000 mg/kg-day exhibited an 11% decrease in maternal body weight gain and a 17% decrease in net weight gain (CIT, 2004a, unpublished report). However, there was no effect of 18 weeks of ETBE exposure at 1,000 mg/kg-day on body weight in males or females when body weight change was calculated over the entire treatment period (CIT, 2004b, unpublished report).

ETBE is cleaved to acetaldehyde and TBA by microsomal CYPs, most likely by CPY2A6, 3A4, or 2B6 in humans and by CYP2B1 in rats. However, acetaldehyde appears to be primarily a point-of-contact toxicant, and it is unlikely that humans will ingest ETBE in quantities sufficient to elicit acetaldehyde-related toxicity in the gastrointestinal tract. There are a number of subchronic and chronic studies of TBA (Archarya et al., 1997, 1995; NTP, 1995; Lindamood et al., 1992).

F344/N rats were treated for 2 years with TBA via drinking water at estimated doses of 0, 90, 200, and 420 mg/kg-day (males) and 0, 180, 330, and 650 mg/kg-day (females) (NTP, 1995). Final body weights were reduced at the high dose to a similar extent in males and females, despite the different dose levels. Mineralization in the kidney was found in males even at the lowest dose and was thought to be related to $alpha_{2u}$-globulin-induced nephropathy. Focal renal

tubule hyperplasia and adenomas were observed in males. Other significant pathology was generally related to malignancies. B6C3F$_1$ mice were treated at 0, 540, 1,040, and 2,070 mg/kg-day (males) and 0, 510, 1,020, and 2,110 mg/kg-day (females). Thyroid follicular gland cell hyperplasia was observed in males at all doses and in females at the two highest doses, although thyroid effects have not been corroborated in other studies.

Acharya et al. (1997, 1995) treated rats for 10 weeks with 0.5% TBA in drinking water. They observed hepatic centrilobular necrosis, vacuolation in hepatocytes, and loss of hepatic architecture. They also detected considerable kidney pathology, such as degeneration of renal tubules, degeneration of the basement membrane of Bowman's capsule, and vacuolation of glomeruli. Lindamood et al. (1992) conducted a 90-day subchronic study of TBA in drinking water in rats and mice at concentrations of up to 4%. The pathologic findings were predominantly in the urinary tract: calculi, renal pelvic dilatation, thickening of the bladder mucosa, and transitional epithelial hyperplasia and inflammation.

The limited published data on oral exposure to ETBE are insufficient to determine if the observed effects of ETBE-exposure, such as increased liver and kidney weight, are related to the parent compound or its metabolites.

An overview of animal studies with oral exposure to ETBE is provided in Table 4-8.

Table 4-8. Oral toxicity studies for ETBE

Species	Dose /duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
Subchronic studies					
Rat (Sprague-Dawley) (24 pregnant females/group)	0, 250, 500, 1,000 mg/kg-day, gavage in corn oil on GDs 5–19	500	1,000	Reduced maternal body weight and weight gain [-11 and -17% net gain]	CIT, 2004a, unpublished
		1,000	Not detected	Fetal toxicity or developmental toxicity	
Rat (Sprague-Dawley) (two generations of 25/sex/group)	0, 250, 500, 1,000 mg/kg-day; gavage in corn oil for 18 wks, including premating, mating, gestation, and weaning	250	500	Reduced body weight gain [-22% in F0 males at 1,000 mg/kg-day and -29% in F0 males at 500 mg/kg-day in last ¼ of treatment (day 85 to 113)]	CIT, 2004b, unpublished
		250	500	Increase in liver weights [(+17% absolute +24% relative in F0 males, +27% absolute +25% relative in F1 males, +10% absolute +9% relative in F1 females at 1,000 mg/kg-day) and (+14% absolute +11% relative in F1 males at 500 mg/kg-day)]; with hepatic centrilobular hypertrophy in F0 and F1 males at 1,000 mg/kg-day	
		Not detected	250	Increase in kidney weights [(+21% absolute +28% relative in F0 males, +58% absolute +58% relative in F1 males, +11% absolute +10% relative in F1 females at 1,000 mg/kg-day) and (+15% absolute +18% relative in F0 males, +22% absolute +19% relative in F1 males at 500 mg/kg-day) and +11% relative in F1 and F0 males at 250 mg/kg-day]; with acidophilic globules in F0 and F1 males at 1,000 mg/kg-day	
		1,000	Not detected	Reproductive and developmental performance	
Chronic studies					
Rat (Sprague-Dawley) (60/sex/group)	0, 250, 1,000 mg/kg-day, 4 days/week for 104 weeks; gavage in olive oil	Not detected	250	Tumor incidence in uterus (malignant)	Maltoni et al., 1999
		250	1,000	Pathologies of oncological interest of the mouth epithelium (total) in males	

4.6.2. Inhalation

There is limited information on the noncancer toxicity of ETBE by the inhalation route in humans and animals. Nihlén et al. (1998b) exposed eight volunteers for 2 hours to ETBE

concentrations of up to 50 ppm (the highest acute dose considered safe in the country where the study was conducted). The exposed volunteers experienced irritation in the throat and airways, nasal swelling, a bad taste in the mouth, and slightly impaired lung function. The authors found some markers of inflammation, but there was no correlation with ETBE exposure levels.

There is evidence for kidney-related effects following inhalation exposure to ETBE, with the majority of the data coming from the 13-week inhalation study by Medinsky et al. (1999) in F344 rats and CD-1 mice. Absolute kidney weight was increased in male and female F344 rats, but not CD-1 mice. The increased kidney weight in females was accompanied by negative histopathology and was not associated with additional evidence of nephrotoxicity, with the exception of a less than twofold increase in cellular proliferative response at early time points (after 1 and 4, but not 13 weeks of exposure). In male rats, the increased kidney weight was associated with hyaline droplet accumulation with alpha$_{2u}$-globulin immunoreactivity, a dose-response in a sustained increase of greater than twofold in cellular proliferation (after 1, 4, and 13 weeks of ETBE exposure) determined by the increased LI, and a dose-response in regenerative foci (Medinsky et al., 1999). The Medinsky et al. (1999) kidney data included a semi-quantitative measure of hyaline droplets, and a weak dose-response in cellular proliferation. Support for these data for a male rat-specific alpha$_{2u}$-globulin-mediated mode of action as presented in the Risk Assessment Forum Technical Panel Report (U.S. EPA, 1991b) is discussed in Section 4.6.3. There is only one additional study that provides evidence relative to the effect of inhalation exposure to ETBE on the kidney. White et al. (1995) found an increase in the absolute kidney weight of male, but not female, Sprague-Dawley rats after 4 weeks of exposure to 4,000 ppm ETBE. Histopathology of the kidney of both males and females was negative.

Several studies demonstrated an effect of inhalation exposure to ETBE on the liver, an effect that was largely restricted to an increase in absolute or relative liver weight. Medinsky et al. (1999) exposed F344 rats and CD-1 mice for 13 weeks to 500–5,000 ppm ETBE. Absolute liver weight was increased in male rats and mice of both sexes at 1,750 ppm, and in mice and rats of both sexes at 5,000 ppm. Absolute liver weight was also increased in male and female Sprague-Dawley rats after 4 weeks of inhalation exposure to 4,000 ppm ETBE (White et al., 1995). Although serum levels of hepatic enzymes were measured and histological observations were made of the livers in both rat studies, there was no additional support for hepatotoxicity in the rat data. The incidence of centrilobular hypertrophy in the livers of both male and female mice exposed to 5,000 ppm was significantly higher than in controls. Hepatic proliferation was also measured in the CD-1 mice as part of the Medinsky et al. (1999) study, although it was not measured in rats. Dose-related increases in the LI were seen in the livers of male mice exposed to 1,750 and 5,000 ppm after 1 and 4 weeks of exposure (but not after 13 weeks) and female mice exposed to 1,750 and 5,000 ppm after 1 and 13 weeks of exposure (but not after 4 weeks); the effect was thought to be consistent with a mitogenic response of the liver to ETBE. Small statistically significant changes in peripheral hematology parameters were also measured in the

Medinsky et al. (1999) study in rats that were not considered to be clinically significant by the authors. There was a 1% increase in MCV in female rats exposed to 5,000 ppm ETBE at the interim sampling point and females exposed to both 1,750 and 5,000 ppm at the 13-week time point. There was a 4% decrease in MCHC in male rats exposed to 5,000 ppm ETBE at the interim sampling point and a 2.5% decrease in males exposed to both 1,750 and 5,000 ppm at the 13-week time point. The range of historical values is not discussed by the authors and no changes in MCV or MCHC were observed in the parallel study in CD-1 mice at any dose. There was no pattern to support a relationship between the small changes in MCV or MCHC and increased liver weight or any other liver data in rats and mice from the available studies. Such a relationship would support acetaldehyde-mediated hepatotoxicity (i.e., via one of the main metabolites of ETBE), as elevated MCV is a good predictor of liver disease in alcoholics (Conigrave et al., 1993). Therefore, in the absence of any histopathological evidence or serum enzyme evidence of hepatotoxicity, and with no evidence of a relationship between the 1% increase in MCV in female rats and observed minimal hepatic effects (e.g., increased liver weight in female rats at 5,000 ppm only compared the 1% MCV at 1,750 and 5,000 ppm or the absence of elevated MCV in male rats or mice of either sex despite increased liver weight in male rats and CD-1 mice of both sexes), the small increase in MCV in female rats only was not considered support for hepatotoxicity.

There was no evidence of neurotoxicity from two studies that performed a FOB in rats after 4 or 13 weeks inhalation exposure at concentrations up to 5,000 ppm (Dorman et al., 1997; White et al., 1995). However, high doses of ETBE (4,000–5,000 ppm) were associated with ataxia and sedation, described by the authors as being associated with transient CNS depression. In both experiments, signs associated with CNS depression were absent within 15 minutes to 1 hour following exposure.

No typical reproductive or developmental toxicity studies of inhalation exposure to ETBE were identified, although the Medinsky et al. (1999) 13-week exposure study of F344 rats did include histology of the gonads and some reproductive tissue. Degenerated spermatocytes were found in the testes of rats from all treatment groups, including controls. The percentage of seminiferous tubules containing degenerated spermatocytes was significantly increased at the two high doses. The authors did not observe any other effects on the testes or reproductive tissue following ETBE exposure from the other data collected (e.g., epididymal histopathology and examination of the seminiferous tubules for a shift in developmental stage or the presence of lumenal debris). However, standard endpoints of spermatocyte function that would be included in a two-generation reproduction and fertility study (e.g., epididymal sperm motility, epididymal sperm count, and epididymal sperm morphology) were not included in the Medinsky et al. (1999) study.

There is no evidence of other systemic effects resulting from inhalation exposure to ETBE from the limited published data available. However, Medinsky et al. (1999) reported a

treatment-related increase in the incidence of congestion in the bone marrow of female rats. No additional description of the observation was provided by the authors. The bone marrow histology was considered not clinically relevant by the authors, as it was not supported by any changes in hematopoietic cell populations.

ETBE is metabolized to acetaldehyde and TBA. Acetaldehyde, primarily a point-of-contact toxicant, is an effective respiratory tract irritant. Although results from inhalation studies with ETBE in humans (Nihlén et al., 1998a, b) provide some evidence for irritant action, data are unavailable to determine if the effect is related to the parent compound or metabolites.

In a subchronic inhalation study of TBA in F344/N rats and B6C3F_1 mice (0, 135, 270, 540, 1,080, or 2,100 ppm, 6 hours/day, 5 days/week), Mahler (1997) reported outward symptoms that were consistent with alcohol toxicity (rough coat, hypoactivity, ataxia, prostration). Absolute and relative kidney weights were increased in both sexes of rats, as were relative liver weights in female rats, at the two highest doses. Male rats displayed dose-related increases in the severity of chronic nephropathy. Gonad histology was unaffected in both sexes.

There are few studies of the toxicity of ETBE following inhalation exposure. Available data suggest hepatic and renal effects. The database is insufficient to determine if the observed effects of ETBE exposure, such as increased liver and kidney weight, are related to the parent compound or its metabolites.

An overview of animal studies with inhalation exposure to ETBE is provided in Table 4-9.

Table 4-9. Subchronic inhalation toxicity studies for ETBE

Species	Dose /duration	NOAEL[a] (ppm)	LOAEL[a] (ppm)	Effect	Reference
Rat (Sprague-Dawley) (10/sex/group)	0, 501, 2,090, and 3,910 ppm, 6 hrs/day, 5 days/wk, for 4 wks	501	2,090	Increase in liver weight [+16.8% absolute 16.1% relative in males and +9.5% abs. +12.5% rel. in females at 3,910 ppm; +10% rel. in females at 2,090 ppm]	White et al., 1995
		2,090	3,910	Increase in kidney weight [+12.8% absolute in males]	
		2,090	3,910	Increase in adrenal weight [+13.7% absolute in males]	
Rat (F344) (48/sex/group)	0, 505, 1,748, and 4,971 ppm, 6 hrs/day, 5 days/wk, for 13 wks	505	1,748	Increase in absolute liver weight [+32.4% in males and +25.8% in females at 4,971 ppm; +14.2% in males at 1,748 ppm]	Medinsky et al., 1999
		505	1,748	Increase in absolute kidney weight [+19.3% in males and +21.3% in females at 4,971 ppm; +9.8% in males and +12.2% in females at 1,748 ppm]	
		Not detected	505	Renal effects in males, i.e., regenerative foci, hyaline droplet accumulation with presence of $alpha_{2u}$-globulin	
		505	1,748	Increase in % seminiferous tubules with spermatic degeneration [+12.7% at 4,971 ppm and +7.8% at 1,748 ppm]	
		1,748	4,971	Increase in absolute adrenal weight [+34.3% in males and +17.8% in females]	
Mice (CD-1) (40/sex/group)	0, 501, 1,754, and 4,962 ppm, 6 hrs/day, 5 days/wk, for 13 wks	501	1,754	Increase in absolute liver weight [+18% in males and +32.6% in females at 4,962 ppm; +12.9% in males and 19% in females at 1,754 ppm]; with hepatic centrilobular hypertrophy in both sexes at 4,962 ppm	Medinsky et al., 1999
Rat (F344) (12 sex/group from Medinsky et al., 1999; Bond et al., 1996a)	0, 505, 1,748, and 4,971 ppm, 6 hrs/day, 5 days/wk, for 13 wks	4,971	Not detected	Transient ataxia immediately after exposure	Dorman et al., 1997

[a]Reported concentrations are non-duration-adjusted animal exposures.

4.6.3. Mode of Action Information

There are only a few studies that have been conducted to evaluate the effects of exposure to ETBE by the oral or inhalation route. There is a single inhalation-exposure study (Medinsky et al., 1999) that was designed to provide information relevant to the determination of alpha$_{2u}$-globulin-associated nephropathy under the assumption that ETBE would lead to renal cytotoxicity based on its structural similarity to MTBE, a compound that has been shown to be a renal toxicant in male rats (Cal EPA, 1999; Prescott-Mathews, et al., 1997). Detailed studies on the potential toxicity of ETBE to any organ system have not been conducted.

Kidney Effects:

Several studies (see Sections 4.2.3.1 and 4.3) have demonstrated that oral or inhalation exposure to ETBE results in kidney effects. For example, ETBE exposure was associated with increased kidney weight in several studies: in male Sprague-Dawley rats in a 4-week inhalation study (White et al., 1995); in male and female F344 rats, but not CD-1 mice, in a 13-week inhalation study (Medinsky et al., 1999); and in male and female Sprague-Dawley rats in an oral two-generation reproduction and fertility study (CIT 2004b, unpublished report). The Medinsky et al. (1999) study focused on the potential role of alpha$_{2u}$-globulin based on the assumption that there would be overt renal effects associated with ETBE because of the renal toxicity found with the methyl analog (MTBE). The data from Medinsky et al. (1999) also showed increased hyaline droplet formation, the presence of alpha$_{2u}$-globulin in the proximal tubules, a twofold increase in LI, and the presence of regenerative foci in male F344 rats but not female rats. Regenerative foci are associated with repair of damaged tubules. Therefore, the presence of regenerative foci represents indirect evidence of necrosis, as some cells of the proximal tubule would have had to have died and the remaining cells undergone regenerative proliferation for regenerative foci to be observed.

General issues concerning the determination of alpha$_{2u}$-globulin-associated nephropathy

Alpha$_{2u}$-globulin derived from hepatic synthesis is unique to male rats; it is not generally found in female rats or in mice or humans of either sex (U.S. EPA, 1991b; Alden, 1986). Although young male rats may have some hyaline droplets in proximal tubules with alpha$_{2u}$-globulin, chemically-induced accumulation of alpha$_{2u}$-globulin in the proximal tubule is restricted to mature male rats. Therefore, data from mice and female rats can be used to demonstrate that chemical-associated nephrotoxicity is male-rat specific. The increased alpha$_{2u}$-globulin accumulation is proposed to result from reduced renal catabolism of the alpha$_{2u}$-globulin-chemical complex. The resulting accumulation is thought to initiate a sequence of events leading to chronic proliferation of the renal tubule epithelium, as well as an exacerbation of chronic progressive nephropathy. The histopathological sequence in mature male rats, potentially leading to the formation of renal tumors consists of the following:

- Excessive accumulation of hyaline droplets containing alpha$_{2u}$-globulin in renal proximal tubules
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium
- Sustained regenerative tubule cell proliferation, providing exposure continues
- Development of intralumenal granular casts from sloughed cell debris associated with tubule dilation and papillary mineralization
- Foci of tubule hyperplasia in the convoluted proximal tubules, and
- Renal tubule tumors

In the absence of information demonstrating that the alpha$_{2u}$-globulin processes is operative (as in criteria 1–3 below), it should be assumed that male rat nephropathy is relevant for risk assessment purposes. Additional categorical guidelines available from the Risk Assessment Forum Technical Panel Report (U.S. EPA, 1991b) outline the data necessary to determine the involvement of alpha$_{2u}$-globulin and the full report should be consulted rather than the summary presented here.

The following information from adequately conducted studies of male rats is used for demonstrating that the alpha$_{2u}$-globulin process may be a factor in any observed renal effects–an affirmative response in each of the three categories is required. If data are lacking for any of the criteria in any one category, the available renal toxicity data should be considered relevant to humans and analyzed in accordance with standard risk assessment principles. The three categories of information and criteria are as follows:

(1) *Increased number and size of hyaline droplets in the renal proximal tubule cells of treated male rats*. The abnormal accumulation of hyaline droplets in the proximal tubule helps differentiate alpha$_{2u}$-globulin inducers from chemicals that produce renal tubule toxicity by other modes of action.

(2) *Accumulating protein in the hyaline droplets is alpha$_{2u}$-globulin*. Hyaline droplet accumulation is a nonspecific response to protein overload and, thus, it is necessary to demonstrate that the protein in the droplet is, in fact, alpha$_{2u}$-globulin.

(3) *Additional aspects of the pathological sequence of lesions associated with alpha$_{2u}$-globulin nephropathy are present*. Typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia. If the response is mild, not all of these lesions may be observed. However, some elements consistent with the pathological sequence must be demonstrated to be present.

The Risk Assessment Forum Technical Panel Report (U.S. EPA, 1991b) also suggests additional information that may be useful for the analysis including sustained cell division in the proximal tubule of the male rat. This relates to a sustained increase in cell replication of the renal tubule at doses used in the studies and a dose-related increase in atypical hyperplasia of the

renal tubule is consistent with the alpha$_{2u}$-globulin process, especially if other laboratory animals were tested and did not show similar responses.

The report specifically states: "If a compound induces alpha$_{2u}$-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate endpoint to determine noncancer (systemic) effects potentially occurring in humans."

ETBE and alpha$_{2u}$-globulin-associated renal effects

Criteria (1): Data from Medinsky et al. (1999) demonstrated a dose-dependent increase in hyaline droplet formation in the proximal tubule of male rats following inhalation exposure to ETBE for 1, 4, and 13 weeks (Table 4-4). Hyaline droplet severity was expressed as a mean grade scored with 1 for minimal, 2 for <10%, 3 for 10–25%, 4 for 25–50%, and 5 for >50% of the cortex involved with Mallory-Heidenhain staining. The Mallory-Heidenhain staining of female rats was negative for all time points. Only the mean scores are presented, without standard errors or statistics. The authors state that there is a concentration-dependent increase in hyaline droplet accumulation in the male rat kidney, although the differences are small on this semi-quantitative scale after 1 week of exposure (Table 4-4: severity of 1.2 in controls, 3.4 at 500 ppm, 4.0 at 1,750 ppm, and 4.6 at 5,000 ppm). A similar difference is also reported after 4 weeks of exposure (Table 4-4: severity of 1.8 in controls, 2.6 at 500 ppm, 3.4 at 1,750 ppm, and 3.8 at 5,000 ppm) and 13 weeks of exposure (Table 4-4: severity of 1.8 in controls, 3.0 at 500 ppm, 3.2 at 1,750 ppm, and 3.8 at 5,000 ppm). Although the data minimally support the first criteria, a quantitative measure of hyaline droplet number and size would represent stronger data.

Criteria (2): Alpha$_{2u}$-globulin immunoreactivity was observed in the hyaline droplets of the renal proximal tubular epithelium of male rats from all exposure groups, and not in female rats (Medinsky et al., 1999). There was no quantitative determination of the alpha$_{2u}$-globulin in the study. The authors state that there is an ETBE-related increase in hyaline droplets and that these droplets are immunoreactive for alpha$_{2u}$-globulin (Medinsky et al., 1999).

Criteria (3): There are two additional aspects of the pathological sequence associated with alpha$_{2u}$-globulin-related effects that are present in male rats from the Medinsky et al. (1999) study: sustained cell proliferation and the presence of regenerative foci. In male rats exposed to ETBE, after 1 or 4 weeks of exposure, a greater than twofold increase in the cell proliferation or LI in the renal tubule occurred at the high dose only (Table 4-5). The increased cell proliferation in high-dose males was sustained through 13 weeks of exposure as determined by the LI. After 13 weeks of exposure, the LI was statistically increased by more than twofold in males relative to control for all doses of ETBE, and, therefore, at all doses demonstrating elevated hyaline droplet severity. However, the value of the LI at 13 weeks, does not demonstrate a dose-response (LI = 0.91, 2.16, 3.40, and 2.47 for 0, 500, 1,750, and 5,000 ppm ETBE). The difference between the LI for the low dose (500 ppm) and the control was significant at $p < 0.05$, while the difference between the two higher doses and the control were significant at $p < 0.001$.

Tubule cell proliferation was not sustained in female rats exposed to ETBE, although cell proliferation was statistically increased in female rats after 1 week of exposure. The less than twofold increase was observed at all doses with no evidence of a dose-response in female rats. The LI remained increased by less than twofold in female rats at the high dose (5,000 ppm) at the 4 week time point. No effect on the renal LI in female rats was seen at any exposure concentration after 13 weeks of exposure to ETBE.

The presence of regenerative foci is also an indication of $alpha_{2u}$-globulin-associated effects. The number of regenerative foci was increased in male rat kidneys in the 1,750 and 5,000 ppm dose groups, respectively, after 4 weeks of exposure (Table 4-4). After 13 weeks of exposure, there was a dose-response relationship in the mean number of regenerative foci observed with 2, 11, 17, and 34 foci for 0, 500, 1,750, and 5,000 ppm ETBE, respectively. The available data do not indicate other aspects of the pathological sequence of lesions associated with $alpha_{2u}$-globulin-related effects such as single-cell necrosis, exfoliation of the epithelial cells into the proximal tubular lumen, granular casts, or linear mineralization of papillary tubules. The Risk Assessment Forum Technical Panel Report (U.S. EPA, 1991b) notes that if the $alpha_{2u}$-globulin response is mild, not all of these lesions may be observed, but states that elements consistent with the pathological sequence must be demonstrated to be present. The absence of any of these typical lesions in the pathological sequence in the available data for ETBE presents considerable uncertainty in the determination of $alpha_{2u}$-globulin as a mode of action for observed effects. It is important to note that the presence of regenerative foci represent indirect evidence of necrosis because regenerative foci are associated with repair of damaged tubules, and therefore, some cells of the proximal tubule would have had to have died and the remaining cells undergone regenerative proliferation for this to occur. However, direct evidence of lesions associated with $alpha_{2u}$-globulin-related pathological sequence would represent stronger data.

There is not enough information to determine if some or all of ETBE-induced kidney effects are caused by the parent compound or its metabolite, TBA. Although there is no evidence of renal cancer in the single study of ETBE carcinogenicity, the nephropathy and subsequent cancer incidence in male rats exposed to TBA has been postulated to be mediated by $alpha_{2u}$-globulin accumulation (Williams and Borghoff, 2001; Takahashi et al., 1993). However, the dose associated with an increase in the severity of chronic nephropathy in male rats in a 13-week inhalation study (Mahler, 1997) is below doses associated with accumulation of protein droplets in the kidney either in that study (Mahler, 1997) or in a 10-day exposure (Borghoff et al., 2001), suggesting that $alpha_{2u}$-globulin may not be the mechanism or the only mechanism for TBA-associated nephropathy. More detailed evaluation of nephrotoxicity associated with TBA and data on the relationship between ETBE metabolism and toxicity will be required to answer this question.

ETBE and renal effects in female rats

As stated above, ETBE exposure was associated with increased kidney weight in female F344 rats in a 13-week inhalation study (Medinsky et al., 1999) and female Sprague-Dawley rats from an unpublished oral two-generation reproduction and fertility study (CIT 2004b, unpublished report), but not in female Sprague-Dawley rats in a 4-week inhalation study (White et al., 1995), or in female CD-1 mice in a 13-week inhalation study (Medinsky et al., 1999). There was no evidence of renal lesions, and both Mallory-Heidenhain staining and $alpha_{2u}$-globulin immunoreactivity of kidneys from female rats were negative for all time points (1, 4, and 13 weeks) in the 13-week inhalation study (Medinsky et al., 1999). Although renal tubule cell proliferation was statistically increased in all female rats exposed to ETBE after 1 week of exposure, the increase was less than twofold, and the increase was not sustained in female rats exposed to ETBE. The LI remained elevated by less than twofold in female rats at the high dose (5,000 ppm) at the 4 week time point and no effect on renal cell proliferation was seen at any exposure concentration in female rats after 13 weeks of exposure to ETBE. The short-term increase in proliferation in females (and in the absence of $alpha_{2u}$-globulin) suggests that a non-$alpha_{2u}$-globulin mechanism may be responsible for this short-term effect in females. No increase in LI was seen in female rats after similar short-term (10-day) inhalation exposure to TBA (Borghoff et al., 2001) at concentrations up to 1,750 ppm or MTBE (Prescott-Mathews et al., 1997) at concentrations up to 3,013 ppm.

Summary

There are data indicating renal toxicity in male and female rats following ETBE exposure. ETBE exposure causes an increase in renal weight in both male and female rats and a semi-quantitative increase in hyaline droplet formation in the proximal tubule of male rats (and not female rats). The presence of $alpha_{2u}$-globulin in the hyaline droplets has been confirmed in male rats and not female rats by immunoreactivity studies, sustained cell proliferation in cells of the renal tubule has been shown to occur in male rats with a transient increase observed in female rats, and regenerative foci have been identified in male rats. The presence of regenerative foci indicates that cellular repair mechanisms are responding to cell death. Typical lesions in the pathological sequence associated with $alpha_{2u}$-globulin nephropathy include: evidence of single cell necrosis, exfoliation of epithelial cells into the proximal tubule lumen, the presence of granular casts in the renal tubule, linear mineralization of tubules within the renal papilla, and tubule hyperplasia. These effects have not been reported in the available studies. Female rats have been shown to exhibit an increase in kidney weight and an increase in renal tubule cell proliferation that was not sustained following ETBE exposure. Given the available data, a determination cannot be made as to whether $alpha_{2u}$-globulin accumulation is the mode of action or the only mode of action for renal effects observed in male and female rats associated with ETBE exposure.

All of the data supporting alpha$_{2u}$-globulin-associated kidney effects are from a single subchronic study (Medinsky et al., 1999). A well-conducted chronic study to examine the effects of ETBE exposure on the kidney of rats and mice of both sexes would be beneficial to determine the role of alpha$_{2u}$-globulin in observed renal toxicity associated with ETBE.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight-of-Evidence

No epidemiological evidence is available that allows for the assessment of the carcinogenic potential of ETBE in humans. ETBE has not been shown to act as a genotoxicant in any of the available assays of ETBE, although some studies did not control for potential evaporative loss of ETBE during exposure. In addition, a G/ETBE mixture exposure study reported equivocal results in the micronucleus test. In the only available animal cancer bioassay, Maltoni et al. (1999) reported an increased incidence of four tumors or combined tumors and precancers reported as pathologies of oncological interest in Sprague-Dawley rats exposed to 0, 250, and 1,000 mg/kg-day of ETBE by gavage 4 days/week for 104 weeks. The authors reported a statistically significant increase in the total pathologies of oncological interest of the mouth epithelium in males at the high dose of ETBE and total malignant uterine tumors (a classification that included vaginal schwannomas) in females that was restricted to the low dose of ETBE only. Nonsignificant increases in hemolymphoreticular neoplasias in both sexes and total pathologies of oncological interest of the forestomach in males that was restricted to the low dose of ETBE only were also reported. No other tumor types were found to be elevated in this study. The Maltoni et al. (1999) study utilized two dose levels and both doses tested caused increased mortality at early time points.

The Maltoni et al. (1999) study showed early mortality associated with both doses of ETBE. The ETBE-associated mortality presents a complication on the ability to interpret increased tumor incidences that were reported to be restricted to the low-dose group (i.e., total malignant uterine tumors in females and total pathologies of oncological interest of the forestomach in males) based on the possibility that the increased mortality in high-dose animals could potentially mask an observation of increased tumor incidence that might otherwise be seen at the high dose. Individual animal data were not provided, and therefore, survival analyses or a time to tumor analysis could not be conducted. It is not known if the increased mortality was secondary to toxic or carcinogenic effects.

No criteria were defined for the determination of dysplasia or for potential grading of lesions, which introduces uncertainty in the reported increase in the total pathologies of oncological interest for the forestomach and the mouth epithelium where dysplasias represent high portions of those totals (i.e., 58 and 69% of the total pathologies in the forestomach were squamous cell dysplasias and 100 and 73% of the total pathologies in the mouth epithelium were squamous cell dysplasias at 250 and 1,000 mg/kg respectively) (Table 4-1). The grade or rating

of dysplasia is particularly important to the predictive value of these precancers, as the majority of lesions with mild to moderate dysplasia do not progress into cancer (Rosin et al., 2000; Schepman et al., 1998). Precancerous lesions identified as dysplasias represent the majority of lesions that the authors include within their categories of total pathologies of oncological interest for both the mouth epithelium and forestomach, which limits the ability to interpret the relevance of the increased incidences of these two categories of lesions.

Maltoni et al. (1999) did not indicate the pathological information used to delineate tumors associated with the tongue from other oral pathologies, or the grouping of tissues of the uterus and vagina. These tissues were also listed separately and when the vaginal schwannomas are removed from the uterine tumors, the total malignant tumors of the uterus (Table 4-1) were not statistically increased relative to control at either dose by χ^2 test (the same test used by the authors on the grouped data).

The mode(s) of action of ETBE for the increased incidence of tumors or combined tumors and precancers reported in Maltoni et al. (1999) are unknown. It is unclear whether or not observed effects are associated with the parent compound or the metabolites. A limited number of unpublished in vivo and in vitro genotoxicity tests demonstrate no DNA damage resulting from exposure to ETBE and all available assays of genotoxicity of ETBE were negative. Carcinogenicity data from the metabolites of ETBE (TBA and acetaldehyde) or MTBE, the methyl analog of ETBE, indicate dissimilar tumor types for these compounds. The available carcinogenicity data for MTBE support the possibility that ETBE may cause an increase in lymphohemoreticular neoplasias, as there is evidence that exposure to MTBE is associated with an increase in lymphohematopoietic cancer in female rats (Belpoggi et al., 1998, 1997, 1995). However, as discussed in Sections 4.2.3 and 4.6.2, the increase in these tumors following ETBE exposure is slight, not statistically significant, and shows no evidence of dose response (3/60, 8/60, and 6/60 in males and 3/60, 6/60, and 5/60 in females, at 0, 250, and 1,000 mg/kg-day respectively).

The data reported in Maltoni et al. (1999) indicated that exposure to ETBE is associated with a statistically significant increased incidence in total uterine tumors in females and a combined total pathologies of oncological interest of the mouth epithelium in males. Although the total pathologies of oncological interest in the mouth epithelium includes precancers as well as tumors, the total uterine tumors does not include any precancers. Maltoni et al. (1999) also report a nonstatistical increase in hemolymphoreticular neoplasias in both sexes and total pathologies of oncological interest of the forestomach in males. The increased incidence of uterine tumors in female rats was restricted to the low dose of ETBE, and the individual animal data are not presented to allow a time to tumor analysis to evaluate if the increased mortality in the high-dose females prevented a dose-response in uterine tumors. In addition, uterine tumors alone are not statistically increased, and it is only when tumors from additional tissues (i.e., vaginal schwannomas) are included that the low dose increase is statistically significant. The

reported increase in pathologies of oncological interest of the mouth epithelium in males at the high dose of ETBE contains considerable uncertainty because of the dependence on the inclusion of squamous cell dysplasias (which represent 73% of the total at the high dose) without the histopathological criteria necessary to evaluate the predictive value of these precancers. The increase in lymphohemoreticular neoplasias and total pathologies of oncological interest of the forestomach in males are not statistically significant and showed no evidence of a dose response.

Thus, under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *suggestive evidence of human carcinogenicity* of ETBE based on the only oral cancer bioassay in Sprague-Dawley rats (Maltoni et al., 1999). The classification is consistent with an example provided in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) which suggests *suggestive evidence of human carcinogenicity* when a statistically significant increase is seen at one dose only, but no significant response at the other doses, and no overall trend.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

No studies are available that assess the carcinogenic potential of ETBE in humans. In the single chronic cancer bioassay evaluating the carcinogenic potential of ETBE, Maltoni et al. (1999) exposed 8-week-old Sprague-Dawley rats (60/group) to 0, 250, and 1,000 mg/kg-day ETBE by gavage 4 days/week for 104 weeks. Animals were allowed to die a natural death; upon death, animals were necropsied and tissues were preserved in 70% ethyl alcohol for later histopathological evaluation and classification. The tumor data were presented according to dose group, tumor site, and histiotype, and in some cases, malignant tumors were distinguished from benign or total tumors (Table 4-1). There was a statistically significant increase in the incidence of total pathologies of oncological interest of the mouth epithelium (including oral cavity, tongue, and lips) in males at the high dose (1,000 mg/kg-day) of ETBE and total malignant uterine tumors (a classification that included vaginal schwannomas) in females at the low dose (250 mg/kg-day) of ETBE only. No other tumor types were found to be significantly elevated in this study; however, the authors also reported nonsignificant increases in hemolymphoreticular neoplasias in both sexes and total pathologies of oncological interest of the forestomach in males at the low dose of ETBE.

The authors reported limitations associated with the design and conduct of the study, including the use of only two dose levels, testing only a single animal species, and the fact that both test doses of ETBE caused increased mortality of ETBE-exposed rats.

The increased mortality of animals at both doses of ETBE presents a limitation in the study and ability to interpret the results from a quantitative perspective. Mortality differed for each dose and sex such that, for some weeks of the study, mortality was increased in treated animals relative to controls, whereas for other weeks, there was no difference in mortality, or treated ETBE-exposed animals had lower mortality. Digitization of the data from the survival curves in Maltoni et al. (1999) shows an approximately 8–20% increase in mortality of high dose

females relative to control animals from weeks 56 to 88. A similar increased in mortality (8–30%) was also observed in the high-dose males relative to controls from weeks 56 to 120. Low-dose males and females exhibited a smaller increase in mortality (e.g., mortality in males was increased by 7–17% in low-dose males and 11% in low-dose females). The increased mortality in low dose males and females was observed at fewer time points than animals exposed to the high dose of ETBE. For example, low-dose females only displayed increased mortality at 88 weeks of age relative to controls. Low-dose males exhibited increased mortality relative to controls from weeks 56 to 88 and were similar to controls at other time points. The authors suggested that due to the increased mortality in the ETBE exposed rats, some of the carcinogenic effects were only observed at the low dose and not at the high dose.

In the absence of that data on tumor incidence and survival of individual animals, a quantitative time to tumor analysis cannot be performed. In one of the examples where an effect is reported at the low exposure dose only, the authors describe an increase in the total incidence of pathologies of oncological interest (acanthomas, squamous cell dysplasias, and squamous cell carcinomas) of the forestomach in males at the low dose (250 mg/kg-day), while the incidence at the high dose (1,000 mg/kg-day) was identical to controls (13/60, 24/60, and 13/60, at 0, 250, and 1,000 mg/kg-day respectively). The increased mortality associated with high-dose males after 40 weeks of age, provides some support for the possibility that an effect at the high dose of ETBE is obscured by increased mortality in this group; however, that suggestion cannot be evaluated without individual animal data on mortality and tumor incidences. Similarly, Maltoni et al. (1999) reported a statistical increase in total malignant tumors (carcinomas and sarcomas) of the uterus (that includes tumors of the uterus and vagina) at the low dose (250 mg/kg-day), while the tumor incidence at the high dose was identical to controls (2/60, 10/60, 2/60, respectively). Based on the survival curves, the appearance of malignant uterine tumors would need to be correlated to weeks 56–88 to support a dose response because the mortality of the high dose females was only increased relative to controls during that time period. The decreased mortality of females in the high-dose group relative to controls after 88 weeks of age would make the detection of tumors more likely in females exposed to the high dose during this time period, not less likely.

Maltoni et al. (1999) reported dysplasias as one of the pathologies of oncological interest for both the mouth epithelium and the forestomach. The increase in total pathologies of oncological interest was only statistically significant for the grouped lesions the mouth epithelium, not for any individual tumor type or precancer. The authors report the pathologies of oncological interest of the forestomach as nonsignificantly increased. The criteria for the determination of dysplasia or for potential grading of lesions were not described for either the forestomach or the mouth epithelium. No rating of dysplasias was reported for the forestomach, and two grades of dysplasia were included within the total pathologies of oncological interest of the mouth epithelium. The inclusion of a separate, more severe classification for squamous cell

dysplasias borderline with in situ carcinoma in the mouth epithelial data as a distinct category from squamous cell dysplasias suggests that the criteria for general squamous cell dysplasias used by Maltoni et al. (1999) for both the mouth epithelium and forestomach may have included mild to moderate dysplasias, which, as described below, may not be predictive for cancer. This more severe lesion was only reported in one of the male rats at any dose and the majority of the total pathologies of oncological interest of the mouth epithelium were general squamous cell dysplasias (which represented 83, 100, and 73% of the total at 0, 250, and 1,000 mg/kg-day respectively). The rating of dysplasia is particularly important to the predictive value of these precancers. Although there is a good correlation between severe dysplasia and eventual carcinoma, the majority of lesions with mild to moderate dysplasia do not progress into cancer (Rosin et al., 2000; Schepman et al., 1998). The use of squamous cell dysplasia data without explicit criteria or the relative number of mild to moderate dysplasia for both the total pathologies of oncological interest of the mouth epithelium and the forestomach is problematic to the interpretation of data from Maltoni et al. (1999). In both cases, that the study authors suggested that there was an increase in total pathologies of interest in male rats, dysplasias represent high portions of that total (i.e., 58 and 69% of the total pathologies in the forestomach were squamous cell dysplasias and 100 and 73% of the total pathologies in the mouth epithelium were squamous cell dysplasias at 250 and 1,000 mg/kg respectively) (Table 4-1). Therefore, the fact that the precancerous lesions identified as dysplasias represent the majority of lesions that the authors include within their categories of total pathologies of oncological interest for both the mouth epithelium and forestomach, limits the ability to interpret the relevance of the increased incidences of these two categories of lesions reported in Maltoni et al. (1999).

As discussed above, the pathologies of oncological interest in both the mouth epithelium and forestomach included both tumors and precancers. The pathologies of oncological interest of the mouth epithelium listed by the authors consisted of several organs including the tongue, lips, and oral cavity. Of note in this grouping is the issue that tongue cancer is different from other cancers of the oral cavity and should be considered separately (Sathyan et al., 2006). In addition, tissues of the uterus and vagina were combined within the category of total malignant tumors of the uterus, one of the two tumor types listed as statistically increased by Maltoni et al. (1999). Although, the authors indicated that the incidence of total malignant tumors of the uterus were increased at the low dose of 250 mg/kg-day (and not at 1,000 mg/kg-day), it appears that four malignant schwannomas of the uterus-vagina were included in the eight total uterine sarcomas at 250 mg/kg-day. When the vaginal schwannomas are removed from the uterine tumors, the total malignant tumors of the uterus (Table 4-1) were not statistically increased relative to control at either dose by χ^2 test (the same test used by the authors on the grouped data).

There is some evidence of carcinogenic potential for the two primary metabolites of ETBE; TBA, as indicated in a 2-year study conducted by the National Toxicology Program

(NTP, 1995; Cirvello et al., 1995), and acetaldehyde (WHO, 1995). The NTP study (NTP, 1995; Cirvello et al., 1995) concludes that chronic exposure to TBA is associated with an increased incidence of combined adenoma or carcinoma in the kidney of male F344 rats and thyroid follicular cell adenoma in female $B6C3F_1$ mice with equivocal evidence of thyroid follicular cell adenoma or carcinoma in male mice. The World Health Organization (WHO) assessment suggests that the carcinogenicity of acetaldehyde (increased incidence of nasal tumors in male and female rats and laryngeal tumors in hamsters) is associated with the irritancy of acetaldehyde, as it is only seen at concentrations above the NOEL for irritation.

There is also evidence for the carcinogenicity of MTBE, the methyl analogue of ETBE, which appears to be a multi-site and multi-species carcinogen (Cal EPA, 1999; Mennear, 1997). Exposure to MTBE is associated with increased incidence of tumors in both sexes of two species of animals. Specifically, hepatocellular tumors in both male and female mice (Bird et al., 1997; Burleigh-Flayer et al., 1992), renal tumors in male rats (Bird et al., 1997; Chun et al., 1992), Leydig cell tumors in male rats (Belpoggi et al., 1998, 1997, 1995; Bird et al., 1997; Chun et al., 1992), and lymphohematopoietic cancer in female rats (Belpoggi et al., 1998, 1997, 1995) have been observed following exposure to MTBE. The Belpoggi et al. (1998, 1997, 1995) studies were conducted in the same laboratory as the single, preliminary report of results of the ETBE cancer bioassay (Maltoni et al., 1999). The mode(s) of action for carcinogenicity for ETBE are unknown for any of the tumor types reported in Maltoni et al. (1999). It is also unclear whether or not observed effects are associated with the parent compound or the metabolites. The small database for ETBE contributes to the uncertainty, but carcinogenicity data from the metabolites of ETBE (TBA and acetaldehyde) or MTBE, the methyl analog of ETBE, are not similar to either of the tumor types that were statistically increased in Maltoni et al. (1999) (i.e., total malignant uterine tumors in females at the low dose and total pathologies of oncological interest of the mouth epithelium in males) as they were not observed with these structurally related chemicals. The evidence for the carcinogenicity of MTBE supports the possibility that ETBE may cause an increase in lymphohemoreticular neoplasias, as there is evidence that exposure to MTBE is associated with an increase in lymphohematopoietic cancer in female rats (Belpoggi et al., 1998, 1997, 1995). However, as discussed above and in Section 4.2.3, the increase in these tumors following ETBE exposure is slight, not statistically significant, and shows no evidence of dose response.

4.7.3. Mode of Action Information

ETBE has not been shown to act as a genotoxicant in most of the tests conducted, with equivocal results in the micronucleus test. Medinsky et al. (1999) concluded that the increased hepatocyte labeling indices observed at several time points and all doses tested during a 13-week subchronic inhalation study in mice might indicate a mitogenic response to ETBE in the liver.

However, there was no evidence of hepatic tumors in the only chronic ETBE study (i.e., Maltoni et al., 1999).

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

The limited data on ETBE (presented in Section 4.3) are insufficient to determine if ETBE is a teratogen or a developmental toxicant. General patterns of enzymes levels associated with development suggest that humans at early age may have diminished ability to metabolize ETBE because of decreased levels or reduced activity of the CYP enzymes that metabolize ETBE. Direct evidence of enzyme differences from ETBE exposure studies in humans is not available. Therefore, the limited available developmental toxicity data on ETBE are inadequate to determine if children are more susceptible to potential ETBE toxicity.

The primary enzymes likely to be involved in ETBE metabolism in humans based on in vitro studies using liver microsomes are CYP2A6, 3A4, and 2B6, in rank order of their contribution to the initial step in metabolism of ETBE from the parent compound to TBA and acetaldehyde. Much of the knowledge concerning childhood levels of CYPs comes from studies on drug clearance. Dorne and coworkers (Dorne et al., 2005; Dorne, 2004) have published evaluations of factors associated with drug clearance vs. ethnicity or age. These data indicate that clearance of CYP3A4-metabolized drugs should be threefold lower in neonates than in adults, while in children 1–16 years of age, it should be about 1.4-fold higher. Data on the activity of CYP3A4 from the drug clearance data may be relevant to potential age-associated susceptibility to ETBE, as CYP3A4 is one of the three main CYPs likely to contribute to ETBE metabolism; however, other enzyme activities relevant to ETBE metabolism were not presented by Dorne et al. (2005). Alcorn and McNamara (2002) also used a mathematical approach to assess hepatic and renal clearance in children. They produced scaling factors derived from known enzyme activities and physical parameters (body, liver weight). According to their calculations, clearance of CYP3A4-metabolized drugs in fetuses of less than 30 weeks should be approximately 4% that of adults, climbing to about 17% immediately after birth, and to 50% within the first 2 months of life. This was essentially confirmed by findings of de Wildt et al. (1999). However, these findings may be inconsistent with a report by Blanco et al. (2000), who investigated CYP3A4 activities in human liver microsomes between birth and 75 years of age. A linear regression of their data revealed no change of CYP3A4 activity with age, but it is not evident that any of their microsome preparations came from donors less than 1 year of age.

Hines and McCarver (2002) assembled data on metabolic activities of human CYPs with developmental age. They reported that CYP2A6 activity in nasal mucosa of children 13–18 weeks old was readily detectable, but that liver-specific activity was only 1–5% of adult values. Age-related differences in CYP2A6 may be particularly relevant to ETBE because data from Le Gal et al. (2001) from liver microsome samples suggested that CYP2A6 may account

for the majority of ETBE metabolism in the human liver. Hines and McCarver (2002) concluded that in general, there was no evidence that isozymes from the CYP2A family were expressed in fetal liver. By 1 year of age, however, specific activities had come close to adult levels. There are limited data on the developmental profile of CYP2B6, one of the secondary enzymes that may catalyze the metabolism of ETBE to TBA and acetaldehyde in humans based on data from liver microsomes from Le Gal et al. (2001). Hines and McCarver (2002) reported that CYP2B6 was not detectable in 11- or 24-week-old fetal livers, but that CYP2B6 levels reached about 10% of adult levels during the first year of life. Similarly, CYP3A4, one of the other secondary enzymes that may catalyze the metabolism of ETBE based on liver microsome data from Le Gal et al. (2001), is not expressed in fetal liver; although there is a fetal form for the CYP3A family, CYP3A7. However, its catalytic activity has not been well researched and nothing is known about whether it may be involved in the metabolism of ETBE. It appears that the change from CYP3A7 to CYP3A4 begins immediately after birth. While CYP3A7 is still expressed for some time during early postnatal life, CYP3A4 attains adult activity levels by 1 year of age.

In summary, general patterns of developmental changes in cytochrome P-450 levels indicate that humans at early age may have diminished ability to metabolize ETBE; however, direct evidence of enzyme differences from ETBE exposure studies in humans is not available. The limited available developmental toxicity data on ETBE are inadequate to determine if children are at an increased risk from ETBE exposure. The generation and publication of developmental toxicity data from oral and inhalation studies of ETBE in multiple species would provide data necessary to predict if children are at an increased risk from ETBE exposure.

4.8.2. Possible Gender Differences

The various studies in humans conducted by Nihlén and coworkers (Nihlén et al., 1998a, b), and Dekant and coworkers (Dekant et al., 2001a, b; Amberg et al., 2000; Bernauer et al., 1998), include a low number of individuals and are inadequate to assess gender differences in human susceptibility to ETBE-induced toxicity. Reports of studies in animals (e.g., CIT, 2004b, unpublished report; Medinsky et al., 1999) suggest that male rats are more sensitive to renal effects than females. The available data also suggest greater sensitivity for hepatic effects in rats exposed to ETBE, but not in mice. For example, as part of an oral two-generation reproduction and fertility study, liver weight increases were observed in male rats in the F0 and F1 generation at lower doses (500 mg/kg-day) than females (1,000 mg/kg-day in F1 generation only) (see Table 4-7; CIT, 2004b, unpublished report). However, in an inhalation exposure study in CD-1 mice, hepatic effects including increased liver weight, centrilobular hypertrophy, and increased cellular proliferation were observed in males and females at the same doses (Medinsky et al., 1999). In addition, several studies in animals reported lesser responses in females at the same dose as given to males (e.g., 34% increased adrenal weight in male rats compared to 18%

increase in females after inhalation exposure in Medinsky et al., 1999). It is not clear if this observation is related to differences in metabolism between males and females.

Rademaker (2001) stated that women have a 40% higher activity of CYP3A4 than men, making them potentially more sensitive to toxicants, such as ETBE, that are activated by this enzyme. Studies with human microsomes, however, have not indicated that this is the case (Bebia et al., 2004; Parkinson et al., 2004).

4.8.3. Self-reported Sensitive Individuals

The existence of individuals who are sensitive to MTBE has been suggested in several studies and this may also apply to ETBE. However, most of the data for MTBE are anecdotal and no studies of potential ETBE-sensitive individuals were found in the literature. In one of the few controlled exposure studies of self-reported MTBE-sensitive individuals, Fiedler et al. (2000) compared the performance of 12 subjects that were self-reported as sensitive to MTBE exposure with 19 controls in psychophysiologic and neurobehavioral responses as well as their rating of 42 symptoms previously reported to be associated with exposure to MTBE in gasoline, solvent exposure, anxiety, and depression. Subjects were exposed for 15 minutes to clean air, gasoline, gasoline with 11% MTBE (G/11MTBE), and gasoline with 15% MTBE (G/15MTBE) and symptoms and psychophysiologic and neurobehavioral response were measured before, during, and after exposure. Individuals reported significantly more symptoms than controls during the pre-exposure period, suggesting heightened reporting by these individuals independent of exposure. Individuals reported significantly more symptoms than controls when exposure to a G/15MTBE mixture was contrasted to clean air or G/11MTBE ($p = 0.02$). Although nonsignificant, MTBE-sensitive individuals tended to report more symptoms than controls when exposure to G/15MTBE was contrasted to gasoline alone ($p = 0.08$). No MTBE-related changes in psychophysiologic or neurobehavioral responses were observed. Neither group could distinguish whether MTBE was present at 11 or 15% and a majority of both groups indicated that MTBE was present when they were exposed to gasoline only. Separate analysis of the 42 symptom scores by subclass (i.e., MTBE-, anxiety-, depression-, breathing-, solvent-, and environment-related symptoms) did not confirm the symptom specificity for MTBE exposure suggested by the epidemiologic literature. The data from Fiedler et al. (2000) support the possibility of a MTBE-sensitive population, but did not support a dose-response to MTBE exposure. MTBE-sensitive individuals may also be sensitive to ETBE or there may be a similar ETBE-sensitive population. The physiologic basis for the MTBE-sensitive individuals is unknown; however, Hong et al. (2001) looked for polymorphisms in the enzymes responsible for MTBE metabolism as a possible explanation (see Section 4.8.4 below).

4.8.4. Other—Aging; Gene Polymorphisms

It is generally assumed that human's drug metabolizing capacity is diminished with age. Loss of the activities of three main enzymes that are most likely to be responsible for the metabolism of ETBE to TBA and acetaldehyde (CYP2A6, 3A4, and 2B6) might be expected to occur in the elderly, but no relevant information was found. Dorne and coworkers (Dorne et al., 2005; Dorne, 2004) estimated that renal clearance of drugs metabolized by CYP2A6 or 3A4 would be decreased by 20–30 or 20–50%, respectively, in the elderly (i.e., >70 years of age). However, Blanco et al. (2000) did not find evidence that CYP3A4 activity in human liver decreased with age up to 75 years. These authors did not present any data on CYP2A6 or 2B6.

The knowledge of polymorphic genes has been emerging rapidly with the introduction of molecular biology and gene sequencing techniques. A gene mutation can affect the coding or noncoding region of a gene. Substitution of one nucleotide, if within the limits of the degenerate genetic code, would establish a variant for a gene expression without affecting biological activity. If the base substitution results in an amino acid substitution, the affected protein may have any amount of change in activity from negligible to totally inactive, and it may also be more or less stable towards degradation. If the mutation affects the noncoding region of a gene, in particular the promoter region, the result may be a change in the level of transcription, a frameshift mutation with an entirely inactive gene product, or the introduction of an early stop codon and release of an incomplete gene product (Hong and Yang, 1997). Multiple nucleotide substitutions also have been identified. The nomenclature for polymorphic genes mostly uses the common gene name, such as CYP2A6 for the cytochrome P-450 2A6 gene locus, and attaches an asterisk with a subsequent numeral. If one variant exists with subvariants, a capital letter follows the numeral, beginning with A. Given the large number of possible heterozygous combinations of polymorphic CYPs involved in ETBE metabolism and the preliminary stage of current knowledge, a definitive assessment cannot be made to what extent gene polymorphism affects the sensitivity of humans toward ETBE exposure. Activity and variants for the main enzymes that are most likely to be responsible for the metabolism of ETBE and for the metabolism of the ETBE metabolite, acetaldehyde, are discussed below.

As pointed out in Section 3.3, CYP2A6 is likely to be the lead enzyme in humans to cleave the ether bond in ETBE. It exists in an array of variants, and although not all of the variants have been characterized with respect to their biological activity, it is clear that at least one variant (2A6*4) has no catalytic activity (Fukami et al., 2004). Hong et al. (2001) identified three novel CYP2A6 gene variants in a total of 23 individuals who self-identified as sensitive to MTBE exposure. The activity of two of the variants was reduced (2.1 and 2.0 relative to 2.6 pmol/minute/pmol CYP for the wildtype CYP2A6) and the third variant showed a total loss in ether-metabolizing activity. An overview of some of the CYP2A6 variants, with ethnic frequencies and catalytic activities, where available, is presented in Table 4-10. As described in Section 3.3, CYP3A4 generally has lower catalytic activity toward ETBE than CYP2A6;

however, CYP3A4 has the highest abundance of all CYPs in human liver. In humans, the activity of CYP3A4 varies as much as 20-fold. Detailed data on the frequency of the individual CYP3A4 alleles were not available. It may be concluded from the report by Hsieh et al. (2001) that the low- or zero-activity variants are rare, and thus, CYP3A4 polymorphism may not play a role in ETBE toxicity unless it is paired with a low- or zero-activity variant of CYP2A6. CYP2B6 may have the lowest catalytic activity toward ETBE among the three enzymes most likely to be responsible for metabolism of ETBE in humans (CYP2A6, 3A4, and 2B6) (see Section 3.3). At least 13 single nucleotide polymorphisms have been described for this enzyme, making for eight variants, CYP2B6*2–*9, with several subvariants (Jacob et al., 2004). Jinno et al. (2003) reported (in percent of wild-type activity): CYP2B6*2, 160%; *3, 150%; *4, 200%; and *5, 170%. With respect to ETBE toxicity, higher catalytic activity would signify potentially higher risk. Polymorphisms in aldehyde dehydrogenase (ALDH), the enzyme that oxidizes acetaldehyde to acetic acid, may also affect potential ETBE toxicity. The virtually inactive form, ALDH2*2, is responsible for alcohol intolerance and is found in about one-half of all Asians (Ames et al., 2002). This variant is associated with slow metabolism of acetaldehyde, and hence, extended exposure to a possible human carcinogen. With respect to ETBE exposure, the ALDH2*2 variant should increase any type of risk associated with acetaldehyde exposure, since it would allow prolonged exposure to the ETBE metabolite, acetaldehyde.

Table 4-10. Frequencies of gene polymorphisms of human CYP2A6

	Percent of population affected						Catalytic activity
Variant	Caucasian	Turkish	African-American	Asian	Black African	Brazilian[a]	
2A6*1A	66			43	80.5		Wild-type
2A6*1B	30			40	11.9	29.9	↑
2A6*1F	1.8		0				
2A6*1G	1.2		13.3				
2A6*1H	3.1	5.2					
2A6*2	1–3		3	n.d.–0.7	n.d.	1.7	↓↓
2A6*4A	3		n.d.				
2A6*4D	n.d.		0.5	15–20	1.9	0.5	none
2A6*5	n.d.			n.d.–0.1	n.d.		
2A6*6				0.4	n.d.		↓↓
2A6*7					n.d.		
2A6*8					n.d.		
2A6*9	5.2	7.2			5.7	5.7	↓
2A6*10					n.d.		
2A6*11					n.d.		
2A6*17	n.d.		9.4				↓

[a]Average value; according to the authors, frequency of *1B was white > mixed race > black.
n.d. = Variant not detected.
Empty cell = no data.
↑ = Increased catalytic activity.
↓ = Reduced catalytic activity.
↓↓ = Strongly reduced catalytic activity.

Sources: Gyamfi et al. (2005); Vasconcelos et al. (2005); Fukami et al. (2004); Nakajima et al. (2004, 2001); von Richter et al. (2004); Yoshida et al. (2003); Oscarson (2001); Paschke et al. (2001); Pitarque et al. (2001); Chen et al. (1999).

5. DOSE RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect – with Rationale and Justification

The database for oral exposure to ETBE is limited in the number and scope of available studies. There are no available human occupational or epidemiological studies of oral exposure to ETBE. The animal toxicity data associated with oral exposure to ETBE include a single chronic, gavage cancer bioassay in Sprague-Dawley rats (Maltoni et al., 1999), a 2-week oral exposure study evaluating oocyte quality in Simonson rats (Berger and Horner, 2003), and several reproductive and developmental toxicity studies from CIT (2004a, b, 2003 unpublished reports) in Sprague-Dawley and F344 rats. Most of the oral toxicity data on ETBE come from the CIT (2004a, b, 2003 unpublished reports) studies that were specifically designed to assess the reproducibility of histopathological changes in the testes of F344 rats (increased percent of seminiferous tubules with degenerated spermatocytes) observed in a subchronic inhalation study (Medinsky et al., 1999). Therefore, the database for oral ETBE toxicity is unusual in that it contains a two-generation reproduction and fertility study, but lacks a subchronic or chronic oral exposure study with the full range of basic toxicological endpoints such as histology from a standard array of organs and organ systems.

Liver and kidney organ weight increases are the primary noncancer health effects associated with oral exposure to ETBE based on limited available animal data. Increased liver and kidney weights were observed following oral exposure in both the parent and offspring generations and both sexes of rats in a two-generation reproductive toxicity study (CIT 2004b, unpublished report). Additional effects observed in the available studies also included mortality and decreased body weight gain. Further consideration was given to these endpoints as potential critical effects for the determination of the point of departure (POD) for derivation of the oral RfD. BMD modeling, if the data were amenable, was performed and is discussed in detail in Section 5.1.2 and Appendix B.

Data suggesting kidney toxicity in the available oral ETBE exposure studies include the demonstration of increased kidney weight in Sprague Dawley rats of both sexes in the parental (F0) and F1 generations from a two-generation reproductive toxicity study (CIT 2004b, unpublished report). Relative kidney weights were increased in F0- and F1-generation male rats exposed to 250, 500, and 1,000 mg/kg-day ETBE and F1 generation female rats exposed to 1,000 mg/kg-day ETBE. Absolute kidney weights were increased in F0 and F1 generation male rats exposed to 500 and 1,000 mg/kg-day ETBE, F1 males exposed to 250 mg/kg-day ETBE, and F1 generation female rats exposed to 1,000 mg/kg-day ETBE. The LOAEL for increased relative kidney weight was 250 mg/kg-day for oral exposure to ETBE and both values apply to males of either the parental (F0) generation of F1 generation. As 250 mg/kg-day was the lowest dose utilized in the study, a NOAEL was not available for increased relative kidney weight in

either the F0 or F1 generation males. Slight to moderate centrilobular hypertrophy was observed in all of the high-dose animals examined (five total males [3/3 F0 and 2/2 F1]) and was not seen in the single control F0 generation male examined. Only the livers from these six males were examined histologically out of all of the rats in all three generations. Histology was only performed if abnormal morphology was detected at necropsy, and, therefore, there are limited histological data to support nephrotoxicity (histology was only performed on the kidneys from 12 males out of all of the rats in all three generations in the study).

Data suggesting liver toxicity in the available oral ETBE exposure studies were limited to the demonstration of increased liver weight in Sprague-Dawley rats of both sexes in the parental (F0) and F1 generations from a two-generation reproductive toxicity study (CIT 2004b, unpublished report). Absolute and relative liver weights were increased in F0-generation male rats exposed to 1,000 mg/kg-day ETBE, F1 generation animals of both sexes exposed to 1,000 mg/kg-day ETBE, and F1 generation males exposed to 500 mg/kg-day ETBE (LOAEL in male rats). The NOAEL for increased liver weight was 250 mg/kg-day for oral exposure to ETBE during development as the NOAEL for males in the F1 generation was lower than the NOAEL for the parental generation. Acidophilic globules were observed in 5/6 F0 and 3/4 F1 generation males at the high dose. In addition, tubular basophilia (4/6), peritubular fibrosis (3/6), and proteinaceous casts (1/6) were observed in the F0 males at the high dose (1,000 mg/kg-day). Histology was also performed on kidneys from six F1 males (four high-dose, one mid-dose [500 mg/kg-day], and one control) due to the presence of macroscopic lesions in these animals. Tubular basophilia was observed in the control male, the mid-dose male, and two of the four high-dose males. Peritubular fibrosis was also observed in the control male, the mid-dose male, and two of the four high-dose males. The one mid-dose male examined also had sloughed degenerated/necrotic cells in the tubular lumen. Histology was only performed if abnormal morphology was detected at necropsy, and, therefore, there are limited histological data to support hepatotoxicity (histology was only performed on the livers from six males out of all of the rats in all three generations in the study).

As discussed in Section 4.7, ETBE-exposure of Sprague-Dawley rats in a chronic cancer bioassay was associated with increased mortality relative to controls in animals of both sexes 56–88 weeks of age and increased survival relative to controls in females ≥104 weeks of age (Maltoni et al., 1999). At 56 weeks of age, 250 mg/kg is the LOAEL in males for a 7% increase in mortality. Although all rats were necropsied and tissue/organs were taken for microscopic examination, no additional information on related toxicity or pathology associated with the observed increase in mortality was provided. Given the frank effect, lack of quantitative mortality data, and a consistent dose-response trend, this endpoint was not considered ideal for the derivation of the RfD and was not amenable to BMD modeling. However, a LOAEL of 250 mg/kg-day for mortality in males at 56 weeks exposure was considered as a possible POD.

There were several examples of reduced body weight gain from the prenatal and two-generation reproductive toxicity studies in Sprague-Dawley rats (CIT, 2004a, b, unpublished reports). In a prenatal toxicity study (CIT, 2004a, unpublished report), pregnant Sprague-Dawley dams in the 1,000 mg/kg-day ETBE group (LOAEL in female rats) had an 11% decreased maternal body weight gain and a 17% decrease in net weight gain when animals were euthanized on GD 20. The NOAEL for maternal toxicity was 500 mg/kg-day based on decreased maternal weight gain. There was no effect of ETBE treatment on body weight of females in the two-generation reproduction and fertility study (CIT, 2004b, unpublished report) at the same doses over longer treatment periods). Male rats in the F0 generation at 500 and 1,000 mg/kg-day showed significantly lower body weight gains (-29 and -22%, respectively) in the last quarter of the treatment period. The authors suggest a NOAEL of 250 mg/kg-day for this endpoint, although the weight gain in F0 generation males was not different from controls when analyzed over the entire treatment period (days 1–113) (for additional details see Section 4.5.1 of the Toxicological Review).

Oral exposure to ETBE had no effect on oocyte quality, sperm parameters, mating, fertility, gestation, fecundity, or delivery in rats (CIT 2003, 2004a, b, unpublished reports; Berger and Horner, 2003).

CIT (2004b, unpublished report) was chosen as the principal study for derivation of the RfD because the increased kidney and liver weights reported in this study represent the most sensitive effects identified in the database. The LOAEL for increased relative kidney weight was 250 mg/kg-day for oral exposure to ETBE and both values apply to males of either the parental (F0) generation of F1 generation. The LOAEL for increased liver weight was 500 mg/kg-day and the NOAEL for increased liver weight was 250 mg-kg-day for males in the F1 generation. There are limited histological data to support nephrotoxicity or hepatotoxicity in the oral two-generation reproductive toxicity study because histology was only performed if abnormal morphology was detected at necropsy (CIT, 2004b, unpublished report). Increased kidney weight was also observed in F1 generation females at higher doses. Increased liver weight was also observed at higher doses in F1 generation females and F0 generation males. The data for increased organ weight of the kidneys and livers in males were subjected to BMD modeling (Section 5.1.2 and Appendix B) because the effects in males were more pronounced and occurred at lower doses than in females. The data for increased organ weight of the kidneys in females were subjected to BMD modeling for comparison purposes. Data on both absolute and relative organ weights were modeled. The ETBE database contains additional support for the kidney and liver as target organs as determined by increased kidney and liver weights and some related effects seen in inhalation toxicity studies of ETBE (Medinsky et al., 1999; White et al., 1995). Data suggesting kidney toxicity associated with oral exposure to ETBE are limited to increased kidney weights in male and female rats (at higher doses), with limited histopathological support as described above. A number of kidney effects are also reported after

inhalation exposure to ETBE, suggesting that the kidney is a target organ of ETBE exposure. Additional kidney effects observed after inhalation exposure to ETBE include: histological evidence of cellular necrosis (increased incidence of regenerative foci) in the kidneys of male rats, sustained increase in kidney cellular proliferation in male rats, increased kidney cellular proliferation in female rats after 1 and 4 weeks, and increased kidney weights in male and female rats (Medinsky et al., 1999; White et al., 1995). A similar situation is presented by the data on ETBE-related liver effects. Oral exposure to ETBE is associated with increased liver weights in male and female rats (at higher doses), with limited histopathological support as described above. A number of hepatic effects are also reported after inhalation exposure to ETBE, suggesting that the liver is a target organ of ETBE exposure. Additional hepatic effects observed after inhalation exposure to ETBE include: hepatic centrilobular hypertrophy in male and female mice, sustained increase in hepatic cellular proliferation in female mice, increased hepatic cellular proliferation in male mice after 1 and 4 weeks, increased liver organ weight in male and female mice, and increased liver organ weight in male and female rats (Medinsky et al., 1999).

Table 5-1 summarizes the BMD modeling results of the available data (see Section 5.1.2 for complete discussion). The benchmark response (BMR) levels and the PODs are identified in Table 5-1 for each effect. The BMR levels represent a change of one SD from the control mean for continuous variables and are presented as the percent difference from controls. The range of the PODs (approximately 100–900 mg/kg-day) is less than a factor of 10.

Table 5-1. Summary of BMD modeling results of ETBE oral toxicity studies for selection of principal study in Sprague-Dawley rats

	Organ	Endpoint	Sex	Dosing duration (days)	"Best-fit" model	Goodness of fit *p*-value	AIC	BMD[a] (mg/kg-day)	BMDL (mg/kg-day)	Study reference
BMD analyses	Body	Dam body weight change	F	14	Power (non-constant variance[b])	0.35	554.3	1,015	879	CIT, 2004a
		Net dam body weight change	F	14	Linear (constant variance)	0.49	476.6	1,063	696	CIT, 2004a
		F0 body weight change (days 85–113)	M	120	Power – Highest Dose Dropped (non-constant variance[b])	0.73	479.0	492	385	CIT, 2004b
	Liver	F1 liver weight (absolute)	M	120	Hill (non-constant variance)	0.96	329.7	482	294	CIT, 2004b
		F1 liver weight (relative)	M	120	All continuous models exhibited significant lack-of-fit	No data[c]	No data[c]	No data[c]	No data[c]	CIT, 2004b
	Kidney	F0 kidney weight (absolute)	M	120	Hill (constant variance)	0.81	-40.1	381	167	CIT, 2004b
		F0 kidney weight (relative)	M	120	Hill (constant variance)	0.94	-453.0	227	143	CIT, 2004b
		F1 kidney weight (absolute)	M	120	All continuous models exhibited significant lack-of-fit	No data[c]	No data[c]	No data[c]	No data[c]	CIT, 2004b
			F	120	Linear (constant variance)	0.30	-180.2	1,016	687	CIT, 2004b
		F1 kidney weight (relative)	M	120	All continuous models exhibited significant lack-of-fit	No data[c]	No data[c]	No data[c]	No data[c]	CIT, 2004b
			F	120	Linear (non-constant variance[b])	0.10	-412.3	898	562	CIT, 2004b

Table 5-1. Summary of BMD modeling results of ETBE oral toxicity studies for selection of principal study in Sprague-Dawley rats

LOAEL NOAEL / Analyses	Organ	Endpoint	Sex	Dosing duration (days)	LOAEL (mg/kg-day)	NOAEL (mg/kg-day)	Study reference
		Mortality	M	728[d]	250	NA[e]	Maltoni et al., 1999
	Liver	F1 liver weight (relative)	M	120	500	250	CIT, 2004b
	Kidney	F1 kidney weight (absolute)	M	120	500	250	CIT, 2004b
		F1 kidney weight (relative)	M	120	250	NA[f]	CIT, 2004b

[a]All data were modeled as a continuous variable, and a BMR of a change of one SD from the control mean was employed.
[b]Variance model employed (i.e., variance modeled as a power function of the mean) failed to adequately address nonconstant variance.
[c]No data because all models in the software exhibited significant lack-of-fit.
[d]Increased mortality determined at 56 weeks of a 104-week treatment.
[e]NA = not applicable as both doses of ETBE (1,000 and 250 mg/kg-day) resulted in increased mortality and therefore a NOAEL was not determined (Maltoni et al., 1999).
[f]NA = not applicable as all doses of ETBE (1,000, 500, and 250 mg/kg-day) resulted in increased relative kidney weight and therefore, a NOAEL was not determined (CIT 2004b, unpublished report).

BMDL = lower 95% confidence limit on the benchmark dose; F= female; M = Male

5.1.2. Methods of Analysis

The increased liver and kidney weights in Sprague-Dawley rats from the two-generation reproductive toxicity study of ETBE were treated as continuous variables for dose-response modeling (CIT, 2004b, unpublished report). The reduced body weight gain in Sprague-Dawley rats from the prenatal developmental and two-generation reproductive toxicity studies of ETBE were also treated as continuous variables for dose-response modeling (CIT, 2004a, b, unpublished reports). All available models for continuous variables in U.S. EPA's Benchmark Dose Software (BMDS, version 1.4.1) were fit to the data in accordance with U.S. EPA (2000b) BMD methodology. The BMR was defined as a change of one SD from the control mean. For these datasets, this BMR corresponds to an approximate 7–13% change in organ weights and a 14–21% change in body weights. The lower 95% confidence limit on the benchmark dose (BMDL) estimates for the best fitting models for increased liver and kidney weights and reduced body weight gain from CIT (2004a, b, unpublished report) are presented in Table 5-1, and detailed discussion of the modeling of each endpoint is presented in Appendix B. For each endpoint modeled, overall goodness-of-fit tests (χ^2) and determined the Akaike's Information Criterion (AIC) were determined. The chi-square goodness-of-fit test is a measure of how well the model fits the observed data. Models with χ^2 *p*-values ≥0.1 were considered to have adequate fits. The AIC is a measure of the model fit based on the log-likelihood at the maximum likelihood estimates for the parameters. Within the subset of models that exhibit adequate fit, models with lower AIC values are preferred. The "best-fit" model selection criteria are presented in Appendix B and described in detail in EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b).

The increased liver and kidney weights of male rats following oral exposure to ETBE were evaluated as potential PODs. As described above and in Section 4.3, liver and kidney weight was increased in males and females, and the organ weight in males increased at lower doses and to a greater degree. Absolute and relative liver weights of F1 generation males and absolute and relative kidney weights of F0 and F1 generation males were subjected to BMD modeling.

As shown in Appendix B, a somewhat limited set of the available continuous models in BMDS provided adequate fits to the data for absolute and relative liver weights of F1 generation males and absolute and relative kidney weights of F0 and F1 generation males (CIT 2004b, unpublished report). For liver, the Hill model provided the best fit to the increase in absolute liver weight in F1 males. None of the continuous models available in BMDS adequately fit the relative liver weight in F1 males, so a NOAEL of 250 mg/kg-day was retained as a potential POD. For kidney, the Hill model provided the best fit to both absolute and relative kidney weights in F0 males. None of the continuous models available in BMDS adequately fit the absolute or relative kidney weight in F1 males so a NOAEL of 250 mg/kg-day was retained as a potential POD for absolute kidney weight in F1 males, and a LOAEL of 250 mg/kg-day was

retained as a potential POD for relative kidney weight in F1 males. The linear model provided the best fit to both absolute and relative kidney weights in F1 females. Additionally, the models that exhibited adequate fit also showed good fits to the incidence data at low doses (i.e., in the vicinity of the BMR) as evidenced by examining the χ^2 scaled residuals, and the visual fit of the model to the data in the plots from the BMDS output. Thus, the Hill model was selected to estimate the BMDL or POD for absolute liver weight in F1 males and for absolute and relative kidney weights in F0 males. The BMDL associated with a change of one SD from the control mean for an increase in absolute liver weight in F1 males was 294 mg/kg-day. The BMDLs associated with a change of one SD from the control mean for an increase in absolute and relative kidney weights in F0 males were 167 and 143 mg/kg-day, respectively. The BMDLs associated with a change of one SD from the control mean for an increase in absolute and relative kidney weights in F1 females were 687 and 562 mg/kg-day, respectively.

As shown in Appendix B, several of the available continuous models in BMDS provided adequate fits to the data for decreased body weight gain and net body weight gain in dams in CIT (2004a, unpublished report) and decreased body weight gain in F0 males during the last quarter of the treatment period in CIT 2004b, unpublished report). The power model provided the best fit to the body weight gain in pregnant dams, the linear model provided the best fit to the net body weight gain in pregnant dams, and the power model (with the highest dose group dropped) provided the best fit to the body weight gain F0 generation males during the last quarter of treatment period, as assessed by AIC. Thus, the power model was selected to estimate the BMDL or POD for body weight gain in the pregnant dams, the linear model was selected to estimate the POD for net body weight gain in the pregnant dams, and the power model (with the highest dose group dropped) was selected to estimate the POD for body weight gain in the F0 generation males during the last quarter of treatment from CIT studies (2004a, b, unpublished reports). The BMDL associated with a change of one SD from the control mean for body weight gain in pregnant dams was 879 mg/kg-day. The BMDL associated with a change of one SD from the control mean for net body weight gain in pregnant dams was 696 mg/kg-day. The BMDL associated with a change of one SD from the control mean for net body weight gain in F0 generation males during the last quarter of treatment was 385 mg/kg-day.

As discussed in Section 5.1.1, the increased mortality relative to controls observed in Sprague-Dawley rats exposed to ETBE by gavage in a chronic cancer bioassay (Maltoni et al., 1999) was not amenable to BMD modeling. Mortality represents a frank effect, there are a lack of quantitative mortality data from Maltoni et al. (1999), and the data also do not display a consistent dose-response trend. However, a LOAEL of 250 mg/kg-day for mortality in males at 56 weeks exposure was considered as a possible POD.

CIT (2004b, unpublished report) was selected as the principal study (Section 5.1.1) and increased relative kidney weight in F0 generation males as the critical effect because it resulted in the lowest BMDL, 143 mg/kg-day. Data suggesting kidney toxicity associated with oral

exposure to ETBE are limited to increased kidney weights in males and females (at higher doses), with limited histopathological support as described in Section 5.1.1. Increased kidney weight may be considered an adverse effect and may also be an early indicator of more overt kidney toxicity. The designation of kidney toxicity as the critical effect is supported by a number of kidney effects reported after inhalation exposure to ETBE. In addition to increased kidney weights in male and female rats, additional effects observed after inhalation exposure to ETBE include: histological evidence of cellular necrosis (increased incidence of regenerative foci) in the kidneys of male rats, sustained increase in kidney cellular proliferation in male rats, and increased kidney cellular proliferation in female rats after 1 and 4 weeks (Medinsky et al., 1999; White et al., 1995). Based on application of the criteria from the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b) as described above, the Hill model provided the best fit to the kidney weight data.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

Considering the uncertainties in the ETBE database, which are described in Appendix C, the total composite UF is 10,000, consisting of four areas of maximum uncertainty. In the report, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the technical panel concluded that, in cases where maximum uncertainty exists in four or more areas of extrapolation, or when the total UF is 10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Therefore, in lieu of an RfD, Appendix C contains a derivation of an oral minimal data value for ETBE using an UF of 10,000. Use of this minimal data value is not recommended except in limited circumstances, for example, in screening level risk assessments or to rank relative risks. Any use of this value should include a discussion of the uncertainty associated with its derivation.

5.1.4. Previous RfD

An oral assessment for ETBE was not previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect – with Rationale and Justification

The inhalation toxicity database for ETBE is from a limited number of studies which generally report relatively mild effects such as increased organ weights. Data on the effects of ETBE in humans is limited to several 2-hour inhalation studies at doses up to 50 ppm (Nihlen et al., 1998b; Vertrano, 1993, unpublished report; TRC Environmental Corp., 1993). Healthy subjects exposed to ETBE experienced irritation in throat and airways, nasal swelling, a bad taste in the mouth, and slightly impaired lung function (about 3% reduction in vital capacity and forced vital capacity). No chronic inhalation studies are available, although there are several subchronic studies in mice and rats (Medinsky et al., 1999; White et al., 1995; see Section 4.5.2

and Table 4-9). Data from these inhalation toxicity studies of ETBE indicated that among the minimal effects associated with ETBE exposure, the kidney and liver appear to be target organs for ETBE toxicity as determined by increased kidney and liver weights and some additional effects described below. Concordant support for kidney and liver effects resulting from exposure to ETBE is gained through oral exposure studies, which are described more fully in Sections 4.3; 4.5.1; and 5.1.1 (CIT, 2004b, unpublished report).

In a subchronic inhalation study, CD-1 mice and F344 rats were exposed to 0, 500, 1,750, and 5,000 ppm for 13 weeks (Medinsky et al., 1999). Increased liver weight was observed in female rats at 5,000 ppm and in male rats at 1,750 and 5,000 ppm. Increased liver weight was also observed in male and female mice at 1,750 and 5,000 ppm. Hepatic centrilobular hypertrophy was associated with ETBE-induced liver weight increases in the mice, but not the rats. Mice were also tested for a hepatic mitogenic response to ETBE exposure. Dose-related increases in the hepatic LI were seen in male and female mice at 1,750 and 5,000 ppm at different time points. The LI was increased in males at 1 and 4 weeks (not 13 weeks) and in females at 1 and 13 weeks (not 4 weeks). The authors did not find any histological evidence of hepatic lesions or elevated serum enzymes characteristic of hepatotoxicity. A 1% increase in MCV was observed in female rats and a 2.5% decrease in MCHC was noted in male rats exposed to 1,750 and 5,000 ppm ETBE after 13 weeks of exposure, but there was no pattern to support hepatotoxicity as there was no relationship between the small changes in MCV or MCHC and increased liver weight or any other liver data in rats and mice from the available studies (Medinsky et al., 1999). No additional evidence of irregular pathology in the liver was observed in this study. However, there are a number of hepatic endpoints that could constitute a LOAEL: (1) the observed increased liver weights in male and female CD-1 mice and male rats at 1,750 ppm, (2) the increased incidence of centrilobular hypertrophy at 5,000 ppm in male and female CD-1 mice, and (3) the increased LI which was sustained in female CD-1 mice at 13 weeks at 1,750 ppm. Increased liver weight was also observed in Sprague-Dawley rats in a 4-week inhalation study (White et al., 1995).

In a 4-week inhalation study, Sprague-Dawley rats were exposed to 0, 500, 2,000, and 4,000 ppm ETBE (White et al., 1995). The only treatment associated responses from the 4-week subchronic study were increased liver weight in females at 2,000 and 4,000 ppm and increased liver, kidney, and adrenal weight in males at 4,000 ppm. No histopathological findings were reported in the over 40 tissues examined, including the liver, kidney, and adrenal gland. No indications of neurotoxicity were detected in a FOB consisting of 19 parameters covering sensory perception, reflex response, body temperature, and neuromuscular function. Transient ataxia and sedation, overt signs of CNS depression, were seen following exposure termination in the 4,000 ppm group, but ETBE-exposed animals appeared normal within 15 minutes. There were also no significant effects of ETBE exposure observed in serum chemistry (including hepatic enzymes) or hematology evaluations.

In addition to the liver, Medinsky et al. (1999) observed effects of inhalation ETBE exposure in the kidney of male and female rats. Specifically, increased kidney weight was detected in male rats exposed to 1,750 and 5,000 ppm and in female rats exposed to 5,000 ppm as part of the same 13-week inhalation study (Medinsky et al., 1999). In male rats, the increased kidney weight was associated with hyaline droplet accumulation with alpha$_{2u}$-globulin immunoreactivity, the presence of regenerative foci, and greater than twofold increase in cellular proliferation as determined by the increased LI after 1 and 4 weeks of exposure at 5,000 ppm and at all doses after 13 weeks of exposure (Medinsky et al., 1999). Regenerative foci are associated with repair of damaged tubules, and some cells of the proximal tubule would have had to have died and the remaining cells undergone regenerative proliferation for regenerative foci to be observed. However, no other renal effects were noted in this study or in the available subchronic studies. Alpha$_{2u}$-globulin derived from hepatic synthesis is unique to male rats; it is not found in female rats or in mice or humans of either sex (U.S. EPA, 1991b). Based on the available data for ETBE, alpha$_{2u}$-globulin accumulation may be associated with the observed kidney effects in male rats; however, there is considerable uncertainty because of the lack of evidence of typical lesions in the pathological sequence of lesions associated with alpha$_{2u}$-globulin nephropathy. Therefore, a determination cannot be made as to whether alpha$_{2u}$-globulin accumulation is the mode of action or the only mode of action for renal effects associated with ETBE exposure (U.S. EPA, 1991b). The increased kidney weight observed in females was not accompanied by significant histopathology except for a less than twofold increase in LI (i.e., proliferation) at early time points (after 1 and 4 weeks of exposure, not after 13 weeks). Although statistically significant, this small increase in LI that was not sustained over the study period was of questionable biological significance. It is also useful to note that there is insufficient information to determine if some or all of ETBE-induced renal effects are caused by the parent compound or its metabolite, TBA. Some authors have proposed that the nephropathy and subsequent cancer incidence in male rats exposed to TBA is mediated by an excessive accumulation of alpha$_{2u}$-globulin in proximal tubular cells (Williams and Borghoff, 2001; Takahashi et al., 1993). However, the dose of TBA associated with an increase in the severity of chronic nephropathy in male rats in a 13-week inhalation study (Mahler, 1997) is below doses associated with accumulation of protein droplets in the kidney either in that study (Mahler, 1997) or in a 10-day exposure (Borghoff et al., 2001), suggesting that alpha$_{2u}$-globulin may not be the mechanism or the only mechanism for TBA-associated nephropathy. Although some data support alpha$_{2u}$-globulin accumulation as a mode of action for renal effects associated with ETBE exposure in male rats, sufficient data are not available to determine if alpha$_{2u}$-globulin accumulation is the only mode of action for renal effects associated with ETBE exposure (U.S. EPA, 1991b).

The 13-week inhalation exposure study (Medinsky et al., 1999) also included histological evidence that the percentage of seminiferous tubules with spermatocyte degeneration was slightly increased at 1,750 and 5,000 ppm (+7.8 and +12.7%, respectively) in F344 rats.

Degenerated spermatocytes were also found in the testes of control rats. Spermatocyte degeneration is a widely examined histopathological outcome in the testis, and these data are often presented as a percentage of total tubules; however, quantitation of this type provides crude information about the nature and severity of potential spermatogenic damage (Russell et al., 1990).

Several additional effects were also observed in F344 rats in the 13-week inhalation exposure study of ETBE (Medinsky et al., 1999) including increased adrenal weight in males and females, increased heart weight in females, and increased incidence of bone marrow congestion in females. An increase in the adrenal weight was observed in both male and female F344 rats at 5,000 ppm, but there were no associated histopathological findings. A similar increase in adrenal weight was observed in male Sprague-Dawley rats following 4-week inhalation exposure to 4,000 ppm ETBE (White et al., 1995), also without any associated pathological findings in the histology performed on the adrenal gland. Increased heart weight was observed in female F344 rats exposed by inhalation to 500 and 5,000 ppm ETBE, but not to an intermediate dose of 1,750 ppm for 13 weeks. Histological evidence of bone marrow congestion was seen in tissue from female rats at 1,750 and 5,000 ppm. The reported increase in bone marrow congestion was not supported by any changes in hematopoietic cell populations and was, thus, judged to be not clinically relevant by the authors. Also, there were no effects of ETBE exposure in serum chemistry or hematology judged to be clinically relevant by the authors.

In summary, the Medinsky et al. (1999) study was adequately designed with several acceptable dose groups and an adequate number of animals. Sensitive endpoints identified in this study included a number of effects in the liver of mice and rats (i.e., sustained proliferation in the liver indicated by a sustained increase in the LI in mice, increased centrilobular hypertrophy in male and female mice, and increased liver weight in mice and rats). A number of effects were identified in the kidney including increased kidney weight in male and female rats as well as increased incidence and mean number of regenerative foci and sustained proliferation in the kidney indicated by a sustained increase in the LI in male rats. Additional effects were identified in rats including an increased percentage of seminiferous tubules with degenerated spermatocytes in males, increased adrenal gland weight in males and females, and increased heart weight and incidence of bone marrow congestion in females. After consideration of all available endpoints, the mean number of regenerative foci in the kidneys was determined to be the most sensitive and biologically significant effect detected in these studies. As described previously, regenerative foci are associated with repair of damaged kidney tubules and some cells of the proximal tubule would have had to have died and the remaining cells undergone regenerative proliferation for regenerative foci to be observed. Therefore, EPA considers regenerative foci to be indicators of cellular necrosis and a biomarker of an adverse effect. Furthermore, the ETBE database includes additional evidence for the liver and kidney as target organs of ETBE toxicity as determined by increased liver and kidney weights seen in male and

female Sprague-Dawley rats in an oral two-generation reproduction and fertility study (CIT, 2004b, unpublished report).

Therefore, Medinsky et al. (1999) was chosen as the principal study for derivation of the RfC because the kidney effects (i.e., regenerative foci) observed in this study represent the most sensitive effects identified in the database evaluating exposure through the inhalation route to ETBE. White et al. (1995) and CIT (2004b, unpublished study; oral exposure route only) provided supporting data for this endpoint; however, kidney effects were observed in these studies at higher doses.

5.2.2. Methods of Analysis

As described in Section 4.5.2, the database of inhalation toxicity studies on ETBE is limited with the majority of the data from a 4-week inhalation study in Sprague-Dawley rats (White et al., 1995) and a 13-week inhalation study in F344 rats and CD-1 mice (Medinsky et al., 1999). The effects observed in these studies included organ weight changes and effects in the liver, kidney, heart, adrenal, and testes. Further consideration was given to these endpoints for the selection of the critical effect as described below.

Selected endpoints that were treated as continuous variables (i.e., LI of liver, liver weight, LI of kidney, kidney weight, mean number of regenerative foci in kidney, percent seminiferous tubules with degenerated spermatocytes, adrenal gland weight, and heart weight) were modeled employing all available continuous models in U.S. EPA's BMDS (version 1.4.1) in accordance with U.S. EPA (2000b) BMD methodology. The BMR was defined as a change of one SD from the control mean. For these datasets, this BMR corresponds to an approximate 6–19% change in organ weights. All available dichotomous models in the U.S. EPA's BMDS (version 1.4.1) were fit to endpoints treated as quantal (i.e., incidence of centrilobular hypertrophy in the liver of male and female CD-1 mice, incidence of regenerative foci in the kidney of male rats, and incidence of bone marrow congestion in female F344 rats). For dichotomous models, the BMR was defined as a 10% increase in extra risk because there was no clear biological rationale for selecting an alternate BMR for these data. The lower 95% confidence limit on the benchmark concentration (BMCL) estimates for the best fitting models for the selected data sets are presented in Table 5-2, and a detailed discussion of the modeling of each endpoint is presented in Appendix B.

Table 5-2. Summary of BMD modeling results of ETBE inhalation toxicity studies for selection of principal study

Organ	Endpoint	Species strain	Sex	Dosing duration(wks)	"Best-fit" model	Goodness of fit *p*-value	AIC	BMC (ppm)	BMCL[c] (ppm)	$BMCL_{HEC}$[d] (mg/m^3)	Reference
Liver	LI[a]	CD-1 Mice	F	13	Linear (constant variance)	0.57	31.7	2040	1307	975	Medinsky et al., 1999
	Centrilobular hypertrophy[b]	CD-1 Mice	M	13	Log-Probit	0.998	25.8	1602	957	714	Medinsky et al., 1999
			F	13	Logistic	0.24	44.3	1826	1255	937	Medinsky et al., 1999
	Liver weight[a] (absolute)	CD-1 Mice	M	13	2° Polynomial (constant variance)	0.99	- 76.8	1754	936	699	Medinsky et al., 1999
			F	13	2° Polynomial (constant variance)	0.32	- 111.5	1109	709	529	Medinsky et al., 1999
		F344 Rats	M	13	Linear (non-constant variance[e])	0.39	27.9	1648	1260	932	Medinsky et al., 1999
			F	13	Linear (constant variance)	0.99	- 19.7	1663	1300	962	Medinsky et al., 1999
		Sprague-Dawley Rats	M	4	Linear (constant variance)	0.55	49.7	2309	1616	1196	White et al., 1995
			F	4	2° Polynomial (constant variance)	0.97	- 22.4	1593	779	576	White et al., 1995
	Liver weight[a] (relative)	Sprague-Dawley Rats	M	4	2° Polynomial (constant variance)	0.80	- 81.7	2678	1619	1198	White et al., 1995
			F	4	Linear (non-constant variance[e])	0.30	- 89.4	1600	997	738	White et al., 1995

Table 5-2. Summary of BMD modeling results of ETBE inhalation toxicity studies for selection of principal study

Organ	Endpoint	Species strain	Sex	Dosing duration(wks)	"Best-fit" model	Goodness of fit *p*-value	AIC	BMC (ppm)	BMCL[c] (ppm)	$BMCL_{HEC}$[d] (mg/m^3)	Reference
Kidney	Kidney weight[a] (absolute)	F344 Rats	M	13	Linear (constant variance)	0.23	-130.4	2,169	1,632	1,208	Medinsky et al., 1999
			F	13	2° Polynomial (constant variance)	0.83	-191.2	717	494	366	Medinsky et al., 1999
		Sprague-Dawley Rats	M	4	Linear (constant variance)	0.87	-54.7	3,010	1,960	1,450	White et al., 1995
	LI[a]	F344 Rats	M	13	2° Polynomial (non-constant variance[c])	0.12	15.4	160	81	60	Medinsky et al., 1999
	Regenerative foci[a,b]	F344 Rats	M	13	Hill[a] (non-constant variance[e])	0.14	82.4	40	23	17	Medinsky et al., 1999
		F344 Rats	M	13	Logistic[b]	1.0	25.1	47	24	18	Medinsky et al., 1999
Testes	Percent seminiferous tubules with degenerated spermatocytes[a]	F344 Rats	M	13	Linear (non-constant variance[e])	0.41	145.7	397	268	198	Medinsky et al., 1999
Adrenal gland	Adrenal gland weight[a] (absolute)	F344 Rats	M	13	Linear (constant variance)	0.40	-387.3	3,214	2,223	1,645	Medinsky et al., 1999
			F	13	Linear (constant variance)	0.54	-406.7	3,576	2,394	1,771	Medinsky et al., 1999
		Sprague-Dawley Rats	M	4	Power (constant variance)	0.14	-351.3	3,367	2,220	1,643	White et al., 1995

Table 5-2. Summary of BMD modeling results of ETBE inhalation toxicity studies for selection of principal study

Organ	Endpoint	Species strain	Sex	Dosing duration(wks)	"Best-fit" model	Goodness of fit *p*-value	AIC	BMC (ppm)	BMCL[c] (ppm)	$BMCL_{HEC}$[d] (mg/m^3)	Reference
Bone marrow	Bone marrow congestion[b]	F344 Rats	F	13	3° Multistage	0.98	17.5	987	401	297	Medinsky et al., 1999
Heart	Heart weight[a,f] (absolute)	F344 Rats	F	13	All continuous models exhibited significant lack-of-fit	No data	No data	No data	No data	No data	Medinsky et al., 1999

[a]Continuous data.
[b]Dichotomous data.
[c]For continuous variables, a BMR of a change of one SD from the control mean was employed, while for dichotomous variables, a BMR of a 10% change relative to controls was used.
[d]The BMCL (in ppm) was converted to a $BMCL_{HEC}$ (in mg/m^3) using the following three steps: (1) the BMCL (in ppm) was converted to standard units of mg/m^3 using the equation, mg/m^3 = (ppm x molecular weight)/24.45, where molecular weight for ETBE = 102.18; (2) the BMCL (in mg/m^3) was duration-adjusted to a 24 hour/day, 7 day/week exposure by multiplying by 6 hours/24 hours and 5 days/7 days; and (3) assuming ETBE is a Category 3 gas, this 24 hour/day, 7 day/week BMCL (in mg/m^3) was converted to a HEC for extra-respiratory effects by multiplying it by the ratio of animal to human blood:gas partition coefficients, which was 1.0 for mice and 11.6/11.7 or 0.99 for rats.
[e]Variance model employed (i.e., variance modeled as a power function of the mean) failed to adequately address non-constant variance.
[f]Heart weight was increased in female rats exposed to 500 or 5,000 ppm, not 1,750 ppm; therefore, the response is not amenable to BMD modeling or the LOAEL/NOAEL approach.

For each endpoint modeled, BMDS performed a χ^2 goodness-of-fit test and determined the AIC. The goodness-of-fit test is a measure of how well the model fits the observed data. Models with χ^2 *p*-values ≥ 0.1 were considered to exhibit adequate fits. The AIC is a measure of the model fit based on the log-likelihood at the maximum likelihood estimates for the parameters. Within the subset of models that exhibit adequate fit, models with lower AIC values are preferred. The "best-fit" model selection criteria are presented in Appendix B and described in detail in EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b).

Medinsky et al. (1999) was selected as the principal study (Section 5.2.1) and regenerative foci in the kidneys of male rats as the critical effect. Based on application of the criteria from the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b) as described above the Hill model provided the best fit to the mean number of regenerative foci data.

5.2.2.1. *Adjustment to a Human Equivalent Exposure Concentration*

Because the RfC is a standard applicable to a continuous lifetime human exposure but derived from animal studies featuring intermittent, subchronic exposure, EPA guidance (U.S. EPA, 1994a) provides mechanisms for: (1) adjusting experimental exposure concentrations to a value reflecting continuous exposure duration and (2) determining a human equivalent concentration (HEC) from the animal exposure data. The former employs an inverse concentration-time relationship to derive a health-protective duration adjustment to time-weight the intermittent exposures used in the studies. The BMCL for each endpoint in ppm was converted to standard units (mg/m^3) using the equation mg/m^3 = (ppm x molecular weight /24.45, where molecular weight for ETBE = 102.18. This animal exposure in standard units is then adjusted to reflect a continuous exposure by multiplying it by (6 hours/day)/(24 hours/day) and (5 days/week)/(7 days/week) as follows:

$$BMCL_{ADJ} = BMCL\ (mg/m^3) \times 6/24 \times 5/7$$

The RfC methodology provides a mechanism for deriving a human equivalent concentration from the duration-adjusted POD ($BMCL_{ADJ}$) determined from the animal data. The approach takes into account the extra-respiratory nature of the toxicological responses and accommodates species differences by considering blood:air partition coefficients for ETBE in the laboratory animal (rat or mouse) and humans. According to the RfC guidelines (U.S. EPA, 1994b), ETBE is a Category 3 gas because it is largely inactive in the respiratory tract, is rapidly transferred between the lungs and blood, and the toxicological effects observed are extra-respiratory. Therefore, the duration-adjusted $BMCL_{ADJ}$ is multiplied by the ratio of animal/human blood:air partition coefficients (L_A/L_H). As detailed in Section 3.2, the values

reported in the literature for these parameters include an L_A of 11.6 for Wistar rats (Kaneko et al., 2000) and an L_H in humans of 11.7 (Nihlén et al., 1995). No data were available on blood:gas partitioning of ETBE in the mouse; therefore, the default ratio of 1.0 (U.S. EPA, 1994b) was used for the mouse data. This allowed a $BMCL_{HEC}$ to be derived as follows:

For rat data:

$BMCL_{HEC}$ = $BMCL_{ADJ}$ (mg/m^3) × (L_A/L_H) (interspecies conversion)
= $BMCL_{ADJ}$ (mg/m^3) × (11.6/11.7)
= $BMCL_{ADJ}$ (mg/m^3) × (0.1368)

For mouse data:

$BMCL_{HEC}$ = $BMCL_{ADJ}$ (mg/m^3) × 1 (interspecies conversion)

Table 5-2 shows the $BMCL_{HEC}$ values calculated for each of the endpoints modeled. The $BMCL_{HEC}$ value of 17 mg/m^3 for mean number of regenerative foci in the kidneys of male F344 rats was used as the POD to derive the RfC for ETBE.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs)

The $BMCL_{HEC}$ of 17 mg/m^3 for the mean number of regenerative foci in the kidneys of male F344 rats exposed to ETBE for 13 weeks was used as the POD for the RfC.

A total UF of 3,000 was applied to the POD of 17 mg/m^3: 3 for interspecies extrapolation from animals to humans (UF_A); 10 for human intraspecies variability (UF_H); 10 to extrapolate from a subchronic to a chronic study (UF_S); and 10 to account for database deficiencies (UF_D).

A threefold UF was used to account for uncertainties in extrapolating from laboratory animals to humans. This value is adopted by convention where an adjustment from an animal-specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. Application of a full UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method.

A 10-fold UF was used to account for variation in susceptibility among members of the human population (i.e., interindividual variability). Insufficient information is available to predict potential variability in human susceptibility.

A 10-fold UF was used to extrapolate from subchronic to chronic exposure. The $BMCL_{HEC}$ of 17 mg/m^3 for the mean number of regenerative foci from Medinsky et al. (1999) was observed in the kidneys of male F344 rats exposed to ETBE for 13 weeks. Therefore UF_S of 10 was applied to extrapolate from subchronic to chronic exposure.

A UF_D of 10 was used to account for deficiencies in the toxicity database of inhalation exposure to ETBE. Data on the effects of ETBE in humans is limited to several 2-hour inhalation studies. The inhalation database contains several subchronic studies in mice and rats. The database for ETBE lacks both a developmental toxicity study and a multigeneration

reproductive toxicity study by inhalation exposure, although oral studies of developmental and reproductive toxicity have been conducted (CIT, 2004a, b, unpublished reports). The lack of an immunotoxicity study of inhalation exposure to ETBE also contributes to the database uncertainty in light of the potential for suppression of the antibody response suggested by the unpublished G/ETBE mixture study (White, 2002, unpublished report).

A UF for LOAEL to NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a change of one SD from the control mean was selected under the assumption that it represents a minimal biologically significant change. Therefore the RfC from the data in Medinsky et al. (1999) was calculated as follows:

$$\begin{aligned} RfC &= BMCL_{HEC} \div UF \\ &= 17\ mg/m^3 \div 3{,}000 \\ &= 0.0057 \text{ or } 6.0 \times 10^{-3}\ mg/m^3 \end{aligned}$$

5.2.4. Previous RfC

An inhalation assessment for ETBE was not previously available on IRIS.

5.2.5. Reference Value Comparison Information

Figure 5-1 presents the POD, applied UFs, and derived chronic reference values (RfVs) for additional effect endpoints that were modeled using EPA BMDS (version 1.4.1) and appear in Table 5-2. This comparison is intended to provide information on additional health effects associated with ETBE exposure.

PODs and chronic RfCs that could be derived from the additional health effects identified in Table 5-2 are presented in Figure 5-1 to allow a comparison with the critical effect. Consideration of the available dose-response data to determine an estimate of inhalation exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime has led to the selection of the Medinsky et al. (1999) and mean number of regenerative foci in male rat kidneys as the principal study and critical effect for deriving the chronic RfC for ETBE.

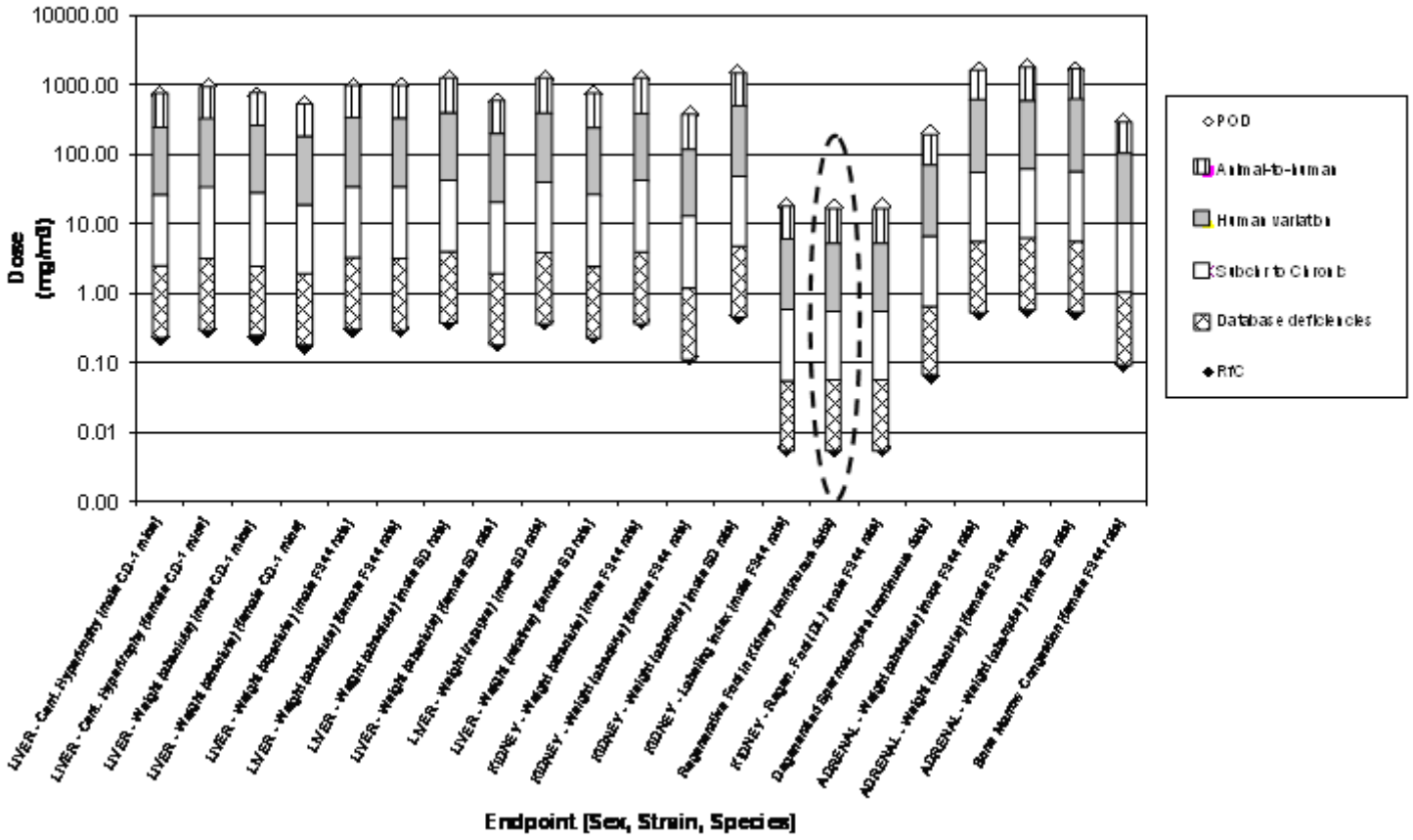


Figure 5-1. RfV comparison array for alternative PODs for inhalation data.

There are a variety of renal effects seen in rats and hepatic effects observed in rats and mice, as well as increased adrenal weight found in rats. Effects observed in the kidney included increased kidney weight in male and female rats, as well as increased mean number and incidence of regenerative foci and sustained proliferation in the kidney indicated by a sustained increase in the LI in male rats. A number of hepatic effects were considered as a potential critical effect including sustained proliferation in the liver indicated by a sustained increase in the LI in mice, increased centrilobular hypertrophy in male and female mice, and increased liver weight in mice and rats. Additional effects considered as potential critical effects included an increased percentage of seminiferous tubules with degenerated spermatocytes in male rats, increased adrenal gland weight in male and female rats, increased heart weight in female rats, and increased incidence of bone marrow congestion in female rats. PODs and chronic RfV that could be derived from the additional health effects identified in Table 5-2 are presented in Figure 5-1 to allow a comparison with the critical effect. For hepatic LI, centrilobular hypertrophy, increased liver weight, increased kidney weight, renal LI, regenerative foci in the kidneys, percentage of seminiferous tubules with degenerated spermatocytes, increased adrenal gland weight, and increased incidence of bone marrow congestion, the total UF applied was 3,000-fold; threefold UF to account for uncertainty in extrapolating from laboratory animals to humans, 10-fold UF to account for variation in susceptibility among members of the human population, 10-fold UF to account for subchronic-to-chronic extrapolation, and 10-fold UF for database deficiencies.

5.3. UNCERTAINTIES IN THE INHALATION REFERENCE CONCENTRATION (RfC)

Risk assessments need to portray associated uncertainty. The following discussion identifies uncertainties associated with the chronic RfC for ETBE. As presented earlier (Sections 5.1.2 and 5.1.3; 5.2.2 and 5.2.3), the UF approach, following EPA practices and RfC and RfD guidance (U.S. EPA, 1994b), was applied to a POD, a $BMDL_{HEC}$ for the chronic RfC. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to extrapolating from an animal bioassay to human exposure, a diverse population of varying susceptibilities, and to account for database deficiencies. These extrapolations are carried out with default approaches given the paucity of experimental ETBE data to inform individual steps.

The database of animal toxicity studies available for the hazard assessment of ETBE, as described throughout the previous section (Chapter 4), is limited. The database of oral toxicity studies includes a prenatal developmental toxicity study, two-generation reproductive toxicity study, and mating and fertility reproductive toxicity study. A single chronic cancer bioassay is available, but the authors did not evaluate or report any noncancer endpoints except mortality. There are no standard subchronic or chronic toxicity studies available for oral exposure to ETBE. Toxicity associated with oral exposure to ETBE is observed as increased organ weight in the

liver and kidneys and decreased body weight gain. The database of inhalation toxicity studies in animals includes two subchronic studies in rats and one subchronic study in mice. No chronic inhalation studies are available. Data from these inhalation toxicity studies of ETBE also indicated that the kidney and liver are target organs for ETBE toxicity as determined by increased kidney and liver weights and additional effects described below. Effects associated with inhalation exposure to ETBE were observed in the liver of mice and rats, kidney and adrenal gland in rats of both sexes, heart and bone marrow in female rats, and testes of male rats. In the liver and kidney, an increase in organ weights, LI, regenerative foci (kidney only), and hepatic centrilobular hypertrophy were reported. Heart and adrenal weights were also increased in rats, but no histopathological lesions were present. Bone marrow congestion was observed in female rats, in the absence of additional hematopoietic effects. An increased percentage of degenerated spermatocytes was also observed in male rats. In addition to the oral and inhalation data are numerous absorption, distribution, metabolism, and excretion references. Critical data gaps have been identified and uncertainties associated with data deficiencies are more fully discussed below.

The critical effect selected for the derivation of the chronic RfC is the mean number of regenerative foci in the kidneys of male rats. Although an increase in liver weights was apparent rats and mice, lesions and serum enzyme levels indicative of liver damage were not evident in rats. Hepatic centrilobular hypertrophy and an increased LI were observed in CD-1 mice. The bone marrow congestion observed in female rats was not considered adverse, as there was no change in the clinical chemistry and hematology parameters.

The selection of the BMD models for the quantitation of the chronic RfC does not lead to significant uncertainty in estimating the PODs since benchmark effect levels were within the range of experimental data. However, the selected model for the RfC, the Hill model, does not represent all possible models one might fit, and other models could be selected to yield more extreme results, both higher and lower than those included in this assessment.

Extrapolating from animals to humans embodies further issues and uncertainties. The effect and the magnitude associated with the concentration at the POD in rodents are extrapolated to human response. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not possible for the toxicity observed following oral and inhalation exposure to ETBE. For the chronic RfC, a factor of 3 was adopted by convention where an adjustment from an animal specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. Application of a full UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a human equivalent concentration as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a

certain degree by the use of the applied dosimetry method and a UF of 3 is retained to account for this component.

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation also needs consideration in extrapolating dose from a subset or smaller sized population, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population. In the absence of ETBE-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation in the derivation of the chronic RfC. Human variation may be larger or smaller; however, ETBE-specific data to examine the potential magnitude of over- or under-estimation are unavailable.

The database of inhalation studies is also of concern due to the lack of a chronic toxicity study, a developmental study, and a multigenerational reproductive toxicity study. The inhalation database contains several subchronic studies in mice and rats. The database for ETBE lacks both a developmental toxicity study and a multigenerational reproductive toxicity study by inhalation exposure, although oral studies of developmental and reproductive toxicity have been conducted (CIT, 2004a, b, unpublished reports). The lack of an immunotoxicity study of inhalation exposure to ETBE also contributes to the database uncertainty in light of the potential for suppression of the antibody response suggested by the unpublished G/ETBE mixture study (White, 2002, unpublished report).

5.4. CANCER ASSESSMENT

Available data indicate there is *suggestive evidence of carcinogenic potential* (U.S. EPA, 2005a) following exposure to ETBE. One oral animal cancer bioassay in rats (Maltoni et al., 1999) is available. Maltoni et al. (1999) exposed Sprague-Dawley rats (60/sex/group) to 0, 250, and 1,000 mg/kg-day of ETBE by gavage 4 days/week for 104 weeks. Statistically significant increases in two tumor types were identified in this study: total pathologies of oncological interest of the mouth epithelium at the high dose (1,000 mg/kg-day) in males and total malignant uterine tumors only at the low dose (250 mg/kg-day) in females. Nonsignificant increases in total pathologies of oncological interest of the forestomach in males (at the lower of the two test doses) and hemolymphoreticular system were reported in both sexes. In all four cases, the total tumors including precancers were listed as increased, and no individual tumor type was reported as increased. This study was reported as a preliminary study and did not include criteria used for the histopathological classification. This is especially relevant to the inclusion of dysplasias as one of the pathologies of oncological interest for both the mouth epithelium and the forestomach. Although there is an association between severe dysplasia and eventual carcinoma, the majority of lesions with mild to moderate dysplasia do not progress to cancer. The significance of the reported increase in total pathologies of oncological interest of the forestomach and mouth epithelium is, therefore, confounded as dysplasias represent high portions (i.e., 58–100%) of the

reported data. The authors grouped tissues, such as total pathologies of oncological interest in the mouth epithelium (including the tongue, lips, and oral cavity), without providing individual tumor information. Tumors of the uterus and vagina were also combined, and the total malignant tumors of the uterus would not be statistically increased relative to control if the vaginal tumors were removed. Knowledge of the historical incidence of tumors in this laboratory would provide further context for the concurrent controls. Historical controls may be particularly relevant to the incidence of hemolymphoreticular neoplasias, which are listed as nonsignificantly increased in the ETBE study. An examination of other oral gavage studies by the same lab suggests that the 5% incidence in control males from Maltoni et al. (1999) may be low relative to historical controls. The total pathologies of oncological interest of the mouth epithelium in males were the only pathologies that exhibited a dose-response or positive dose-related trend. The increased mortality of animals at both doses of ETBE presents a limitation in the study and the ability to interpret the results from a quantitative perspective. For these reasons, an estimate of cancer risks was not quantified.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

ETBE is a colorless liquid with the chemical formula of $C_6H_{14}O$ and a characteristic strong, gasoline-like odor, and a taste that has been described as highly objectionable. It is an oxygenate used as a gasoline additive at amounts up to 17% by weight to reduce the emission of carbon monoxide and ozone into the atmosphere. ETBE is released into the environment through the use and storage of gasoline. Environmental concern surrounding fuel oxygenates is associated with automotive emissions, inhalation and/or dermal exposure while refueling, and oral or dermal exposure from groundwater contamination (largely from leaking underground fuel storage tanks).

Absorption data are only available for exposure to ETBE via inhalation. The percent of the respired dose retained was 32–34%, with a portion of the absorbed dose exhaled, to result in a net uptake via the respiratory tract of about 26% (Nihlén et al., 1998a). Distribution data from ETBE are not available, although data from MTBE, the methyl analog of ETBE, are available along with in vitro experiments comparing MTBE and ETBE partition coefficients in human blood samples. The higher oil:water partition coefficient of ETBE suggests a greater distribution to fat and lipid-rich tissues than MTBE. The major metabolites of ETBE are TBA and acetaldehyde. TBA can be sulfated, glucuronidated, or oxidized to MPD, and subsequently to HBA.

Data on the effects of ETBE in humans are limited to several 2-hour inhalation studies at doses up to 50 ppm. Healthy subjects exposed to ETBE experienced irritation in throat and airways, nasal swelling, a bad taste in the mouth, and slightly impaired lung function (about 3% reduction in vital capacity and forced vital capacity). No chronic inhalation study was available, although there are several subchronic studies in mice and rats.

Liver and kidney toxicity were the primary noncancer health effects of subchronic oral and inhalation exposure to ETBE based on the limited available animal data. Increased liver and kidney weights were observed following oral exposure in rats of both sexes in a two-generation reproductive toxicity study (CIT, 2004b, unpublished report). However, histology was only performed if abnormal morphology was detected at necropsy, and, therefore, there are limited histological data to support nephrotoxicity or hepatotoxicity. Oral exposure to ETBE was also associated with decreased body weight gain in rats (CIT 2004 a, b, unpublished reports). Additional evidence of ETBE-associated kidney toxicity is provided by the ETBE inhalation exposure database. Increased kidney weight was also observed following subchronic inhalation exposure to ETBE in male and female F344 rats (Medinsky et al., 1999) and male Sprague-Dawley rats (White et al., 1995). Data from the inhalation studies demonstrated that increased kidney weight was associated with histopathological changes in male rats (e.g., regenerative foci

indicative of cellular necrosis) in males and sustained increase of greater than twofold in cellular proliferation (Medinsky et al., 1999). The increased kidney weight was not associated with histopathological changes in female rats, although an increased cellular proliferative response of less than twofold was observed at early time points (after 1 and 4, but not 13 weeks of exposure) (Medinsky et al., 1999). Based on the available data for ETBE, there is the suggestion that alpha$_{2u}$-globulin accumulation is the mode of action; however, there is considerable uncertainty because of the lack of evidence of typical lesions in the pathological sequence of lesions associated with alpha$_{2u}$-globulin nephropathy and, therefore, a determination cannot be made as to whether alpha$_{2u}$-globulin accumulation is the mode of action or the only mode of action for renal effects associated with ETBE exposure. Increased liver weight was also observed following subchronic inhalation exposure to ETBE in mice and rats of both sexes (Medinsky et al., 1999; White et al., 1995). No changes in histopathology or serum levels of hepatic enzymes were observed in rats. Dose-related increases in hepatic proliferation were observed in male and female mice at some time periods during a 13-week exposure, and not observed at others (Medinsky et al., 1999).

Inhalation exposure to ETBE was also associated with increased adrenal gland weights in male and female rats, increased percentage of seminiferous tubules with degenerated spermatocytes in male rats, increased heart weight in female rats, and increased incidence of bone marrow congestion in female rats (Medinsky et al., 1999). No sensorimotor dysfunction, neuromuscular dysfunction, or microscopic evidence of neuropathy were detected in F344 rats after 13 weeks of inhalation exposure (Dorman et al., 1997). Evidence of suppressed antibody production from a G/ETBE mixture inhalation immunotoxicity study (White, 2002, unpublished report) suggests that ETBE may have immunosuppressive activity, but there are no data from an immunotoxicity study of ETBE alone.

Under the Guidelines for Carcinogen Risk Assessment (U.S. EPA 2005a) available data for ETBE can be classified as *suggestive evidence of carcinogenic potential,* based on a single oral animal cancer bioassay in rats (Maltoni et al., 1999). Although there is evidence of carcinogenicity of both TBA, the primary metabolite of ETBE (NTP, 1995; Cirvello et al., 1995), and the other primary metabolite, acetaldehyde (WHO, 1995), as well as MTBE (the methyl substituted analog of ETBE reviewed in Ahmed, 2001; Cal EPA, 1999; Mennear, 1997), there is a lack of data on ETBE, its mode(s) of action, and whether the parent compound or metabolites are responsible for observed effects. The genotoxicity data for ETBE were essentially negative.

6.2. DOSE RESPONSE

6.2.1. Noncancer / Oral

Considering the uncertainties in the ETBE database, which are described in Appendix C, the total composite UF is 10,000, consisting of four areas of maximum uncertainty. In the report,

A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002), the RfD/RfC technical panel concluded that, in cases where maximum uncertainty exists in four or more areas of extrapolation, or when the total UF is 10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Appendix C contains a derivation of an oral minimal data value for ETBE using an UF of 10,000. Use of this minimal data value is not recommended except in limited circumstances, for example, in screening level risk assessments or to rank relative risks. Any use of this value should include a discussion of the uncertainty associated with its derivation.

6.2.2. Noncancer / Inhalation

The RfC of 6×10^{-3} mg/m^3 was derived based on increased mean number of regenerative foci in the kidneys in male rats exposed to ETBE for 13 weeks by inhalation (Medinsky et al., 1999). This study was chosen as the principal study because the kidney effects (i.e., regenerative foci) observed in this study represent the most sensitive effects identified in the database evaluating exposure through the inhalation route to ETBE. In addition, EPA considers regenerative foci to be indicators of cellular necrosis and a biomarker of an adverse effect. The RfC is derived by dividing the BMCL of 17 mg/m^3 by a composite UF of 3,000 (factor of 3 for interspecies variability, factors of 10 for interindividual variability, subchronic-to-chronic extrapolation, and database deficiencies). A factor of 3 was selected to account for uncertainties in extrapolating from rats to humans, which is adopted by convention where an adjustment from an animal specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. Insufficient information is available to predict the potential variability in human susceptibility among the population; thus, the human variability UF of 10 was applied. A 10-fold UF was used to account for uncertainty in extrapolating from a subchronic to chronic exposure duration. A 10-fold UF was used to account for deficiencies in the database. The database for ETBE lacks both a developmental toxicity and a multigenerational reproductive toxicity study by inhalation exposure.

The overall confidence in this RfC assessment is medium. Confidence in the principal study (Medinsky et al., 1999) is medium. Confidence in the database is low-to-medium due to the lack of both a developmental toxicity and a multigenerational reproductive toxicity study by inhalation exposure. Reflecting medium confidence in the principal study and low-to-medium confidence in the database, confidence in the RfC is low-to-medium.

6.2.3. Cancer / Oral

Available data that indicate there is *suggestive evidence of carcinogenic potential* (U.S. EPA, 2005a) following exposure to ETBE. One oral animal cancer bioassay in rats (Maltoni et al., 1999) is available. Statistically significant increases in two tumor types were identified in this study: total pathologies of oncological interest of the mouth epithelium at the high dose (1,000 mg/kg-day) in males and total malignant uterine tumors only at the low dose (250 mg/kg-

day) in females. Nonsignificant increases in total pathologies of oncological interest of the forestomach in males (at the lower of the two test doses) and hemolymphoreticular system were reported in both sexes. In all four cases, the total tumors including precancers were listed as increased, and no individual tumor type was reported as increased. This study did not include the criteria used for the histopathological classification, which is especially relevant to the inclusion of dysplasias as one of the pathologies of oncological interest for both the mouth epithelium and the forestomach. Although there is an association between severe dysplasia and eventual carcinoma, the majority of lesions with mild to moderate dysplasia do not progress to cancer. The significance of the reported increase in total pathologies of oncological interest of the forestomach and mouth epithelium is, therefore, confounded as dysplasias represent high portions (i.e., 58–100%) of the reported data. The authors grouped tissues, such as total pathologies of oncological interest in the mouth epithelium (including the tongue, lips, and oral cavity) without providing individual tumor information. Tumors of the uterus and vagina were also combined, and the total malignant tumors of the uterus would not be statistically increased relative to control if the vaginal tumors were removed. Knowledge of the historical incidence of tumors in this laboratory would provide further context for the concurrent controls. Historical controls may be particularly relevant to the incidence of hemolymphoreticular neoplasias, which are listed as nonsignificantly increased in the ETBE study. An examination of other oral gavage studies by the same lab suggests that the 5% incidence in control males from Maltoni et al. (1999) may be low relative to historical controls. The total pathologies of oncological interest of the mouth epithelium in males were the only pathologies that exhibited a dose-response or positive dose-related trend. The increased mortality of animals at both doses of ETBE presents a limitation in the study and in the ability to interpret the results from a quantitative perspective. For these reasons, an estimate of cancer risks was not quantified.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

APPENDIX B. BMD CALCULATIONS FOR THE ORAL MINIMAL DATA VALUE AND RfC

B.1. NONCANCER DOSE-RESPONSE ASSESSMENT FOR ORAL EXPOSURE TO ETBE: BMD MODELING RESULTS

In this appendix, results of BMD modeling are presented for each noncancer endpoint showing significantly elevated means relative to controls following oral exposure to ETBE. For each endpoint, a summary of the dose-response data is presented, followed by a table summarizing the results of the dose-response modeling. Finally, the standard output from the EPA's BMDS (version 1.4.1), for the best-fitting dose-response model is presented.

For these modeling exercises, all continuous models available in BMDS were fit to the corresponding data for each endpoint with the BMR set at one SD above the control mean. To select the "best-fit" model, AIC values were evaluated for all models that did not exhibit a significant lack of fit (i.e., $p < 0.1$), according to the χ^2 goodness-of-fit test. Of these models, the model with the lowest AIC value was typically selected as the best-fit model unless examination of the chi-square scaled residuals indicated another model with a similar AIC exhibited a better fit in the region of the curve where the BMD was estimated. Selection of the BMR and the procedure for selecting the best-fit model are consistent with the EPA's most current *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b).

Organ: Body

Endpoint: Dam Body Weight Change

Species/Gender: Sprague-Dawley Female Rats

(CIT, 2004a, unpublished report)

Table B-1. Mean dam body weight change (and SD) in Sprague-Dawley female rats orally exposed to ETBE on GDs 5 through 20

Administered dose (mg/kg)	Mean dam body weight change, g (SD)
Control (n = 21)	135 (22)
250 (n = 19)	132 (12)
500 (n = 20)	134 (19)
1,000 (n = 22)	120[a] (15)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004a, unpublished report).

Source: CIT, (2004a, unpublished report).

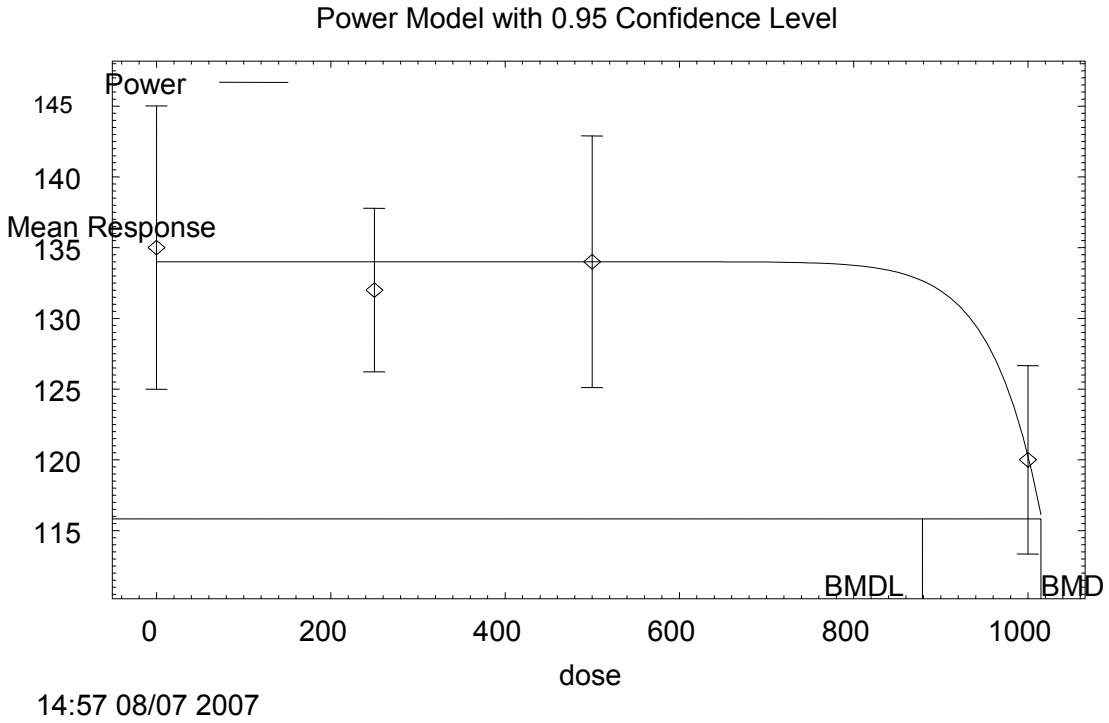
Table B-2. A summary of BMDS (version 1.4.1) modeling results based on mean dam body weight change in Sprague-Dawley female rats orally exposed to ETBE on GDs 5 through 20

Model[a] (non-constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (1°)	0.19	555.6	1,236	796
Power	**0.35**	**554.3**	**1,015**	**879**
Hill	0.15	556.3	1,045	Computation of the lower bound failed

[a]For all models, the variance model employed (i.e., variance modeled as a power function of the mean) failed to adequately address the non-constant variance.

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: CIT (2004a, unpublished report).



Source: CIT (2004a, unpublished report).

Figure B-1. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean dam body weight change in Sprague-Dawley female rats orally exposed to ETBE on GDs 5 through 20.

```
 ================================================================
        Power Model. (Version: 2.14;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_DAMS_BODY_WT_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_DAMS_BODY_WT_CIIT04.plt
                                    Tue Aug 07 14:57:57 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = Dose
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
```

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

Default Initial Parameter Values

lalpha =	5.72308
rho =	0
control =	120
slope =	3.51478
power =	0.222392

Asymptotic Correlation Matrix of Parameter Estimates

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

	lalpha	rho	control	slope
lalpha	1	-1	0.17	-0.31
rho	-1	1	-0.17	0.31
control	0.17	-0.17	1	-0.59
slope	-0.31	0.31	-0.59	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-12.3216	16.6779	-45.0097	20.3664
rho	3.6953	3.42706	-3.02161	10.4122
control	133.717	2.3108	129.188	138.246
slope	-1.37167e-053	3.88616e-054	-2.13334e-053	-6.09996e-054
power	18	NA		

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	21	135	134	22	17.9	0.329
250	19	132	134	12	17.9	-0.418
500	20	134	134	19	17.9	0.0708
1,000	22	120	120	15	14.7	-2.04e-007

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-273.595943	5	557.191887
A2	-269.540413	8	555.080827
A3	-272.114636	6	556.229273
fitted	-273.151218	4	554.302436
R	-278.636681	2	561.273362

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	18.1925	6	0.005769
Test 2	8.11106	3	0.04377
Test 3	5.14845	2	0.07621
Test 4	2.07316	2	0.3547

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1014.9

BMDL = 879.018

Organ: Body

Endpoint: Net Dam Body Weight Change

Species/Gender: Sprague-Dawley Female Rats

(CIT, 2004a, unpublished report)

Table B-3. Mean net dam body weight change (and SD) in Sprague-Dawley female rats orally exposed to ETBE on GDs days 5 through 20

Administered dose (mg/kg)	Mean net dam body weight change, g (SD)
Control (n = 21)	61.8 (13)
250 (n = 19)	59.4 (8.1)
500 (n = 20)	60.0 (11.3)
1,000 (n = 22)	51.5[a] (10.3)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004a, unpublished report).

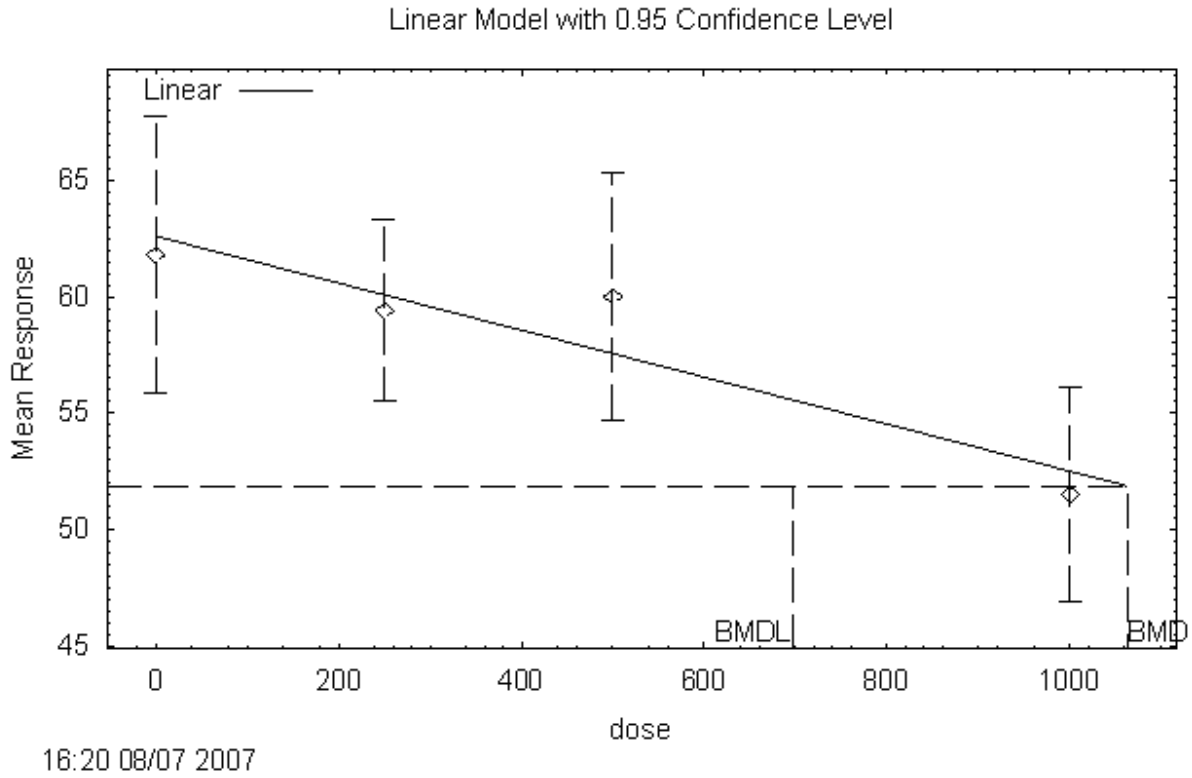
Source: CIT (2004a, unpublished report).

Table B-4. A summary of BMDS (version 1.4.1) modeling results based on mean net dam body weight change in Sprague-Dawley female rats orally exposed to ETBE on GDs 5 through 20

Model (constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (1°)	**0.49**	**476.6**	**1,063**	**696**
Power	0.49	477.7	1,044	748
Hill	NA	479.7	1,044	747

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: CIT (2004a, unpublished report).



Source: CIT (2004a, unpublished report).

Figure B-2. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean dam body weight change in Sprague-Dawley female rats orally exposed to ETBE on GDs 5 through 20.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_DAMS_NET_BODY_WT_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_DAMS_NET_BODY_WT_CIIT04.plt
                                        Tue Aug 07 16:20:42 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
```

Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	118.141	
rho =	0	Specified
beta_0 =	62.54	
beta_1 =	-0.00997714	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	6e-009	5.9e-009
beta_0	6e-009	1	-0.76
beta_1	5.9e-009	-0.76	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	114.378	17.8629	79.3677	149.389
beta_0	62.55	1.83181	58.9597	66.1403
beta_1	-0.0100599	0.00312435	-0.0161835	-0.00393626

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	21	61.8	62.6	13	10.7	-0.321
250	19	59.4	60	8.1	10.7	-0.259
500	20	60	57.5	11.3	10.7	1.04
1,000	22	51.5	52.5	10.3	10.7	-0.434

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-234.596525	5	479.193049
A2	-232.361985	8	480.723971
A3	-234.596525	5	479.193049
fitted	-235.319981	3	476.639962
R	-240.201188	2	484.402375

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	15.6784	6	0.01559
Test 2	4.46908	3	0.2151
Test 3	4.46908	3	0.2151
Test 4	1.44691	2	0.4851

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

```
The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        1063.11


            BMDL =         696.25
```

Organ: Body

Endpoint: F_0 Body Weight Change

Species/Gender: Sprague-Dawley Male Rats

(CIT, 2004b, unpublished report)

Table B-5. Mean F0 body weight change (and SD) in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F0 body weight change, g (SD)
Control (n = 25)	58.0 (15)
250 (n = 25)	56.0 (9)
500 (n = 25)	41.0[a] (19)
1,000 (n = 25)	45.0[a] (5)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

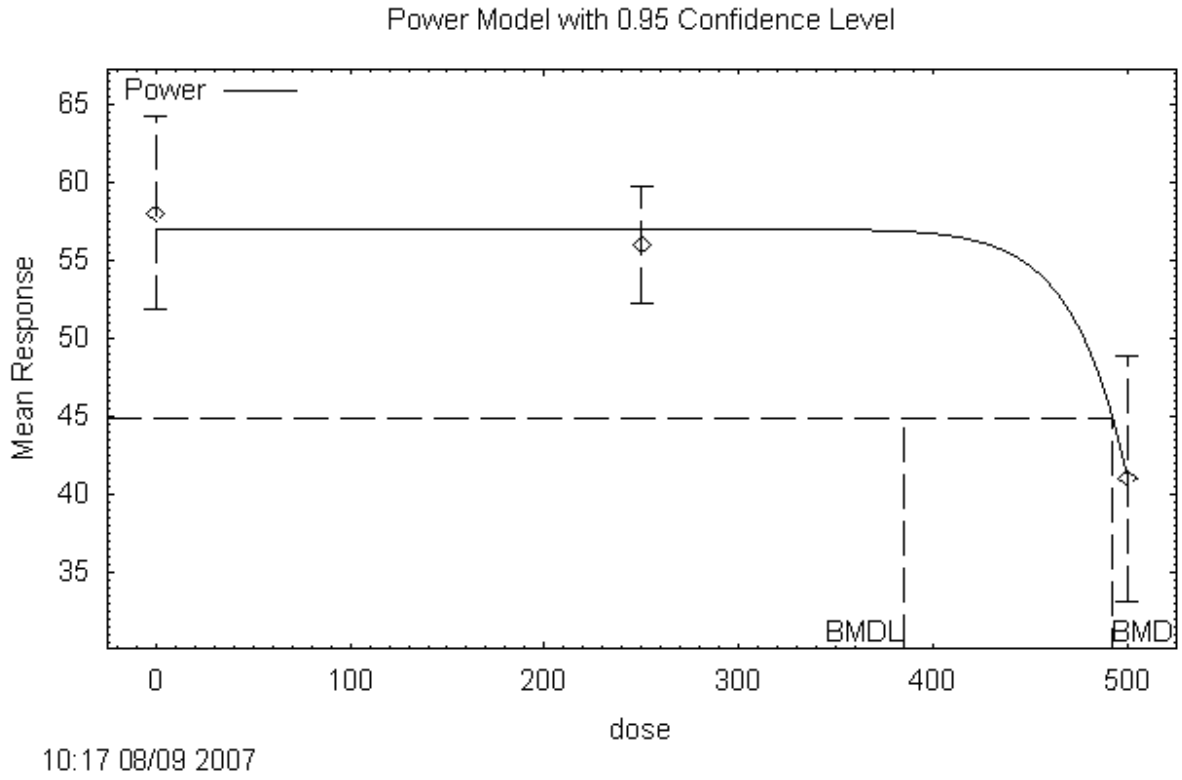
Source: CIT (2004b, unpublished report).

Table B-6. A summary of BMDS (version 1.4.1) modeling results based on mean F0 body weight change in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model[a] (non-constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (1°)	0.01	486.4	405	276
Power	**0.73**	**479.0**	**492**	**385**
Hill[b]	—	—	—	—

[a]For all models, the highest dose group was dropped prior to fitting the model, and the variance model employed (i.e., variance modeled as a power function of the mean) failed to adequately address the non-constant variance.
[b]The Hill model could not be fit to these data as the number of model parameters to be estimated exceeded the number of observations.
Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: CIT (2004b, unpublished report).



Source: CIT (2004b, unpublished report).

Figure B-3. BMDS (version 1.4.1) model output for the best-fit model (i.e., power) based on mean F0 body weight change in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study.

```
================================================================
        Power Model. (Version: 2.14;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F0_BODY_WT_DAYS85_113_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F0_BODY_WT_DAYS85_113_CIIT04.plt
                                        Thu Aug 09 10:17:20 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = Dose
```

The power is restricted to be greater than or equal to 1
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	5.40418
rho =	0
control =	58
slope =	-0.034
power =	1

Asymptotic Correlation Matrix of Parameter Estimates

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

	lalpha	rho	control	slope
lalpha	1	-1	-0.17	0.58
rho	-1	1	0.18	-0.58
control	-0.17	0.18	1	-0.42
slope	0.58	-0.58	-0.42	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	15.447	5.11829	5.41539	25.4787
rho	-2.58484	1.29189	-5.11691	-0.0527773
control	57	1.71977	53.6293	60.3707
slope	-4.19432e-048	1.07511e-048	-6.3015e-048	-2.08714e-048
power	18	NA		

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	25	58	57	15	12.2	0.411

250	25	56	57	9	12.2	-0.411
500	25	41	41	19	18.6	3.09e-006

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-238.625841	4	485.251682
A2	-232.212019	6	476.424038
A3	-235.450799	5	480.901597
fitted	-235.510734	4	479.021468
R	-247.578841	2	499.157682

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	30.7336	4	<.0001
Test 2	12.8276	2	0.001639
Test 3	6.47756	1	0.01092
Test 4	0.11987	1	0.7292

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 492.436

BMDL = 384.702

Organ: Liver

Endpoint: F_1 Liver Weight (absolute)

Species/Gender: Sprague-Dawley Male Rats

(CIT, 2004b, unpublished report)

Table B-7. Mean absolute F1 liver weight (and SD) in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F1 liver weight, g (SD)
Control (n = 24)	18.9 (2.45)
250 (n = 25)	18.9 (2.32)
500 (n = 24)	21.6[a] (4.16)
1,000 (n = 25)	23.9[a] (4.10)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

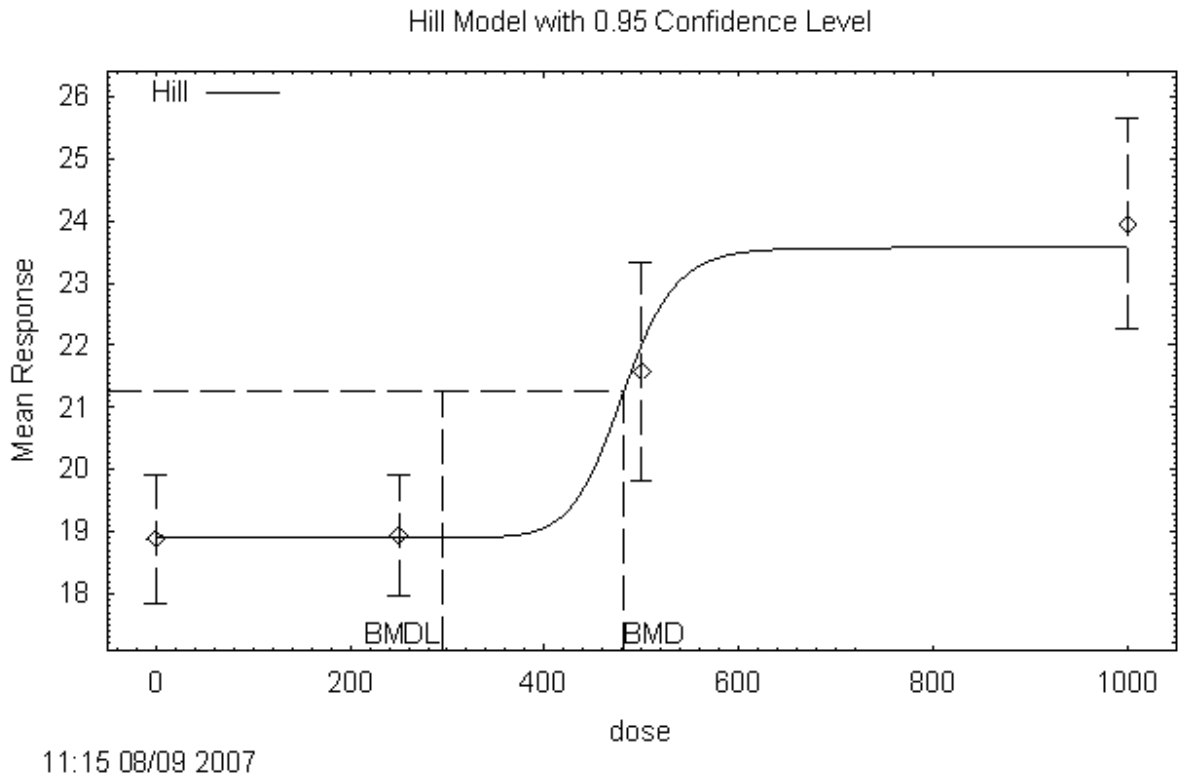
Source: CIT (2004b, unpublished report).

Table B-8. A summary of BMDS (version 1.4.1) modeling results based on mean absolute F1 liver weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model (non-constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (1°)	0.04	334.3	462	337
Power	0.01	335.9	514	345
Hill	**0.96**	**329.7**	**482**	**294**

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: CIT (2004b, unpublished report).



Source: CIT (2004b, unpublished report).

Figure B-4. BMDS (version 1.4.1) model output for the best-fit model (i.e., Hill) based on mean absolute F1 liver weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study.

```
 ================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F1_LIVER_WT_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F1_LIVER_WT_CIIT04.plt
                                        Thu Aug 09 11:15:37 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
```

Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))

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

Default Initial Parameter Values

lalpha =	2.43091
rho =	0
intercept =	18.89
v =	5.06
n =	3.18615
k =	515.152

Asymptotic Correlation Matrix of Parameter Estimates

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

	lalpha	rho	intercept	v	k
lalpha	1	-1	-0.3	0.57	0.26
rho	-1	1	0.29	-0.58	-0.25
intercept	-0.3	0.29	1	-0.41	0.028
v	0.57	-0.58	-0.41	1	0.52
k	0.26	-0.25	0.028	0.52	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-14.7294	5.67709	-25.8562	-3.60247
rho	5.60352	1.87657	1.92552	9.28152
intercept	18.8835	0.338055	18.2209	19.5461
v	4.67469	0.939755	2.8328	6.51658
n	18	NA		
k	480.83	21.3555	438.974	522.685

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	24	18.9	18.9	2.45	2.38	0.0134
250	25	18.9	18.9	2.32	2.38	0.119
500	24	21.6	22	4.16	3.66	-0.577
1,000	25	23.9	23.6	4.1	4.43	0.443

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-166.072486	5	342.144972
A2	-158.990348	8	333.980695
A3	-159.830921	6	331.661841
fitted	-159.832061	5	329.664122
R	-182.852138	2	369.704276

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	47.7236	6	<.0001
Test 2	14.1643	3	0.00269
Test 3	1.68115	2	0.4315
Test 4	0.0022814	1	0.9619

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 481.838

BMDL = 294.036

Organ: Liver

Endpoint: F1 Liver Weight (relative)

Species/Gender: Sprague-Dawley Male Rats

(CIT, 2004b, unpublished report)

Table B-9. Mean relative F1 liver weight (and SD) in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F1 liver weight as percent body weight (SD)
Control (n = 24)	3.20 (0.225)
250 (n = 25)	3.21 (0.245)
500 (n = 24)	3.54[a] (0.317)
1,000 (n = 25)	4.01[a] (0.389)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

Source: CIT (2004b, unpublished report).

Table B-10. A summary of BMDS (version 1.4.1) modeling results based on mean relative F1 liver weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model (non-constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (2°)	0.03	-135.4	418	284
Power	0.05	-136.2	414	295
Hill	NA	-138.0	444	341

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: CIT (2004b, unpublished report).

All continuous dose-response models available in BMDS (version 1.4.1) exhibited significant lack-of-fit (i.e., chi-square *p*-value for goodness-of-fit < 0.1); therefore, no POD could be derived from these data based on the modeling results.

Organ: Kidney

Endpoint: F0 Kidney Weight (absolute)

Species/Gender: Sprague-Dawley Male Rats

(CIT, 2004b, unpublished report)

Table B-11. Mean absolute F0 kidney weight (and SD) in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F0 kidney weight, g (SD)
Control (n = 25)	3.58 (0.413)
250 (n = 25)	3.96[a] (0.446)
500 (n = 25)	4.12[a] (0.624)
1,000 (n = 25)	4.34[a] (0.434)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

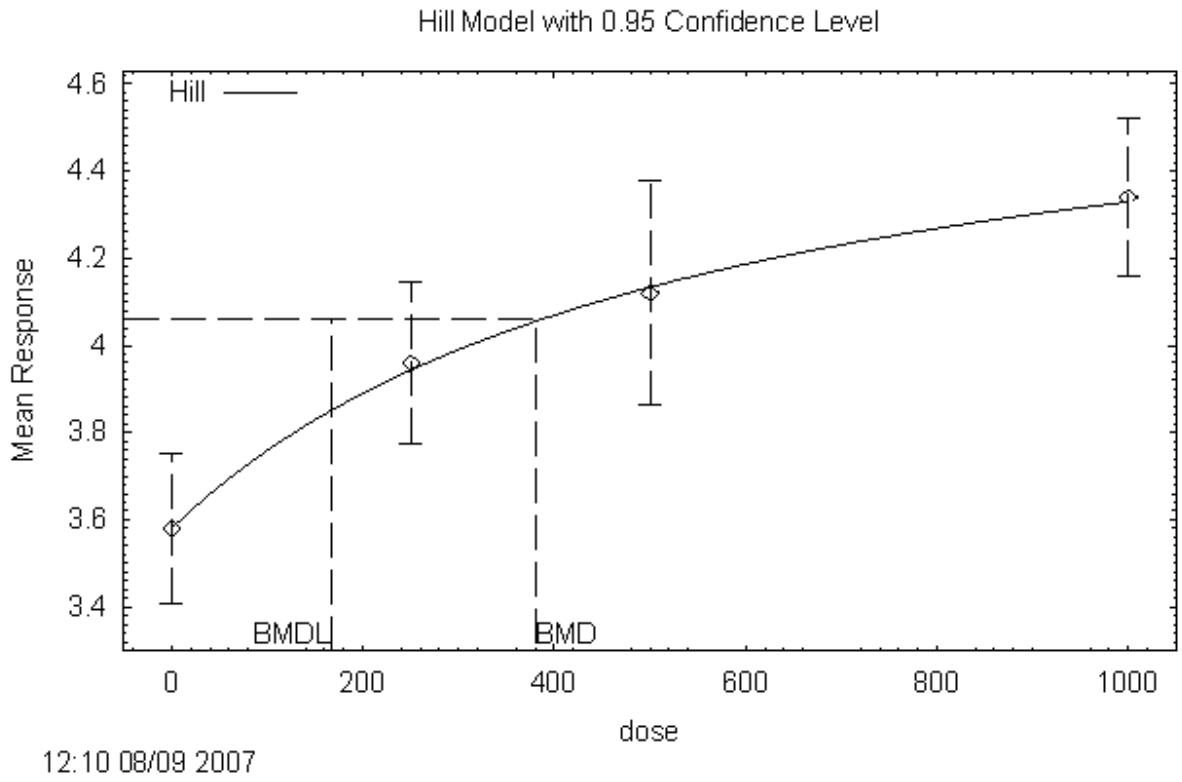
Source: CIT (2004b, unpublished study).

Table B-12. A summary of BMDS (version 1.4.1) modeling results based on mean absolute F0 kidney weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model (constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (2°)	0.58	-39.8	404	250
Power	0.20	-38.9	679	513
Hill	**0.81**	**-40.1**	**381**	**167**

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: CIT (2004b, unpublished report).



Source: CIT (2004b, unpublished report).

Figure B-5. BMDS (version 1.4.1) model output for the best-fit model (i.e., Hill) based on mean absolute F0 kidney weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study.

```
 ================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F0_KIDNEY_WT_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F0_KIDNEY_WT_CIIT04.plt
                                        Thu Aug 09 12:10:32 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
```

Power parameter restricted to be greater than 1
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.236804	
rho =	0	Specified
intercept =	3.58	
v =	0.76	
n =	0.647728	
k =	250	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	intercept	v	k
alpha	1	-7e-008	-1.9e-007	-2.2e-007
intercept	-7e-008	1	0.036	0.42
v	-1.9e-007	0.036	1	0.89
k	-2.2e-007	0.42	0.89	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.227462	0.032168	0.164414	0.29051
intercept	3.58236	0.0952609	3.39565	3.76906
v	1.16337	0.440153	0.300691	2.02606
n	1	NA		
k	548.322	492.789	-417.527	1514.17

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

Table of Data and Estimated Values of Interest

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

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

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	9.481790	2	-14.963581

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	35.0216	6	<.0001
Test 2	5.85084	3	0.1191
Test 3	5.85084	3	0.1191
Test 4	0.057089	1	0.8112

```
The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1.  The modeled variance appears to
be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        380.964

            BMDL =       167.157
```

Organ: Kidney

Endpoint: F0 Kidney Weight (relative)

Species/Gender: Sprague-Dawley Male Rats

(CIT, 2004b, unpublished report)

Table B-13. Mean relative F0 kidney weight (and SD) in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F0 kidney weight, as percent body weight (SD)
Control (n = 25)	0.596 (0.053)
250 (n = 25)	0.662[a] (0.052)
500 (n = 25)	0.706[a] (0.076)
1,000 (n = 25)	0.763[a] (0.063)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

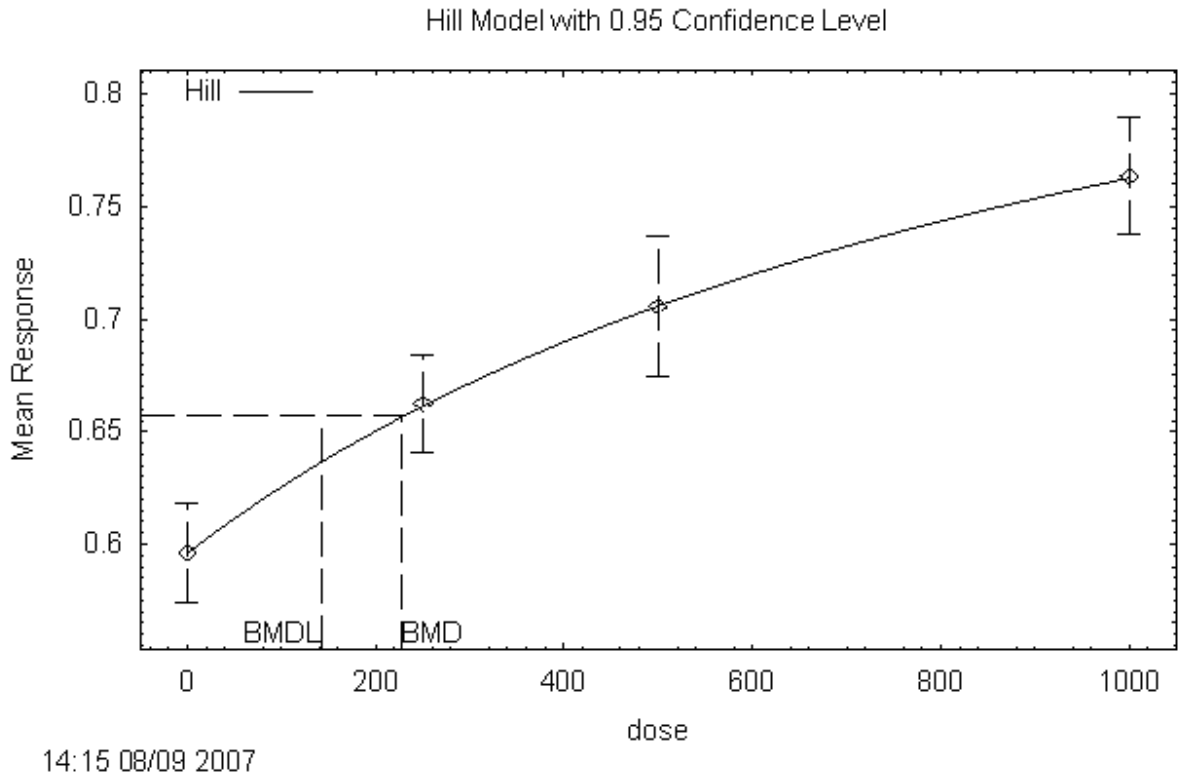
Source: CIT (2004b, unpublished report).

Table B-14. A summary of BMDS (version 1.4.1) modeling results based on mean relative F0 kidney weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model (constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (2°)	0.75	-452.9	243	176
Power	0.13	-450.9	382	317
Hill	**0.94**	**-453.0**	**227**	**143**

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values <0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: CIT (2004, unpublished report).



Source: CIT (2004b, unpublished report).

Figure B-6. BMDS (version 1.4.1) model output for the best-fit model (i.e., Hill) based on mean relative F0 kidney weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study.

```
 ================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F0_REL_KIDNEY_WT_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_MALE_F0_REL_KIDNEY_WT_CIIT04.plt
                                      Thu Aug 09 14:15:52 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.0038145	
rho =	0	Specified
intercept =	0.59628	
v =	0.16713	
n =	0.221145	
k =	649.462	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	intercept	v	k
alpha	1	5.1e-009	-1.6e-008	-1.4e-008
intercept	5.1e-009	1	0.27	0.49
v	-1.6e-008	0.27	1	0.96
k	-1.4e-008	0.49	0.96	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.00366216	0.000517907	0.00264708	0.00467724
intercept	0.596439	0.0119542	0.573009	0.619869
v	0.345284	0.122057	0.106057	0.584512
n	1	NA		
k	1070.39	697.553	-296.786	2437.57

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
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
1,000	25	0.763	0.763	0.063	0.0605	0.0164

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	230.488384	5	-450.976768
A2	232.931535	8	-449.863070
A3	230.488384	5	-450.976768
fitted	230.485140	4	-452.970280
R	195.370878	2	-386.741756

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	75.1213	6	<.0001
Test 2	4.8863	3	0.1803
Test 3	4.8863	3	0.1803
Test 4	0.0064882	1	0.9358

```
The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1.  The modeled variance appears to
be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       227.467

            BMDL =       143.401
```

Organ: Kidney

Endpoint: F_1 Kidney Weight (absolute)

Species/Gender: Sprague-Dawley Male Rats

(CIT, 2004b, unpublished report)

Table B-15. Mean absolute F1 kidney weight (and SD) in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F1 kidney weight, g (SD)
Control (n = 24)	3.38 (0.341)
250 (n = 25)	3.73 (0.449)
500 (n = 24)	4.13[a] (0.640)
1,000 (n = 25)	5.34[a] (5.390)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

Source: CIT (2004b, unpublished report).

Table B-16. A summary of BMDS (version 1.4.1) modeling results based on mean absolute F1 kidney weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model (non-constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (2°)	0.09	80.0	313	218
Power	0.07	80.5	337	240
Hill	NA	82.5	337	Computation of the lower bound failed

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: CIT (2004b, unpublished report).

All continuous dose-response models available in BMDS (version 1.4.1) exhibited significant lack-of-fit (i.e., chi-square *p*-value for goodness-of-fit < 0.1); therefore, no POD could be derived from these data based on the modeling results.

Organ: Kidney

Endpoint: F1 Kidney Weight (absolute)

Species/Gender: Sprague-Dawley Female Rats

(CIT, 2004b, unpublished report)

Table B-17. Mean absolute F1 kidney weight (and SD) in Sprague-Dawley female rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F1 kidney weight, g (SD)
Control (n = 25)	2.24 (0.178)
250 (n = 24)	2.34 (0.242)
500 (n = 25)	2.30 (0.226)
1,000 (n = 23)	2.49[a] (0.284)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

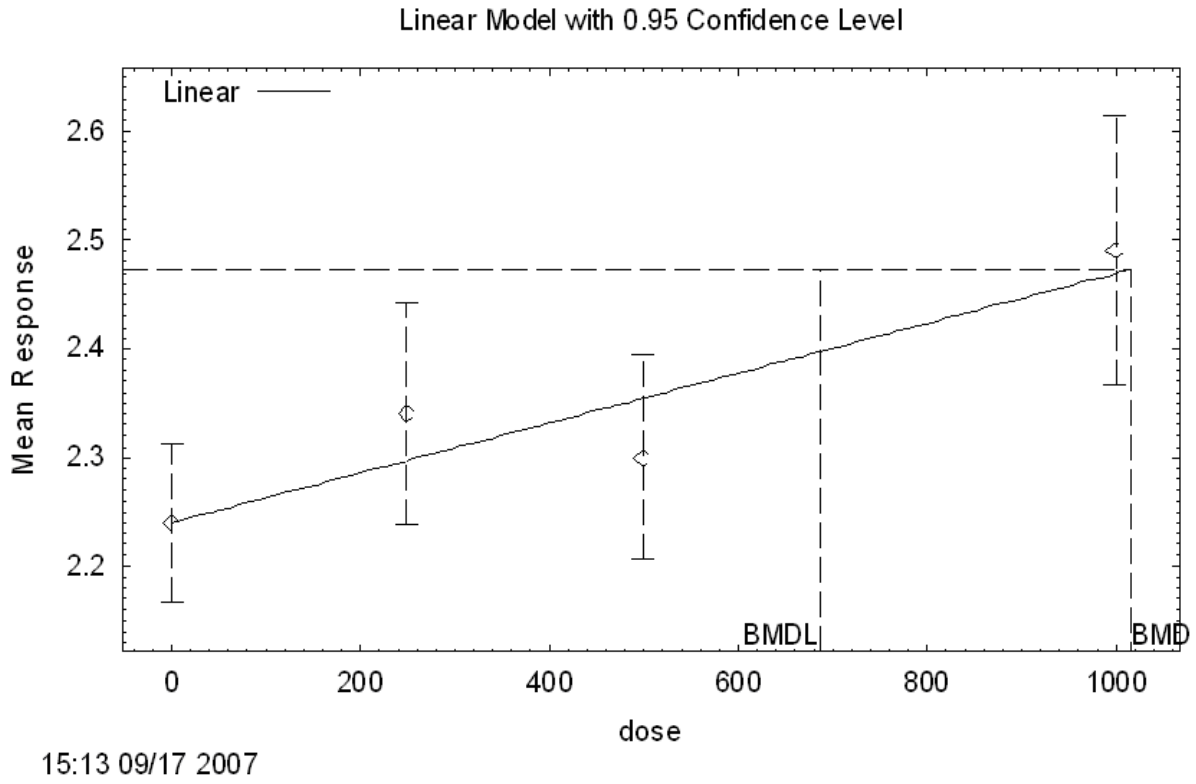
Source: CIT (2004b, unpublished report).

Table B-18. A summary of BMDS (version 1.4.1) modeling results based on mean absolute F1 kidney weight in Sprague-Dawley female rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model (constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (1°)	**0.30**	**-180.2**	**1,016**	**687**
Power	0.14	-178.4	1,033	699
Hill	NA	-176.4	1,033	662

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values <0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: CIT, (2004b, unpublished report).



Source: CIT, (2004b, unpublished report).

Figure B-7. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute F1 kidney weight in Sprague-Dawley female rats orally exposed to ETBE in a two-generation reproductive toxicity study.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_F1_KIDNEY_WT_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_F1_KIDNEY_WT_CIIT04.plt
                                        Mon Sep 17 15:13:50 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.0549209	
rho =	0	Specified
beta_0 =	2.242	
beta_1 =	0.000229714	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	-9e-010	-2.1e-010
beta_0	-9e-010	1	-0.76
beta_1	-2.1e-010	-0.76	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.0539761	0.00775052	0.0387854	0.0691668
beta_0	2.24166	0.0362871	2.17053	2.31278
beta_1	0.000228659	6.44489e-005	0.000102342	0.000354977

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	25	2.24	2.24	0.178	0.232	-0.0356
250	24	2.34	2.3	0.242	0.232	0.868
500	25	2.3	2.36	0.226	0.232	-1.2
1,000	23	2.49	2.47	0.284	0.232	0.406

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	94.282678	5	-178.565356
A2	96.875846	8	-177.751692
A3	94.282678	5	-178.565356
fitted	93.081873	3	-180.163745
R	87.164175	2	-170.328351

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	19.4233	6	0.003505
Test 2	5.18634	3	0.1587
Test 3	5.18634	3	0.1587
Test 4	2.40161	2	0.301

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1016.04

BMDL = 687.185

Organ: Kidney

Endpoint: F1 Kidney Weight (relative)

Species/Gender: Sprague-Dawley Male Rats

(CIT, 2004b, unpublished report)

Table B-19. Mean relative F1 kidney weight (and SD) in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F1 kidney weight, as percent body weight (SD)
Control (n = 24)	0.574 (0.043)
250 (n = 25)	0.634[a] (0.046)
500 (n = 24)	0.684[a] (0.068)
1,000 (n = 25)	0.908[a] (0.958)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

Source: CIT, (2004b, unpublished report).

Table B-20. A summary of BMDS (version 1.4.1) modeling results based on mean relative F1 kidney weight in Sprague-Dawley male rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model (non-constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (2°)	0.004	-318.0	271	194
Power	0.003	-317.8	315	226
Hill	NA	-28.7	13,880	Computation of the lower bound failed

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values <0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: CIT, (2004b, unpublished report).

All continuous dose-response models available in BMDS (version 1.4.1) exhibited significant lack-of-fit (i.e., chi-square *p*-value for goodness-of-fit < 0.1); therefore, no POD could be derived from these data based on the modeling results.

Organ: Kidney

Endpoint: F1 Kidney Weight (relative)

Species/Gender: Sprague-Dawley Female Rats

(CIT, 2004b, unpublished report)

Table B-21. Mean relative F1 kidney weight (and SD) in Sprague-Dawley female rats orally exposed to ETBE in a two-generation reproductive toxicity study

Administered dose (mg/kg)	Mean F1 kidney weight, as percent body weight (SD)
Control (n = 25)	0.692 (0.061)
250 (n = 24)	0.733 (0.075)
500 (n = 25)	0.731 (0.048)
1,000 (n = 23)	0.762[a] (0.097)

[a]Statistically significantly different from control at $p < 0.05$ as reported by CIT (2004b, unpublished report).

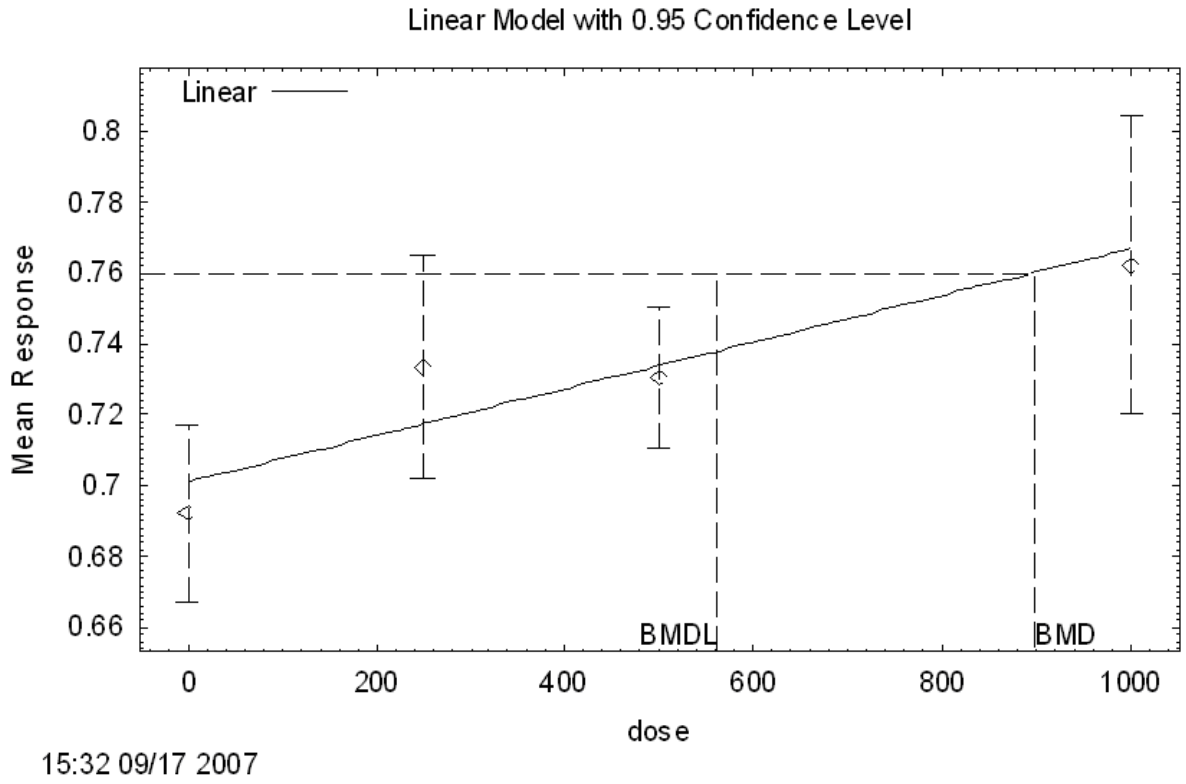
Source: CIT, (2004b, unpublished report).

Table B-22. A summary of BMDS (version 1.4.1) modeling results based on mean relative F1 kidney weight in Sprague-Dawley female rats orally exposed to ETBE in a two-generation reproductive toxicity study

Model[a] (non-constant variance)	χ^2 *p*-value	AIC	BMD_{1SD} (mg/kg)	$BMDL_{1SD}$ (mg/kg)
Polynomial (1°)	**0.10**	**-412.3**	**898**	**562**
Power	0.0001	-398.4	5	Computation of the lower bound failed
Hill	0.03	-410.3	856	Computation of the lower bound failed

[a]For all models, the variance model employed (i.e., variance modeled as a power function of the mean) failed to adequately address the non-constant variance.
Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values <0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: CIT, (2004b, unpublished report).



Source: CIT, (2004b, unpublished report).

Figure B-8. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean relative F1 kidney weight in Sprague-Dawley female rats orally exposed to ETBE in a two-generation reproductive toxicity study

```
 ================================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_F1_REL_KIDNEY_WT_CIIT04.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\ORAL\SD_RATS_FEMALE_F1_REL_KIDNEY_WT_CIIT04.plt
                                          Mon Sep 17 15:32:12 2007
 ================================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = MEAN
Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

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

Default Initial Parameter Values
lalpha = -5.26454
rho = 0
beta_0 = 0.702362
beta_1 = 6.20811e-005

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	0.99	-0.13	0.18
rho	0.99	1	-0.13	0.18
beta_0	-0.13	-0.13	1	-0.72
beta_1	0.18	0.18	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-2.71477	1.3171	-5.29625	-0.1333
rho	8.26635	4.13448	0.162911	16.3698
beta_0	0.700759	0.01,00071	0.681145	0.720373
beta_1	6.59036e-005	2.14377e-005	2.38865e-005	0.000107921

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	25	0.692	0.701	0.061	0.0592	-0.724
250	24	0.733	0.717	0.075	0.0652	1.21
500	25	0.731	0.734	0.048	0.0716	-0.224
1,000	23	0.762	0.767	0.097	0.0858	-0.259

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	208.872773	5	-407.745547
A2	215.204783	8	-414.409567
A3	212.407849	6	-412.815699
fitted	210.131654	4	-412.263308
R	203.219183	2	-402.438367

Explanation of Tests

```
Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)
```

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	23.9712	6	0.0005287
Test 2	12.664	3	0.005422
Test 3	5.59387	2	0.061
Test 4	4.55239	2	0.1027

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

```
            Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        898.036

            BMDL =        561.971
```

B.2. NONCANCER DOSE-RESPONSE ASSESSMENT FOR INHALATION EXPOSURE TO ETBE: BMD MODELING RESULTS

In this appendix, results of BMD modeling are presented for each noncancer endpoint showing significantly elevated incidences (for dichotomous data) or means (for continuous data) relative to controls following inhalation exposure to ETBE. For each endpoint, a summary of the dose-response data is presented, followed by a table summarizing the results of the dose-response modeling. Finally, the standard output from the EPA's BMDS (version 1.4.1), for the best-fitting dose-response model is presented.

For these modeling exercises, all dichotomous or continuous models available in BMDS (version 1.4.1) were fit to the corresponding data for each endpoint with the BMR set at 0.1 (i.e., 10% extra risk) for dichotomous data and one SD above the control mean for continuous data. To select the "best-fit" model, AIC values were evaluated for all models that did not exhibit a significant lack of fit (i.e., $p < 0.1$), according to the chi-square goodness-of-fit test. Of these models, the model with the lowest AIC value was typically selected as the best-fit model unless examination of the chi-square scaled residuals indicated another model with a similar AIC exhibited better fit in the region of the curve where the BMD was estimated. Selection of the BMR and the procedure for selecting the best-fit model are consistent with the EPA's most current *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b).

Organ: Liver
Endpoint: Labeling Index
Species/Gender: CD-1 Female Mice
(Medinsky et al., 1999)

Table B-23. Mean LI (and SD) from the livers of CD-1 female mice exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean LI (SD)
Control (n = 4)	1.92 (2.45)
500 (n = 4)	1.70 (0.88)
1,750 (n = 5)	3.44[a] (0.90)
5,000 (n = 3)	4.97[a] (1.37)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

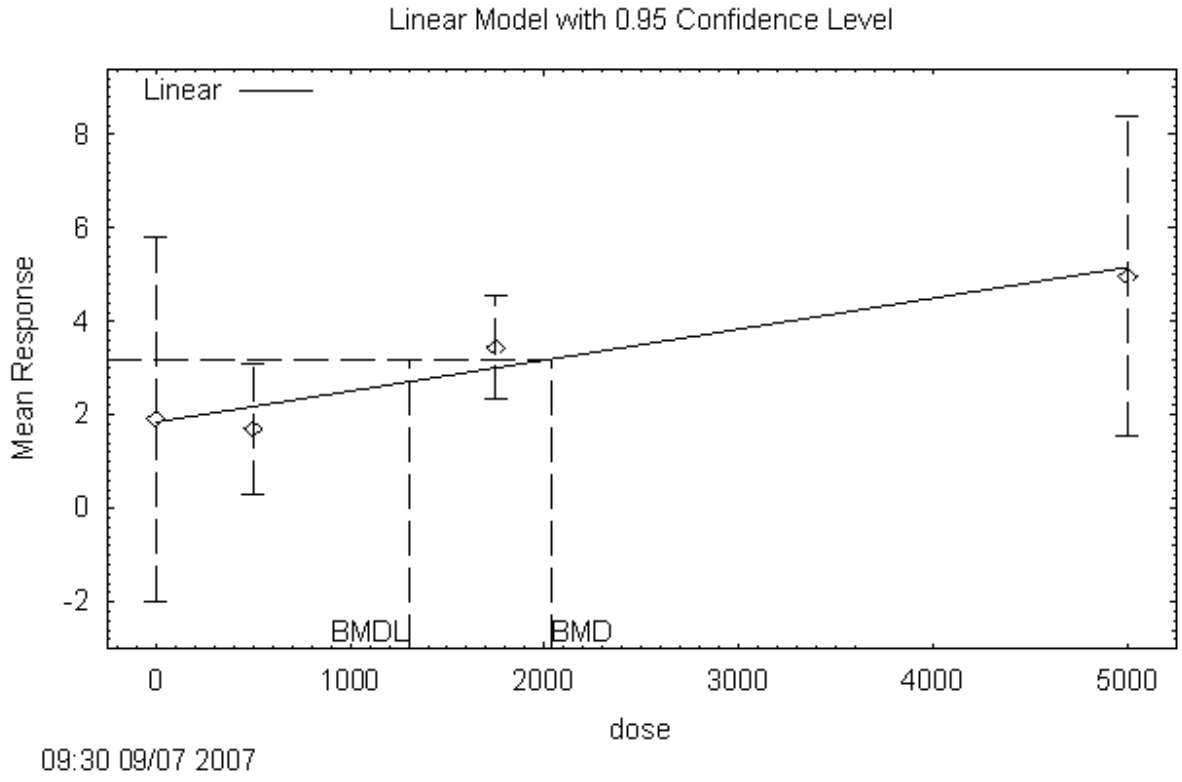
Source: Medinsky et al. (1999).

Table B-24. A summary of BMDS (version 1.4.1) modeling results based on mean LI data from the livers of CD-1 female mice exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.57**	**31.7**	**2,040**	**1,307**
Power	0.57	31.7	2,040	1,307
Hill	NA	34.6	1,699	543

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-9. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean LI data from the livers of CD-1 female mice exposed to ETBE via inhalation for 13 weeks.

```
================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_FEMALES_LIVER_LI_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_FEMALES_LIVER_LI_MEDINSKY99.plt
                                        Fri Sep 07 09:30:35 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	2.27228	
rho =	0	Specified
beta_0 =	1.82498	
beta_1 =	0.00065332	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	-3.7e-009	5.7e-009
beta_0	-3.7e-009	1	-0.67
beta_1	5.7e-009	-0.67	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	1.82871	0.646547	0.561502	3.09592
beta_0	1.84685	0.45746	0.950245	2.74346
beta_1	0.000662928	0.000191491	0.000287613	0.00103824

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	4	1.92	1.85	2.45	1.35	0.108
500	4	1.7	2.18	0.876	1.35	-0.704
1,750	5	3.44	3.01	0.899	1.35	0.723
5000	3	4.97	5.16	1.37	1.35	-0.245

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                     Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1          -12.264824            5      34.529648
            A2           -9.151449            8      34.302898
            A3          -12.264824            5      34.529648
         fitted         -12.828887            3      31.657773
             R          -17.301515            2      38.603029


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

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

   Test 1             16.3001          6         0.01223
   Test 2             6.22675          3          0.1011
   Test 3             6.22675          3          0.1011
   Test 4             1.12812          2          0.5689

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1.  The modeled variance appears to
be appropriate here
```

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2039.89

BMDL = 1306.94

Organ: Liver
Endpoint: Centrilobular Hypertrophy
Species/Gender: CD-1 Male Mice
(Medinsky et al., 1999)

Table B-24. Incidence of centrilobular hypertrophy in the livers of CD-1 male mice exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Incidence
Control	0/15
500	0/15
1,750	2/15
5,000	8/10[a]

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

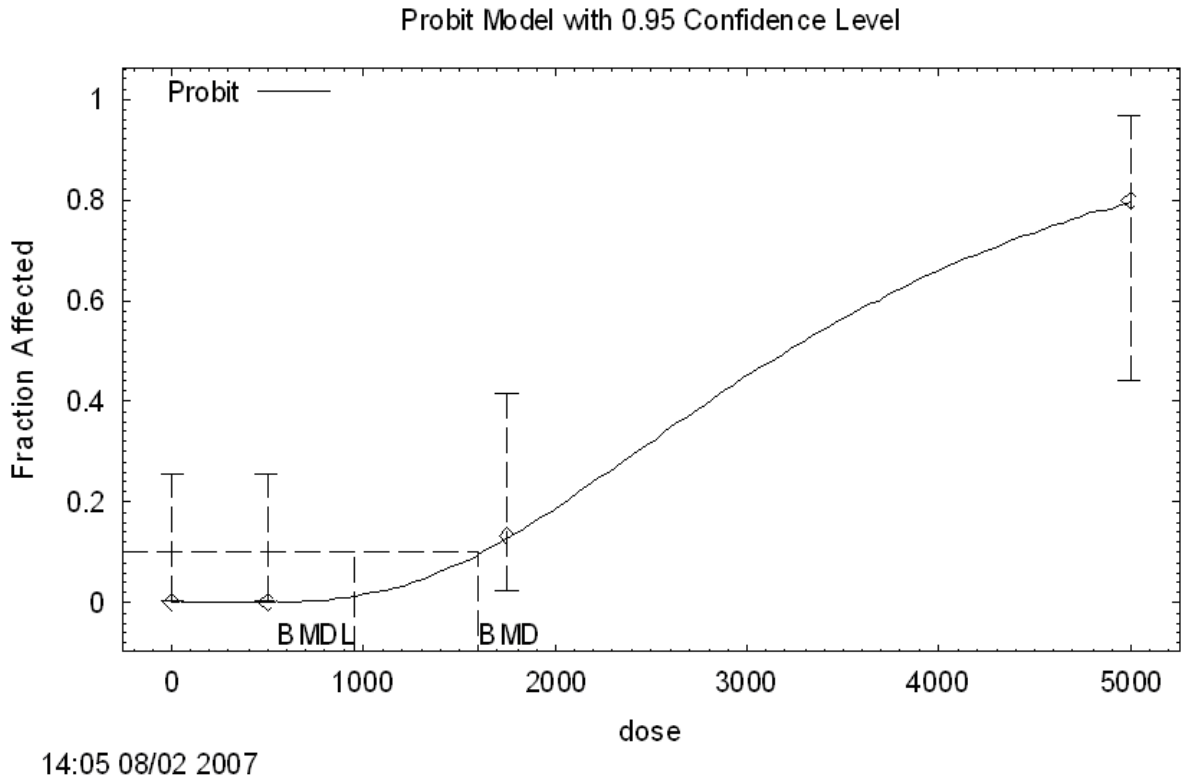
Source: Medinsky et al. (1999).

Table B-25. A summary of BMDS (version 1.4.1) modeling results based on incidence of centrilobular hypertrophy in the livers of CD-1 male mice exposed to ETBE via inhalation for 13 weeks

Model	χ^2 *p*-value	AIC	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
Gamma	0.98	25.9	1,604	901
Logistic	0.62	27.2	1,976	1,304
Log-logistic	0.98	25.9	1,606	943
Multistage (2°)	0.94	24.4	1,351	784
Probit	0.73	26.7	1,847	1,227
Log-probit	**0.998**	**25.8**	**1,602**	**957**
Quantal-linear	0.20	30.0	623	380
Weibull	0.94	26.0	1,612	865

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{10} = Benchmark concentration corresponding to a 10% change relative to controls.
$BMCL_{10}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 10% change relative to controls.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-10. BMDS (version 1.4.1) model output for the best-fit model (i.e., log-probit) based on incidence of centrilobular hypertrophy in the livers of CD-1 male mice exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Probit Model. (Version: 2.8;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_MALES_LIVER_HYPER_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_MALES_LIVER_HYPER_MEDINSKY99.plt
                                        Thu Aug 02 14:05:10 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

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

   where CumNorm(.) is the cumulative normal distribution function
```

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

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

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0
intercept = -8.93692
slope = 1.10637

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept	slope
intercept	1	-1
slope	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-15.0695	4.49702	-23.8835	-6.25553
slope	1.86856	0.565754	0.759699	2.97742

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-10.8941	4			
Fitted model	-10.8984	2	0.00844147	2	0.9958
Reduced model	-26.0777	1	30.367	3	<.0001

AIC: 25.7967

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	15	0.000
500.0000	0.0003	0.004	0	15	-0.064
1,750.0000	0.1321	1.982	2	15	0.014
5000.0000	0.8010	8.010	8	10	-0.008

Chi^2 = 0.00 d.f. = 2 P-value = 0.9978

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1601.9

BMDL = 956.583

Organ: Liver

Endpoint: Centrilobular Hypertrophy

Species/Gender: CD-1 Female Mice

(Medinsky et al., 1999)

Table B-26. Incidence of centrilobular hypertrophy in the livers of CD-1 female mice exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Incidence
Control	0/13
500	2/15
1,750	1/15
5,000	9/14[a]

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

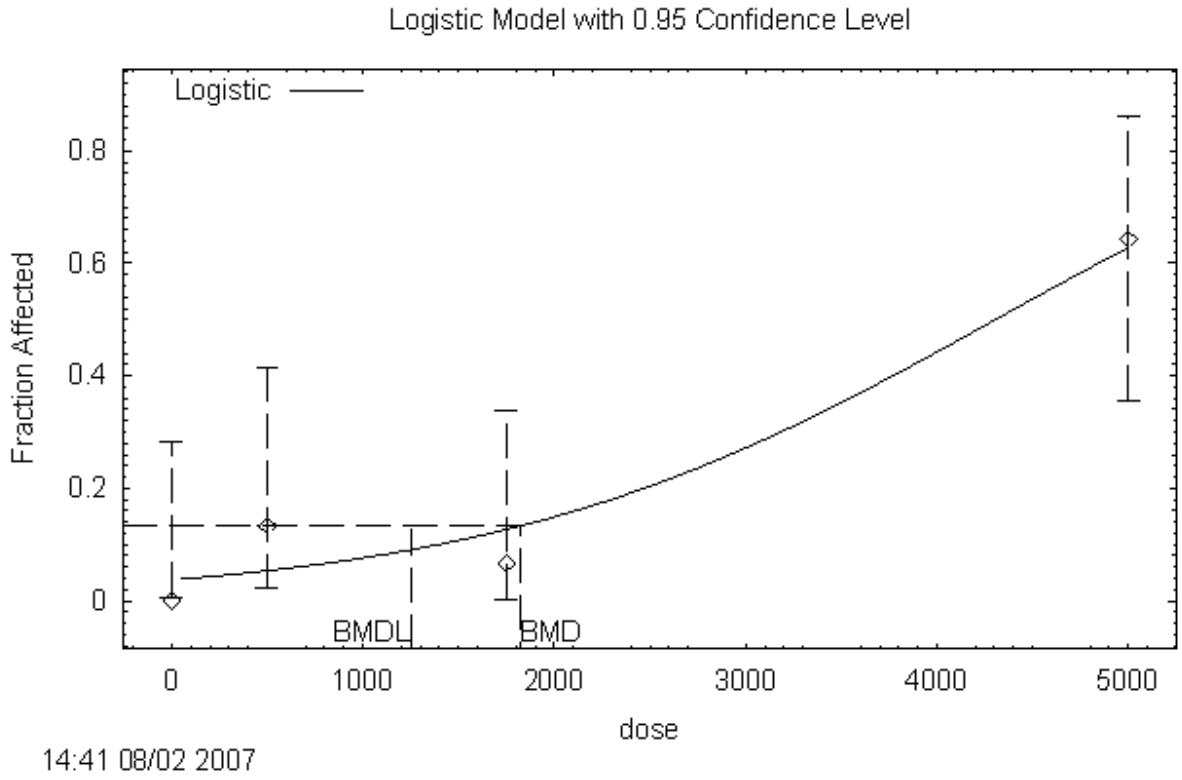
Source: Medinsky et al. (1999).

Table B-27. A summary of BMDS (version 1.4.1) modeling results based on incidence of centrilobular hypertrophy in the livers of CD-1 female mice exposed to ETBE via inhalation for 13 weeks

Model	χ^2 *p*-value	AIC	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
Gamma	0.38	44.0	3,385	511
Logistic	**0.24**	**44.3**	**1,826**	**1,255**
Log-logistic	0.10	46.2	801	335
Multistage (2°)	0.21	44.9	1,722	463
Probit	0.22	44.5	1,644	1,149
Log-probit	0.0.17	46.0	3,661	1,119
Quantal-linear	0.29	43.7	675	430
Weibull	0.17	46.0	4,273	511

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{10} = Benchmark concentration corresponding to a 10% change relative to controls.
$BMCL_{10}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 10% change relative to controls.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-11. BMDS (version 1.4.1) model output for the best-fit model (i.e., logistic) based on incidence of centrilobular hypertrophy in the livers of CD-1 female mice exposed to ETBE via inhalation for 13 weeks.

```
 ======================================================================
        Logistic Model. (Version: 2.9; Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_FEMALES_LIVER_HYPER_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_FEMALES_LIVER_HYPER_MEDINSKY99.plt
                                          Thu Aug 02 14:41:46 2007
 ======================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

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

Dependent variable = Response
Independent variable = Dose
Slope parameter is not restricted

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

Default Initial Parameter Values
background = 0 Specified
intercept = -2.87922
slope = 0.000663794

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept	slope
intercept	1	-0.85
slope	-0.85	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-3.24768	0.760128	-4.7375	-1.75785
slope	0.000754885	0.000202312	0.000358361	0.00115141

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.6887	4			
Fitted model	-20.1599	2	2.94242	2	0.2296
Reduced model	-29.3352	1	21.2931	3	<.0001

AIC: 44.3197

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0374	0.486	0	13	-0.711
500.0000	0.0536	0.805	2	15	1.370
1,750.0000	0.1271	1.907	1	15	-0.703
5000.0000	0.6287	8.802	9	14	0.109

```
 Chi^2 = 2.89      d.f. = 2        P-value = 0.2360


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1826.48

            BMDL =        1255.48
```

Organ: Liver
Endpoint: Liver Weight (absolute)
Species/Gender: CD-1 Male Mice
(Medinsky et al., 1999)

Table B-28. Mean absolute liver weight (and SD) in CD-1 male mice exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute liver weight, g (SD)
Control (n = 15)	2.16 (0.36)
500 (n = 15)	2.26 (0.28)
1,750 (n = 15)	2.44[a] (0.24)
5,000 (n = 10)	2.55[a] (0.25)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

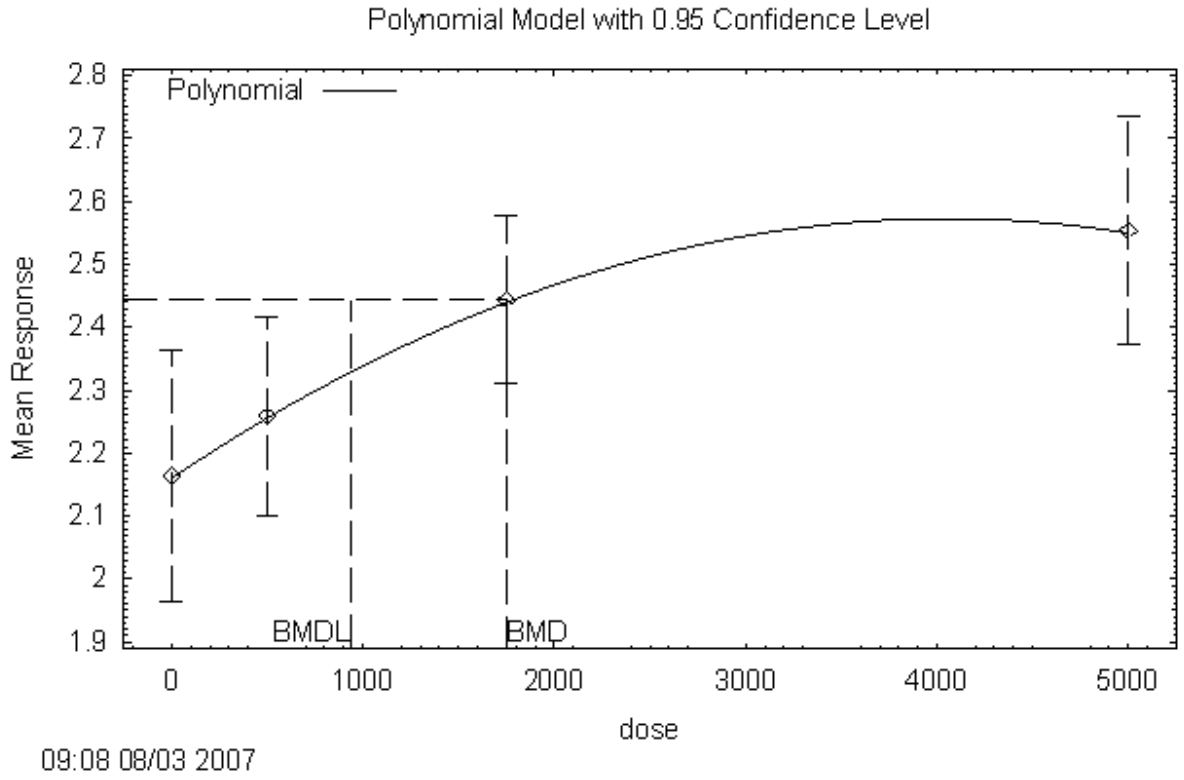
Source: Medinsky et al. (1999).

Table B-29. A summary of BMDS (version 1.4.1) modeling results based on mean absolute liver weight in CD-1 male mice exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	**0.99**	**-76.8**	**1,754**	**936**
Power	0.30	-76.4	3,781	2,521
Hill	NA	-74.8	1,758	598

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-12. BMDS (version 1.4.1) model output for the best-fit model (i.e., 2° polynomial) based on mean absolute liver weight in CD-1 male mice exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_MALES_LIVER_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_MALES_LIVER_WT_MEDINSKY99.plt
                                        Fri Aug 03 09:08:17 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.0849361	
rho =	0	Specified
beta_0 =	2.16369	
beta_1 =	0.000204224	
beta_2 =	-2.52315e-008	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1	beta_2
alpha	1	6.3e-010	-7.1e-010	7.4e-010
beta_0	6.3e-010	1	-0.71	0.6
beta_1	-7.1e-010	-0.71	1	-0.97
beta_2	7.4e-010	0.6	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.078759	0.0150188	0.0493228	0.108195
beta_0	2.16369	0.0622113	2.04175	2.28562
beta_1	0.000204223	8.4892e-005	3.7838e-005	0.000370609
beta_2	-2.52309e-008	1.61469e-008	-5.68783e-008	6.41641e-009

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	15	2.16	2.16	0.359	0.281	0.00434

500	15	2.26	2.26	0.284	0.281	-0.00675
1,750	15	2.44	2.44	0.243	0.281	0.00267
5000	10	2.55	2.55	0.252	0.281	-0.000318

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	42.387507	5	-74.775013
A2	43.831021	8	-71.662041
A3	42.387507	5	-74.775013
fitted	42.387471	4	-76.774942
R	35.748395	2	-67.496791

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	16.1653	6	0.01289
Test 2	2.88703	3	0.4094
Test 3	2.88703	3	0.4094
Test 4	7.16895e-005	1	0.9932

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1754.48

BMDL = 936.067

Organ: Liver
Endpoint: Liver Weight (absolute)
Species/Gender: CD-1 Female Mice
(Medinsky et al., 1999)

Table B-30. Mean absolute liver weight (and SD) in CD-1 female mice exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute liver weight, g (SD)
Control (n = 13)	1.56 (0.21)
500 (n = 15)	1.59 (0.16)
1,750 (n = 15)	1.86[a] (0.19)
5,000 (n = 14)	2.07[a] (0.30)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

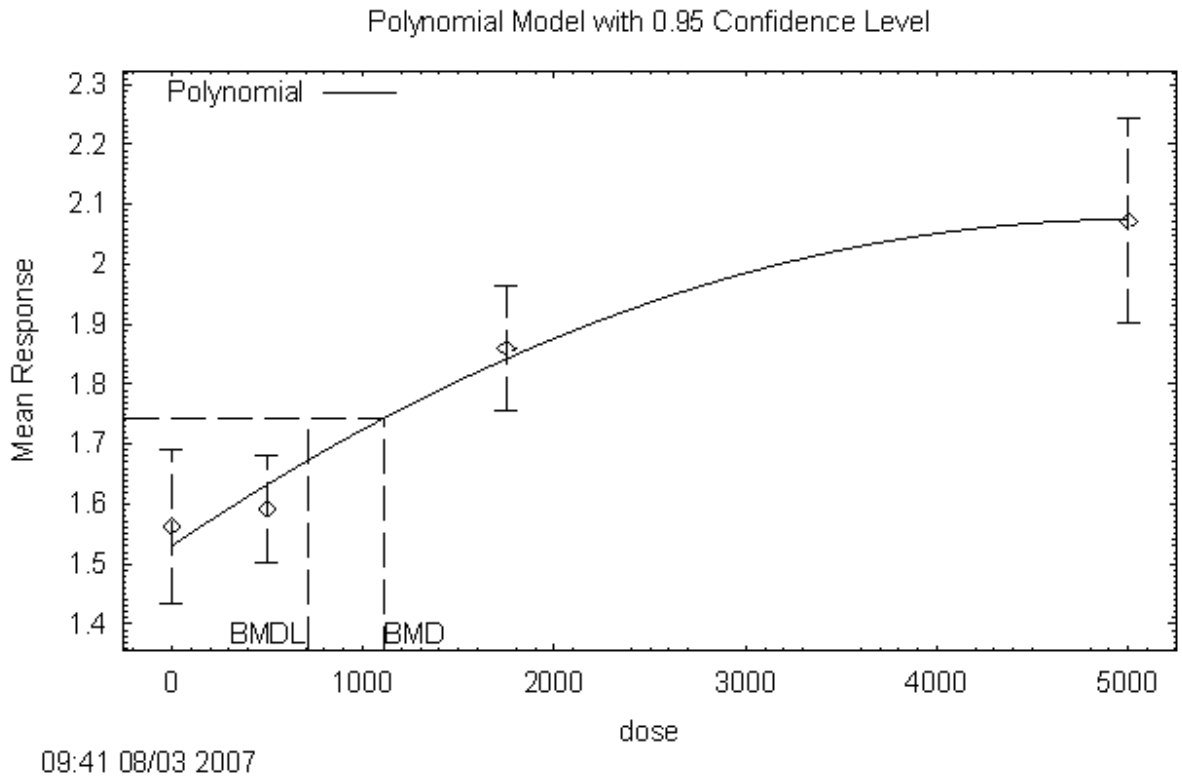
Source: Medinsky et al. (1999).

Table B-31. A summary of BMDS (version 1.4.1) modeling results based on mean absolute liver weight in CD-1 female mice exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	**0.32**	**-111.5**	**1,109**	**709**
Power	0.14	-110.5	2,113	1,644
Hill	NA	-110.5	1,345	704

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-13. BMDS (version 1.4.1) model output for the best-fit model (i.e., 2° polynomial) based on mean absolute liver weight in CD-1 female mice exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_FEMALES_LIVER_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\CD1_MICE_FEMALES_LIVER_WT_MEDINSKY99.plt
                                        Fri Aug 03 09:41:35 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.0477941	
rho =	0	Specified
beta_0 =	1.5348	
beta_1 =	0.000212599	
beta_2 =	-2.09645e-008	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1	beta_2
alpha	1	-7.7e-012	4.8e-012	-8.2e-012
beta_0	-7.7e-012	1	-0.73	0.62
beta_1	4.8e-012	-0.73	1	-0.98
beta_2	-8.2e-012	0.62	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.0452046	0.0084676	0.0286084	0.0618008
beta_0	1.53173	0.0496296	1.43446	1.629
beta_1	0.000215557	6.59293e-005	8.63377e-005	0.000344776
beta_2	-2.14312e-008	1.22684e-008	-4.54768e-008	2.61439e-009

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	13	1.56	1.53	0.211	0.213	0.53
500	15	1.59	1.63	0.162	0.213	-0.768
1,750	15	1.86	1.84	0.189	0.213	0.304
5000	14	2.07	2.07	0.295	0.213	-0.0306

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	60.237958	5	-110.475915
A2	63.184016	8	-110.368033
A3	60.237958	5	-110.475915
fitted	59.751845	4	-111.503689
R	41.070159	2	-78.140319

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	44.2277	6	<.0001
Test 2	5.89212	3	0.117
Test 3	5.89212	3	0.117
Test 4	0.972226	1	0.3241

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

```
The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        1108.52


            BMDL =        708.952
```

Organ: Liver
Endpoint: Liver Weight (absolute)
Species/Gender: F344 Male Rats
(Medinsky et al., 1999)

Table B-32. Mean absolute liver weight (and SD) in F344 male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute liver weight, g (SD)
Control (n = 11)	8.86 (1.19)
500 (n = 11)	9.38 (0.74)
1,750 (n = 11)	10.1[a] (0.49)
5,000 (n = 11)	11.7[a] (0.68)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

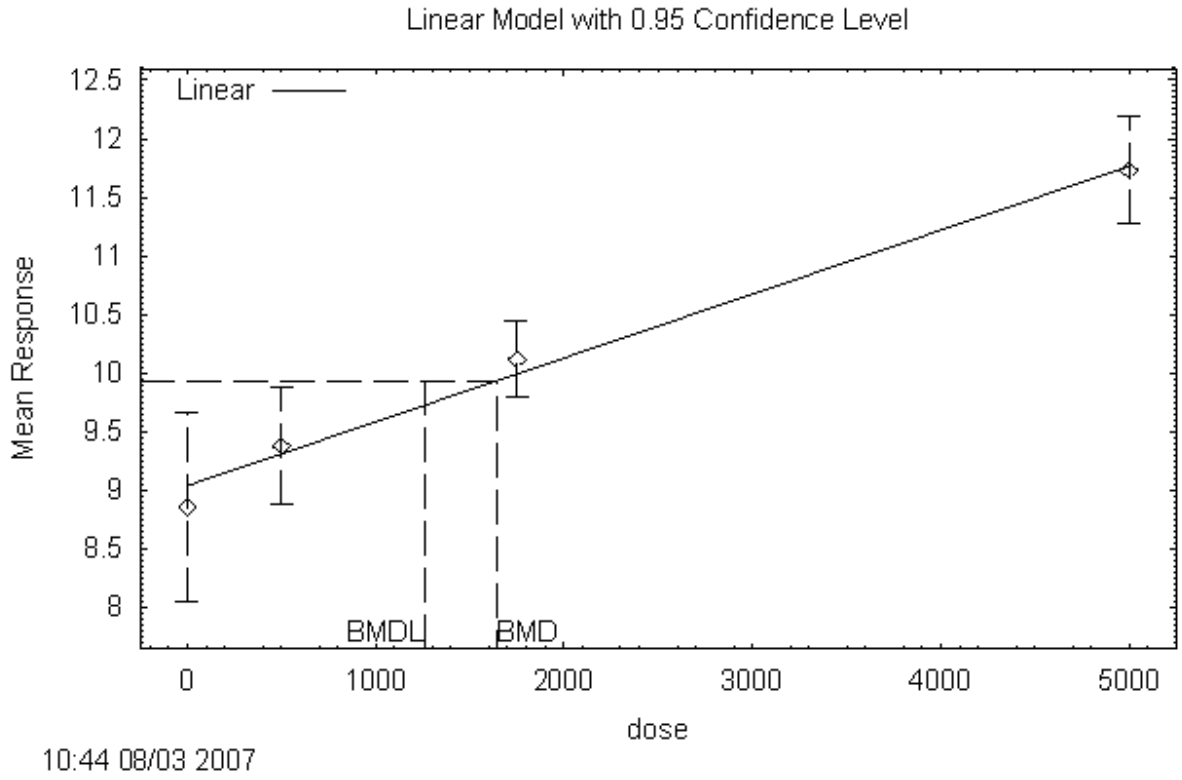
Source: Medinsky et al. (1999).

Table B-33. A summary of BMDS (version 1.4.1) modeling results based on mean absolute liver weight in F344 male rats exposed to ETBE via inhalation for 13 weeks

Model[a] (non-constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.39**	**27.9**	**1,648**	**1,260**
Power	0.30	27.9	1,648	1,260
Hill	0.57	28.3	1,098	645

[a]For all models, the variance model employed (i.e., variance modeled as a power function of the mean) failed to adequately address the non-constant variance.
Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: Medinsky et al. (1999).



Source: (Medinsky et al. (1999).

Figure B-14. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute liver weight in F344 male rats exposed to ETBE via inhalation for 13 weeks

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_LIVER_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_LIVER_WT_MEDINSKY99.plt
                                        Fri Aug 03 10:44:41 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
```

Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

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

Default Initial Parameter Values

lalpha =	-0.406567
rho =	0
beta_0 =	9.01743
beta_1 =	0.000553417

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-1	0.029	-0.039
rho	-1	1	-0.029	0.04
beta_0	0.029	-0.029	1	-0.77
beta_1	-0.039	0.04	-0.77	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	7.20485	4.25062	-1.12621	15.5359
rho	-3.37146	1.84605	-6.98964	0.246732
beta_0	9.03722	0.174598	8.69502	9.37943
beta_1	0.000544548	5.27201e-005	0.000441219	0.000647878

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	8.86	9.04	1.19	0.897	-0.666
500	11	9.38	9.31	0.744	0.853	0.262
1,750	11	10.1	9.99	0.488	0.758	0.559
5000	11	11.7	11.8	0.677	0.576	-0.173

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-10.958703	5	31.917406
A2	-6.371795	8	28.743591
A3	-8.992356	6	29.984711
fitted	-9.935317	4	27.870635
R	-34.689491	2	73.378982

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	56.6354	6	<.0001
Test 2	9.17381	3	0.02707
Test 3	5.24112	2	0.07276
Test 4	1.88592	2	0.3895

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

```
The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        1647.64


            BMDL =        1259.77
```

Organ: Liver
Endpoint: Liver Weight (absolute)
Species/Gender: F344 Female Rats
(Medinsky et al., 1999)

Table B-34. Mean absolute liver weight (and SD) in F344 female rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute liver weight, g (SD)
Control (n = 10)	5.19 (0.44)
500 (n = 11)	5.29 (0.40)
1,750 (n = 11)	5.64 (0.52)
5,000 (n = 11)	6.53[a] (0.52)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

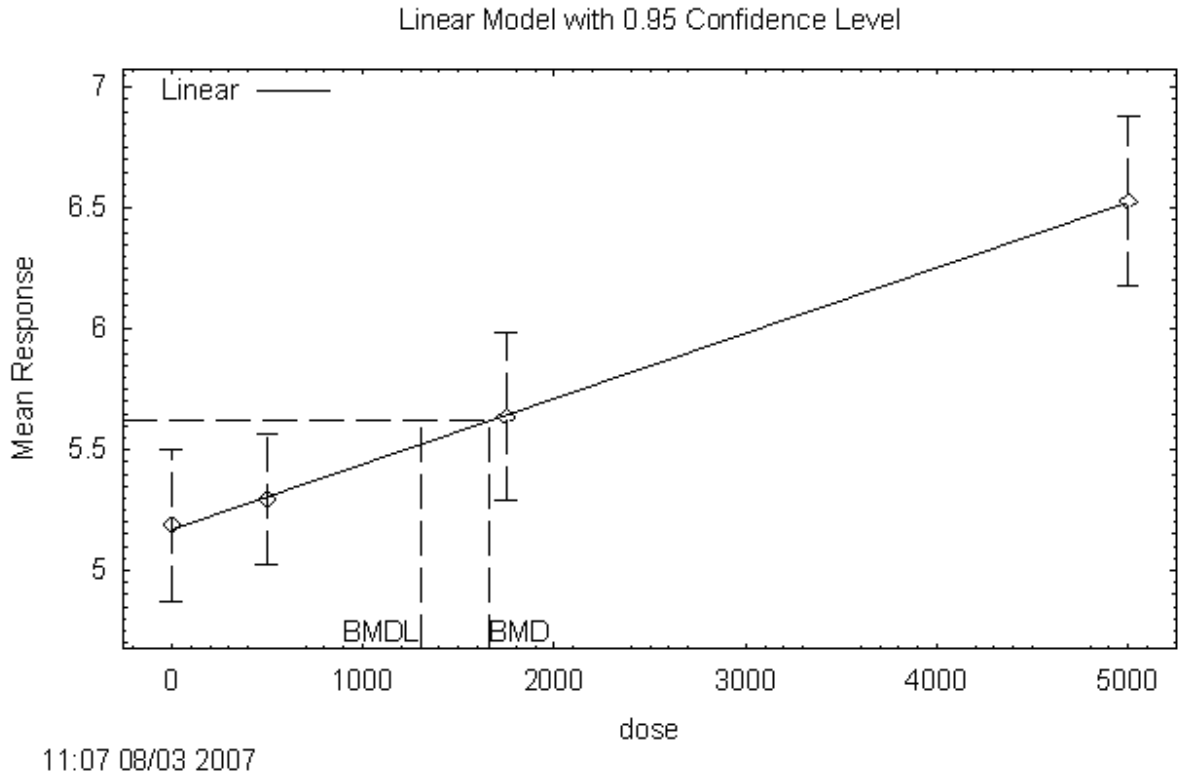
Source: Medinsky et al. (1999).

Table B-35. A summary of BMDS (version 1.4.1) modeling results based on mean absolute liver weight in F344 female rats exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.99**	**-19.7**	**1,663**	**1,300**
Power	0.93	-17.7	1,762	1,301
Hill	NA	-15.7	1,760	888

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-15. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute liver weight in F344 female rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_LIVER_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_LIVER_WT_MEDINSKY99.plt
                                        Fri Aug 03 11:07:08 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
```

Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.223223	
rho =	0	Specified
beta_0 =	5.17267	
beta_1 =	0.000270389	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	-4e-009	3.5e-010
beta_0	-4e-009	1	-0.69
beta_1	3.5e-010	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.202595	0.0436928	0.116959	0.288232
beta_0	5.1719	0.0947258	4.98624	5.35756
beta_1	0.000270586	3.51979e-005	0.000201599	0.000339573

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	5.19	5.17	0.44	0.45	0.127
500	11	5.29	5.31	0.397	0.45	-0.0899
1,750	11	5.64	5.65	0.519	0.45	-0.0621
5000	11	6.53	6.52	0.519	0.45	0.0307

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	12.840251	5	-15.680502
A2	13.399966	8	-10.799932
A3	12.840251	5	-15.680502
fitted	12.825726	3	-19.651451
R	-5.766125	2	15.532250

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	38.3322	6	<.0001
Test 2	1.11943	3	0.7724
Test 3	1.11943	3	0.7724
Test 4	0.0290511	2	0.9856

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

```
The p-value for Test 3 is greater than .1.  The modeled variance appears to
be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


            Benchmark Dose Computation

Specified effect =            1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =         0.95

             BMD =       1663.45


            BMDL =       1299.55
```

Organ: Liver

Endpoint: Liver Weight (absolute)

Species/Gender: Sprague-Dawley Male Rats

(White et al., 1995)

Table B-36. Mean absolute liver weight (and SD) in Sprague-Dawley male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute liver weight, g (SD)
Control (n = 10)	10.2 (1.18)
500 (n = 10)	10.0 (0.62)
2,000 (n = 10)	10.6 (1.24)
4,000 (n = 10)	11.9[a] (1.19)

[a]Statistically significantly different from control at $p < 0.05$ as reported by White et al. (1995).

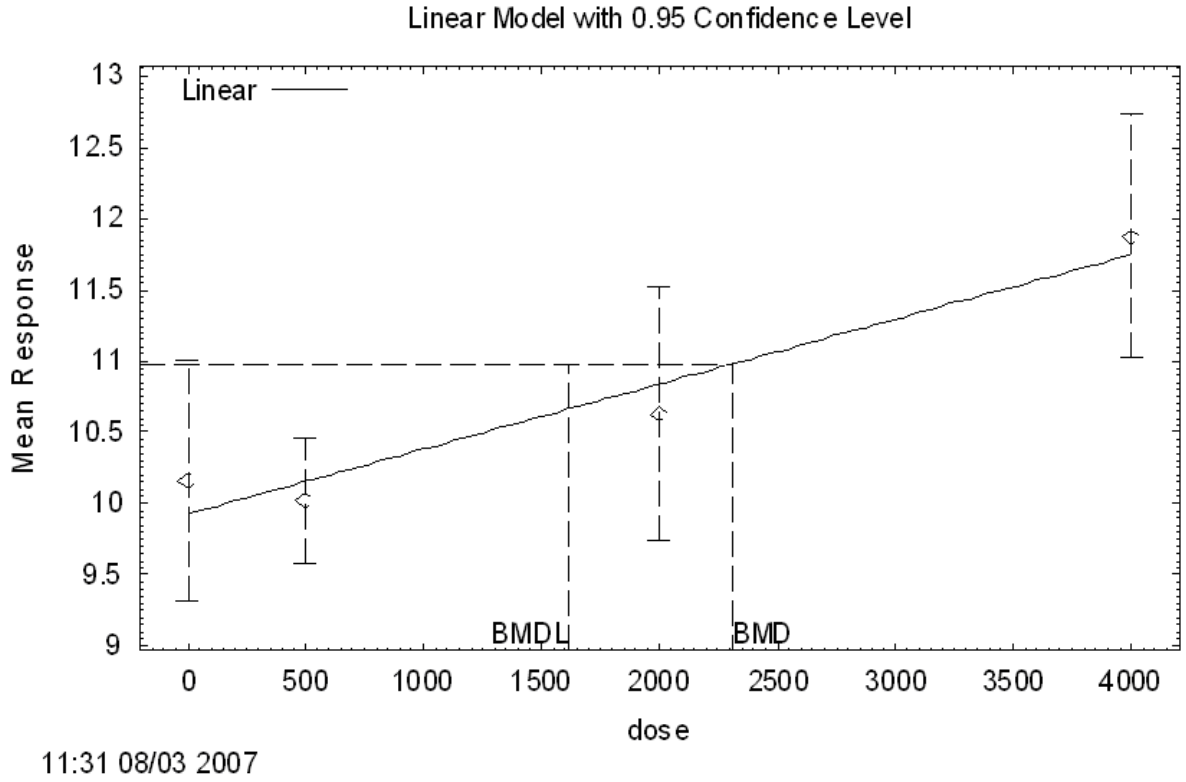
White et al. (1995).

Table B-37. A summary of BMDS (version 1.4.1) modeling results based on mean absolute liver weight in Sprague-Dawley male rats exposed to ETBE via inhalation for 13 weeks

Model(constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.55**	**49.7**	**2,309**	**1,616**
Power	0.68	50.7	2,929	1,728
Hill	NA	52.6	2,189	1,620

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: White et al. (1995).



Source: White et al. (1995).

Figure B-16. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute liver weight in SD male rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_LIVER_WT_WHITE95.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_LIVER_WT_WHITE95.plt
                                        Fri Aug 03 11:31:03 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	1.18263	
rho =	0	Specified
beta_0 =	9.93277	
beta_1 =	0.000453677	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	-9.9e-011	-2e-011
beta_0	-9.9e-011	1	-0.72
beta_1	-2e-011	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	1.09693	0.245282	0.616191	1.57768
beta_0	9.93277	0.239424	9.46351	10.402
beta_1	0.000453677	0.00010641	0.000245117	0.000662238

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	10.2	9.93	1.18	1.05	0.686
500	10	10	10.2	0.62	1.05	-0.422
2000	10	10.6	10.8	1.24	1.05	-0.634
4000	10	11.9	11.7	1.19	1.05	0.37

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-21.247521	5	52.495041
A2	-18.658223	8	53.316446
A3	-21.247521	5	52.495041
fitted	-21.850388	3	49.700776
R	-29.342646	2	62.685293

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.3688	6	0.001575
Test 2	5.1786	3	0.1592
Test 3	5.1786	3	0.1592
Test 4	1.20574	2	0.5472

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2308.57

BMDL = 1616.16

Organ: Liver

Endpoint: Liver Weight (absolute)

Species/Gender: Sprague-Dawley Female Rats

(White et al., 1995)

Table B-38. Mean absolute liver weight (and SD) in Sprague-Dawley female rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute liver weight, g (SD)
Control (n = 10)	6.0 (0.39)
500 (n = 10)	6.2 (0.49)
2,000 (n = 10)	6.5 (0.40)
4,000 (n = 10)	6.6[a] (0.46)

[a]Statistically significantly different from control at $p < 0.05$ as reported by White et al. (1995).

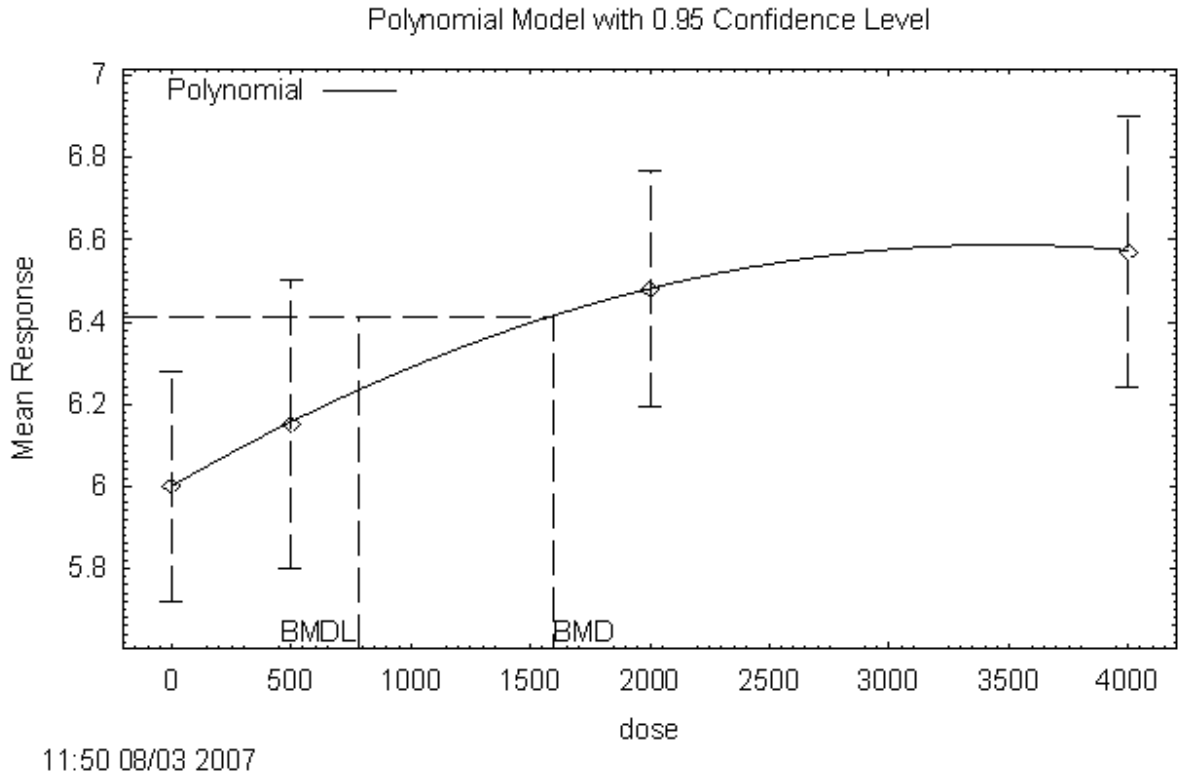
Source: White et al. (1995).

Table B-39. A summary of BMDS (version 1.4.1) modeling results based on mean absolute liver weight in Sprague-Dawley female rats exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	**0.97**	**-22.4**	**1,593**	**779**
Power	0.48	-23.0	2,996	1,953
Hill	NA	-20.4	1,469	406

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: White et al. (1995).



Source: White et al. (1995).

Figure B-17. BMDS (version 1.4.1) model output for the best-fit model (i.e., 2° polynomial) based on mean absolute liver weight in Sprague-Dawley female rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_FEMALES_LIVER_WT_WHITE95.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_FEMALES_LIVER_WT_WHITE95.plt
                                        Fri Aug 03 11:50:46 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.19095	
rho =	0	Specified
beta_0 =	5.99736	
beta_1 =	0.000337626	
beta_2 =	-4.85928e-008	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1	beta_2
alpha	1	7.7e-010	-2.3e-010	-8.1e-011
beta_0	7.7e-010	1	-0.67	0.54
beta_1	-2.3e-010	-0.67	1	-0.97
beta_2	-8.1e-011	0.54	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.171862	0.0384294	0.0965413	0.247182
beta_0	5.99736	0.112462	5.77694	6.21778
beta_1	0.000337626	0.000167084	1.01462e-005	0.000665105
beta_2	-4.85928e-008	3.9939e-008	-1.26872e-007	2.96862e-008

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	6	6	0.39	0.415	0.0201
500	10	6.15	6.15	0.49	0.415	-0.0307
2000	10	6.48	6.48	0.4	0.415	0.0134
4000	10	6.57	6.57	0.46	0.415	-0.00288

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	15.222084	5	-20.444167
A2	15.584990	8	-15.169980
A3	15.222084	5	-20.444167
fitted	15.221315	4	-22.442631
R	9.717433	2	-15.434865

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	11.7351	6	0.06815
Test 2	0.725812	3	0.8671
Test 3	0.725812	3	0.8671
Test 4	0.00153657	1	0.9687

The p-value for Test 1 is greater than .05. There may not be a diffence between responses and/or variances among the dose levels
Modelling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1593.19

BMDL = 778.988

Organ: Liver

Endpoint: Liver Weight (relative)

Species/Gender: Sprague-Dawley Male Rats

(White et al., 1995)

Table B-40. Mean relative liver weight (and SD) in Sprague-Dawley male rats exposed to four different concentrations of ETBE via inhalation for 4 weeks

Administered dose (ppm)	Mean relative liver weight, g (SD)
Control (n = 10)	2.86 (0.21)
500 (n = 10)	2.84 (0.15)
2,000 (n = 10)	2.96 (0.21)
4,000 (n = 10)	3.32[a] (0.25)

[a]Statistically significantly different from control at $p < 0.05$ as reported by White et al. (1995).

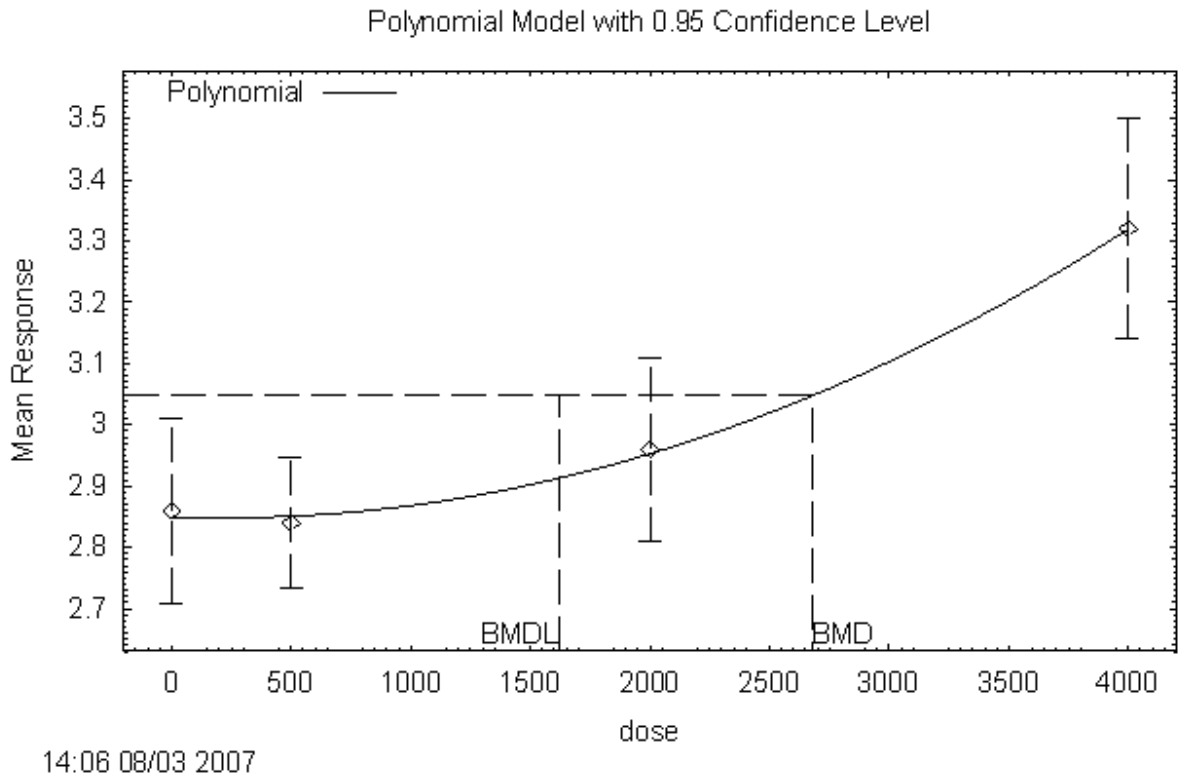
Source: White et al. (1995).

Table B-41. A summary of BMDS (version 1.4.1) modeling results based on mean relative liver weight in Sprague-Dawley male rats exposed to ETBE via inhalation for 4 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	**0.80**	**-81.7**	**2,678**	**1,619**
Power	0.77	-81.7	2,644	1,624
Hill	NA	-79.7	2,150	1,633

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: White et al. (1995).



Source: White et al. (1995).

Figure B-18. BMDS (version 1.4.1) model output for the best-fit model (i.e., 2° polynomial) based on mean relative liver weight in Sprague-Dawley male rats exposed to ETBE via inhalation for 4 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_REL_LIVER_WT_WHITE95.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_REL_LIVER_WT_WHITE95.plt
                                        Fri Aug 03 14:06:06 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
```

Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.0433	
rho =	0	Specified
beta_0 =	2.8517	
beta_1 =	-1.46048e-005	
beta_2 =	3.2994e-008	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1	beta_2
alpha	1	3.3e-010	-5.9e-011	-3.1e-011
beta_0	3.3e-010	1	-0.67	0.54
beta_1	-5.9e-011	-0.67	1	-0.97
beta_2	-3.1e-011	0.54	-0.97	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.0390352	0.00872854	0.0219276	0.0561428
beta_0	2.8517	0.0535977	2.74665	2.95675
beta_1	-1.46048e-005	7.96297e-005	-0.000170676	0.000141466
beta_2	3.2994e-008	1.90343e-008	-4.31244e-009	7.03005e-008

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	2.86	2.85	0.21	0.198	0.133
500	10	2.84	2.85	0.15	0.198	-0.202
2000	10	2.96	2.95	0.21	0.198	0.0886
4000	10	3.32	3.32	0.25	0.198	-0.019

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	44.899263	5	-79.798526
A2	46.154309	8	-76.308617
A3	44.899263	5	-79.798526
fitted	44.865825	4	-81.731649
R	31.476069	2	-58.952138

Explanation of Tests

```
Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)
```

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	29.3565	6	<.0001
Test 2	2.51009	3	0.4735
Test 3	2.51009	3	0.4735
Test 4	0.0668772	1	0.7959

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems

to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2678.38

BMDL = 1618.94

Organ: Liver

Endpoint: Liver Weight (relative)

Species/Gender: Sprague-Dawley Female Rats

(White et al., 1995)

Table B-42. Mean relative liver weight (and SD) in Sprague-Dawley female rats exposed to four different concentrations of ETBE via inhalation for 4 weeks

Administered dose (ppm)	Mean relative liver weight, g (SD)
Control (n = 10)	2.80 (0.17)
500 (n = 10)	2.93 (0.10)
2,000 (n = 10)	3.08[a] (0.22)
4,000 (n = 10)	3.15[a] (0.24)

[a]Statistically significantly different from control at $p < 0.05$ as reported by White et al. (1995).

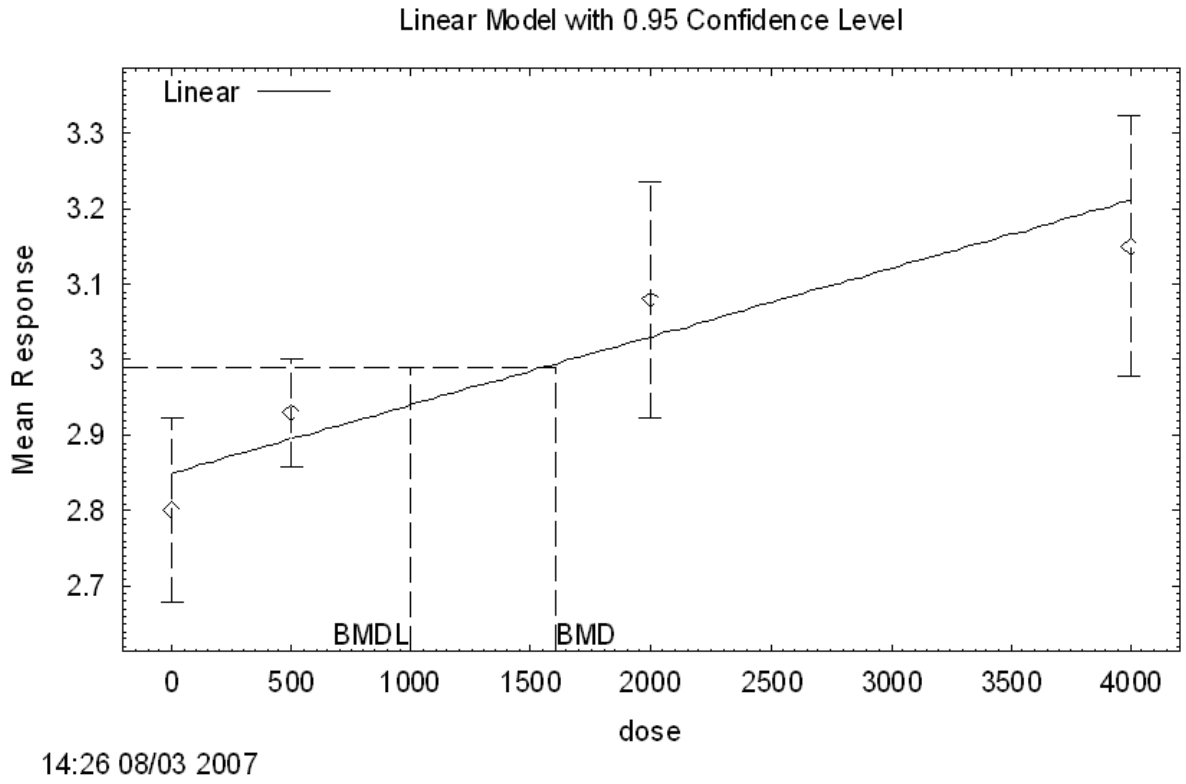
Source: White et al. (1995).

Table B-43. A summary of BMDS (version 1.4.1) modeling results based on mean relative liver weight in Sprague-Dawley female rats exposed to ETBE via inhalation for 4 weeks

Model[a] (non-constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.30**	**-89.4**	**1,600**	**997**
Power	0.30	-89.4	1,600	997
Hill	NA	-87.9	704	240

[a]For all models, the variance model employed (i.e., variance modeled as a power function of the mean) failed to adequately address the non-constant variance.
Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: White et al. (1995).



Source: White et al. (1995).

Figure B-19. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean relative liver weight in Sprague-Dawley female rats exposed to ETBE via inhalation for 4 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_FEMALES_REL_LIVER_WT_WHITE95.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_FEMALES_REL_LIVER_WT_WHITE95.plt
                                        Fri Aug 03 14:26:27 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = MEAN
Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

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

Default Initial Parameter Values

lalpha =	-3.31801
rho =	0
beta_0 =	2.85748
beta_1 =	8.15484e-005

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-1	0.17	-0.26
rho	-1	1	-0.17	0.26
beta_0	0.17	-0.17	1	-0.64
beta_1	-0.26	0.26	-0.64	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-13.0534	5.49755	-23.8284	-2.27837
rho	8.78327	5.01654	-1.04897	18.6155
beta_0	2.84555	0.0355734	2.77583	2.91527
beta_1	9.03565e-005	2.1278e-005	4.86523e-005	0.000132061

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	2.8	2.85	0.17	0.145	-0.996
500	10	2.93	2.89	0.1	0.155	0.802
2000	10	3.08	3.03	0.22	0.189	0.897
4000	10	3.15	3.21	0.24	0.244	-0.737

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)

```
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
    Model A3 uses any fixed variance parameters that
    were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                     Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1           48.467326            5     -86.934652
            A2           52.265071            8     -88.530141
            A3           49.925883            6     -87.851765
        fitted           48.717299            4     -89.434598
             R           39.537229            2     -75.074458


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

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

   Test 1              25.4557          6       0.0002811
   Test 2              7.59549          3         0.05516
   Test 3              4.67838          2         0.09641
   Test 4              2.41717          2          0.2986

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .1.  You may want to consider a
different variance model

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data
```

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1599.81

BMDL = 997.078

Organ: Kidney
Endpoint: Kidney Weight (absolute)
Species/Gender: F344 Male Rats
(Medinsky et al., 1999)

Table B-44. Mean absolute kidney weight (and SD) in F344 male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute kidney weight, g (SD)
Control (n = 11)	1.73 (0.16)
500 (n = 11)	1.85 (0.14)
1,750 (n = 11)	1.90[a] (0.10)
5,000 (n = 11)	2.07[a] (0.12)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

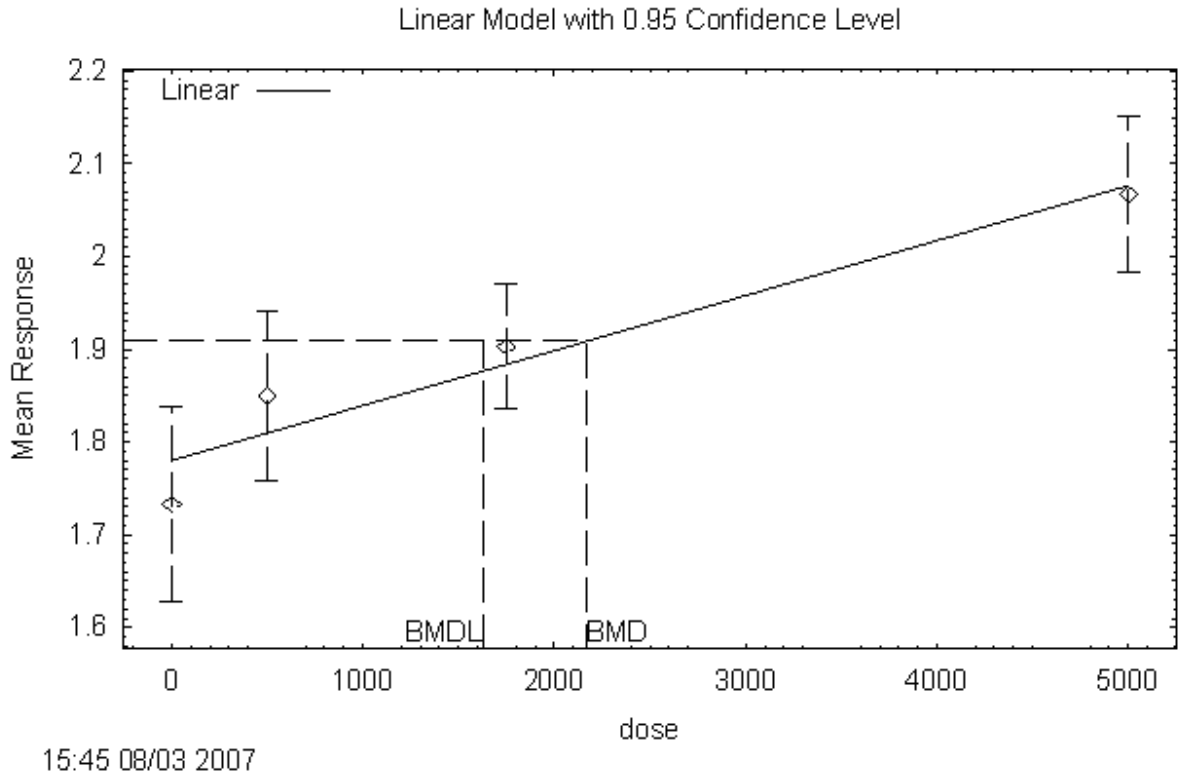
Source: Medinsky et al. (1999).

Table B-45. A summary of BMDS (version 1.4.1) modeling results based on mean absolute kidney weight in F344 male rats exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.23**	**-130.4**	**2,169**	**1,632**
Power	0.23	-130.4	2,169	1,632
Hill	0.24	-130.0	1,099	396

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-20. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute kidney weight in F344 male rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_WT_MEDINSKY99.plt
                                        Fri Aug 03 15:45:16 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 0.0170425
rho = 0 Specified
beta_0 = 1.78068
beta_1 = 5.93491e-005

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	2.4e-010	5.6e-010
beta_0	2.4e-010	1	-0.68
beta_1	5.6e-010	-0.68	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.0165668	0.00353206	0.00964412	0.0234895
beta_0	1.78068	0.0265071	1.72873	1.83263
beta_1	5.93491e-005	9.96331e-006	3.98214e-005	7.88769e-005

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	1.73	1.78	0.155	0.129	-1.23
500	11	1.85	1.81	0.137	0.129	1.02
1,750	11	1.9	1.88	0.1	0.129	0.476
5000	11	2.07	2.08	0.124	0.129	-0.269

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	69.681815	5	-129.363630
A2	70.760620	8	-125.521241
A3	69.681815	5	-129.363630
fitted	68.207768	3	-130.415535
R	55.197968	2	-106.395937

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	31.1253	6	<.0001
Test 2	2.15761	3	0.5403
Test 3	2.15761	3	0.5403
Test 4	2.94809	2	0.229

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2168.73

BMDL = 1632.4

Organ: Kidney
Endpoint: Kidney Weight (absolute)
Species/Gender: F344 Female Rats
(Medinsky et al., 1999)

Table B-46. Mean absolute kidney weight (and SD) in F344 female rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean absolute kidney weight, g (SD)
Control (n = 10)	1.08 (0.07)
500 (n = 11)	1.13 (0.05)
1,750 (n = 11)	1.21[a] (0.08)
5,000 (n = 11)	1.31[a] (0.06)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

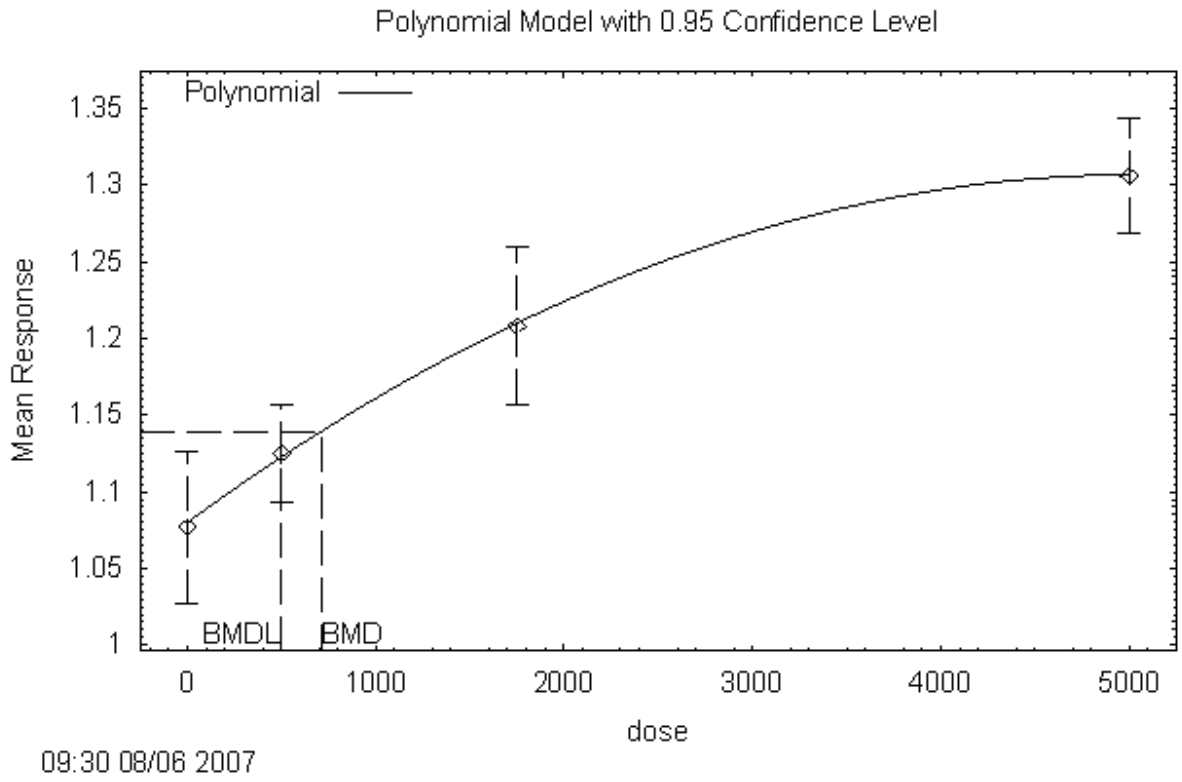
Source: Medinsky et al. (1999).

Table B-47. A summary of BMDS (version 1.4.1) modeling results based on mean absolute kidney weight in F344 female rats exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	**0.83**	**-191.2**	**717**	**494**
Power	0.09	-188.4	1,465	1,162
Hill	NA	-189.2	641	346

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-21. BMDS (version 1.4.1) model output for the best-fit model (i.e., 2° polynomial) based on mean absolute kidney weight in F344 female rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_KIDNEY_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_KIDNEY_WT_MEDINSKY99.plt
                                        Mon Aug 06 09:30:30 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 0.00394613
rho = 0 Specified
beta_0 = 1.07904
beta_1 = 9.00466e-005
beta_2 = -8.93576e-009

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1	beta_2
alpha	1	1.3e-008	-1.8e-008	1.8e-008
beta_0	1.3e-008	1	-0.72	0.62
beta_1	-1.8e-008	-0.72	1	-0.98
beta_2	1.8e-008	0.62	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.00358299	0.000772727	0.00206847	0.0050975
beta_0	1.07919	0.0160403	1.04775	1.11062
beta_1	8.9905e-005	2.14749e-005	4.7815e-005	0.000131995
beta_2	-8.91318e-009	3.99586e-009	-1.67449e-008	-1.08143e-009

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	1.08	1.08	0.069	0.0599	-0.115
500	11	1.13	1.12	0.048	0.0599	0.171
1,750	11	1.21	1.21	0.076	0.0599	-0.0677
5000	11	1.31	1.31	0.055	0.0599	0.00659

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                    Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1           99.602165            5    -189.204331
            A2          100.989890            8    -185.979779
            A3           99.602165            5    -189.204331
        fitted           99.578511            4    -191.157022
             R           75.306055            2    -146.612110


                Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                   Tests of Interest

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

  Test 1             51.3677          6          <.0001
  Test 2             2.77545          3          0.4276
  Test 3             2.77545          3          0.4276
  Test 4           0.0473084          1          0.8278

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here
```

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 716.719

BMDL = 494.126

Organ: Kidney

Endpoint: Kidney Weight (absolute)

Species/Gender: Sprague-Dawley Male Rats

(White et al., 1995)

Table B-48. Mean absolute kidney weight (and SD) in Sprague-Dawley male rats exposed to four different concentrations of ETBE via inhalation for 4 weeks

Administered dose (ppm)	Mean absolute kidney weight, g (SD)
Control (n = 10)	2.73 (0.35)
500 (n = 10)	2.71 (0.18)
2,000 (n = 10)	2.89 (0.28)
4,000 (n = 10)	3.08[a] (0.35)

[a]Statistically significantly different from control at $p < 0.05$ as reported by White et al. (1995).

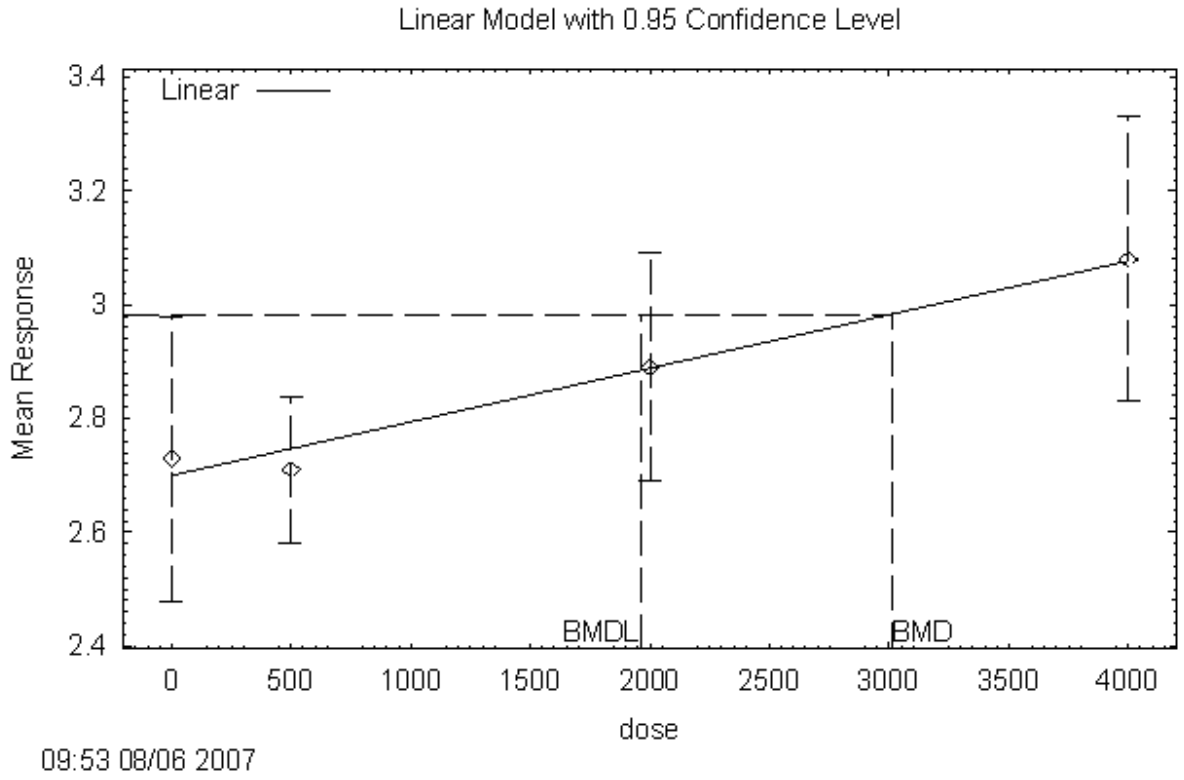
Source: White et al. (1995).

Table B-49. A summary of BMDS (version 1.4.1) modeling results based on mean absolute kidney weight in Sprague-Dawley male rats exposed to ETBE via inhalation for 4 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.87**	**-54.7**	**3,010**	**1,960**
Power	0.65	-52.8	3,186	1,969
Hill	NA	-51.0	2,247	606

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: White et al. (1995).



Source: White et al. (1995).

Figure B-22. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute kidney weight in Sprague-Dawley male rats exposed to ETBE via inhalation for 4 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_KIDNEY_WT_WHITE95.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_KIDNEY_WT_WHITE95.plt
                                        Mon Aug 06 09:53:16 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 0.08895
rho = 0 Specified
beta_0 = 2.69923
beta_1 = 9.43226e-005

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	3.5e-009	-4.2e-009
beta_0	3.5e-009	1	-0.72
beta_1	-4.2e-009	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.0806269	0.0180287	0.0452913	0.115963
beta_0	2.69923	0.0649108	2.572	2.82645
beta_1	9.43226e-005	2.88492e-005	3.77791e-005	0.000150866

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	2.73	2.7	0.35	0.284	0.343
500	10	2.71	2.75	0.18	0.284	-0.405
2000	10	2.89	2.89	0.28	0.284	0.0237
4000	10	3.08	3.08	0.35	0.284	0.0388

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	30.500828	5	-51.001655
A2	32.981294	8	-49.962588
A3	30.500828	5	-51.001655
fitted	30.358450	3	-54.716900
R	25.621610	2	-47.243219

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	14.7194	6	0.02256
Test 2	4.96093	3	0.1747
Test 3	4.96093	3	0.1747
Test 4	0.284755	2	0.8673

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3010.4

BMDL = 1959.5

Organ: Kidney

Endpoint: Labeling Index

Species/Gender: F344 Male Rats

(Medinsky et al., 1999)

Table B-50. Mean LI (and SD) in the kidney of F344 male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean LI (SD)
Control (n = 5)	0.93 (0.30)
500 (n = 5)	2.26[a] (0.86)
1,750 (n = 5)	3.42[a] (0.66)
5,000 (n = 5)	2.59[a] (1.21)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

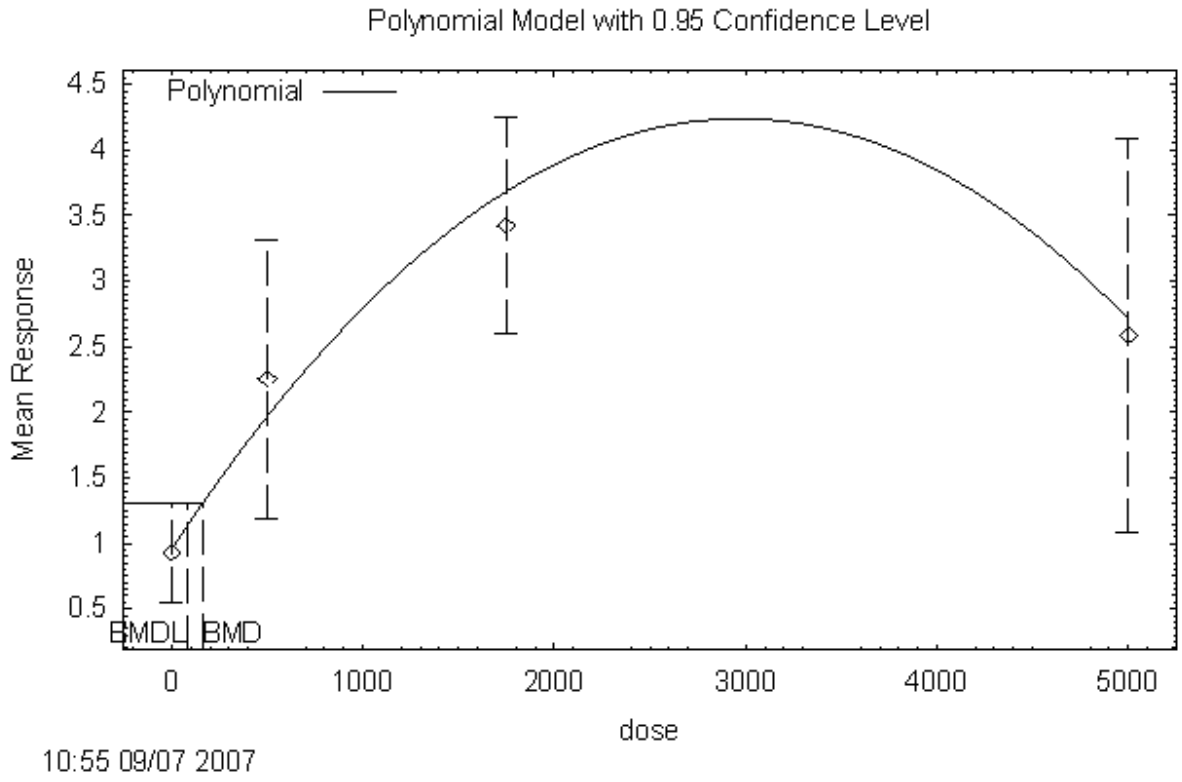
Source: Medinsky et al. (1999).

Table B-51. A summary of BMDS (version 1.4.1) modeling results based on mean LI in the kidney of F344 male rats exposed to ETBE via inhalation for 13 weeks

Model (non-constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	**0.12**	**15.4**	**160**	**81**
Power	<0.0001	30.3	2,300	342
Hill	NA	15.6	406	Computation of the lower bound failed

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-23. BMDS (version 1.4.1) model output for the best-fit model (i.e., 2° polynomial) based on mean LI in the kidney of F344 male rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_LI_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_LI_MEDINSKY99.plt
                                        Fri Sep 07 10:55:09 2007
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 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
```

Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

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

Default Initial Parameter Values

lalpha =	-0.383191
rho =	0
beta_0 =	1.09523
beta_1 =	0.00197542
beta_2 =	-3.35849e-007

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1	beta_2
lalpha	1	-0.88	-0.04	0.025	-0.056
rho	-0.88	1	0.0065	-0.069	0.13
beta_0	-0.04	0.0065	1	-0.42	0.34
beta_1	0.025	-0.069	-0.42	1	-0.98
beta_2	-0.056	0.13	0.34	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-2.061	0.693038	-3.41933	-0.702675
rho	1.81929	0.866479	0.121024	3.51756
beta_0	0.957315	0.146009	0.671144	1.24349
beta_1	0.00220546	0.000415615	0.00139087	0.00302006
beta_2	-3.71512e-007	8.46199e-008	-5.37364e-007	-2.0566e-007

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	0.93	0.957	0.302	0.343	-0.178
500	5	2.26	1.97	0.857	0.66	0.978
1,750	5	3.42	3.68	0.662	1.17	-0.493
5000	5	2.59	2.7	1.21	0.88	-0.282

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-3.936651	5	17.873301
A2	0.098177	8	15.803646
A3	-1.473139	6	14.946277
fitted	-2.705971	5	15.411943
R	-13.002695	2	30.005391

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	26.2017	6	0.0002042
Test 2	8.06966	3	0.04459
Test 3	3.14263	2	0.2078
Test 4	2.46567	1	0.1164

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 159.8

BMDL = 80.847

Organ: Kidney

Endpoint: Regenerative Foci (continuous)

Species/Gender: F344 Male Rats

(Medinsky et al., 1999)

Table B-52. Mean regenerative foci (and SD) in the kidney of F344 male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean regenerative foci (SD)
Control (n = 5)	2.2 (0.84)
500 (n = 5)	11.0[a] (4.53)
1,750 (n = 5)	16.80[a] (7.46)
5,000 (n = 5)	33.80[a] (6.30)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

Source: Medinsky et al. (1999).

Table B-53. A summary of BMDS (version 1.4.1) modeling results based on mean regenerative foci in the kidney of F344 male rats exposed to ETBE via inhalation for 13 weeks

Model (non-constant variance)	χ^2 p-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	0.02	85.5	62	32
Power	0.001	93.1	554	156
Hill	**0.14**	**82.4**	**40**	**23**

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: Medinsky et al. (1999).

BMDS (version 1.4.1) model output for the best-fit model (i.e., Hill) based on mean regenerative foci in the kidney of F344 male rats exposed to ETBE via inhalation for 13 weeks (Medinsky et al., 1999)

```
 ================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_MEAN_REGEN_FOCI_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_MEAN_REGEN_FOCI_MEDINSKY99.plt
                                        Fri Sep 07 11:49:42 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

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


                  Default Initial Parameter Values
                         lalpha =      3.37245
                            rho =            0
                      intercept =          2.2
                              v =         31.6
                              n =     0.147672
                              k =      8020.59
```

Asymptotic Correlation Matrix of Parameter Estimates

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

	lalpha	rho	intercept	v	k
lalpha	1	-0.94	-0.41	0.33	0.3
rho	-0.94	1	0.36	-0.39	-0.31
intercept	-0.41	0.36	1	-0.089	0.012
v	0.33	-0.39	-0.089	1	0.91
k	0.3	-0.31	0.012	0.91	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-1.80823	1.05076	-3.86769	0.251225
rho	1.85265	0.409579	1.04989	2.65541
intercept	2.16744	0.368446	1.4453	2.88958
v	38.0267	8.92164	20.5406	55.5128
n	1	NA		
k	1812.05	797.883	248.224	3375.87

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	5	2.2	2.17	0.837	0.829	0.0878
500	5	11	10.4	4.53	3.54	0.385
1,750	5	16.8	20.8	7.46	6.75	-1.34
5000	5	33.8	30.1	6.3	9.48	0.878

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-41.493114	5	92.986228
A2	-33.681268	8	83.362536
A3	-35.107830	6	82.215660
fitted	-36.175922	5	82.351845
R	-60.533595	2	125.067190

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	53.7047	6	<.0001
Test 2	15.6237	3	0.001354
Test 3	2.85312	2	0.2401
Test 4	2.13618	1	0.1439

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 40.3827

BMDL = 22.9779

Organ: Kidney

Endpoint: Regenerative Foci

Species/Gender: F344 Male Rats

(Medinsky et al., 1999)

Table B-54. Incidence of regenerative foci in the kidneys of F344 male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Incidence
Control	4/11
500	10/11[a]
1,750	11/11[a]
5,000	11/11[a]

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

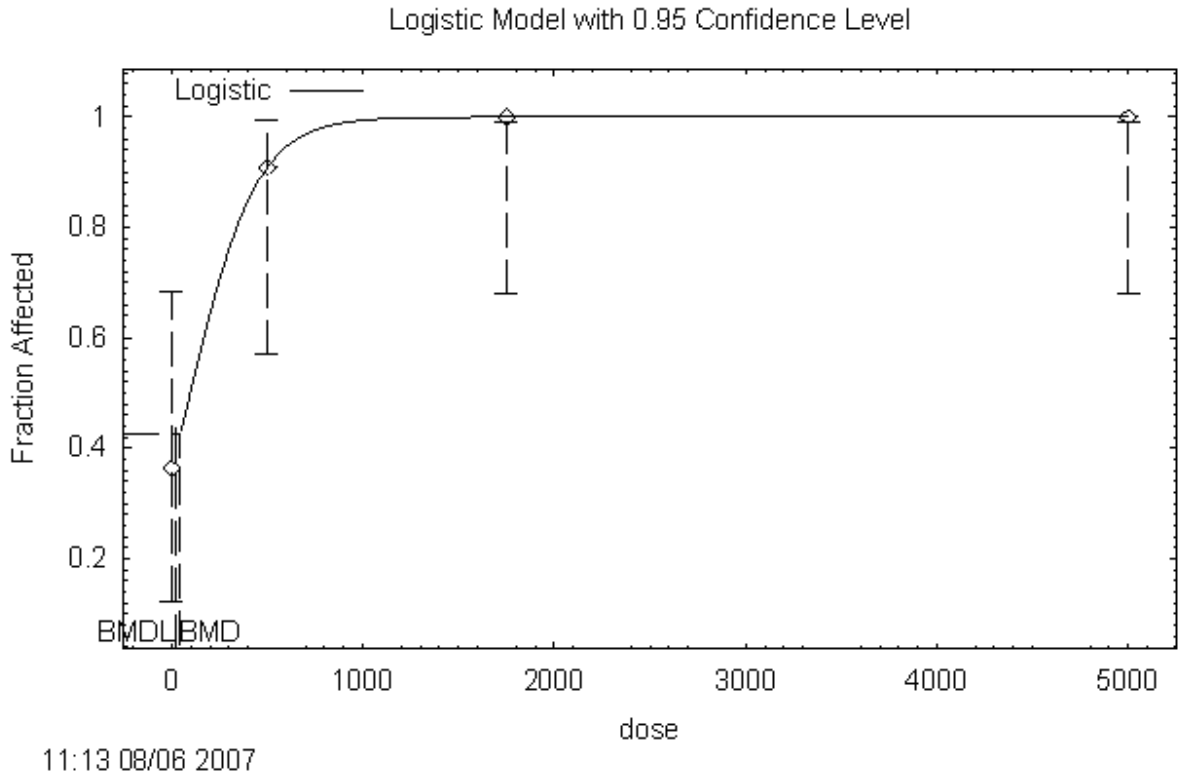
Source: Medinsky et al. (1999).

Table B-55. A summary of BMDS (version 1.4.1) modeling results based on incidence of regenerative foci in the kidneys of F344 male rats exposed to ETBE via inhalation for 13 weeks

Model	χ^2 *p*-value	AIC	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
Gamma	0.999	27.1	175	13
Logistic	**0.9996**	**25.1**	**47**	**24**
Log-logistic	0.9997	27.1	366	1
Multistage (1°)	0.996	25.1	27	13
Probit	1.00	25.1	49	29
Log-probit	0.9997	27.1	257	16
Quantal-linear	0.996	25.1	27	13
Weibull	0.999	27.1	86	13

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{10} = Benchmark concentration corresponding to a 10% change relative to controls.
$BMCL_{10}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 10% change relative to controls.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-24. BMDS (version 1.4.1) model output for the best-fit model (i.e., logistic) based on incidence of regenerative foci in the livers of F344 male rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Logistic Model. (Version: 2.9; Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_REGEN_FOCI_INCIDENCE_MEDINSKY99.(d
)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_KIDNEY_REGEN_FOCI_INCIDENCE_MEDINSKY99.pl
t
                                        Mon Aug 06 11:13:21 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

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

Dependent variable = Response
Independent variable = Dose
Slope parameter is not restricted

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

Default Initial Parameter Values
background = 0 Specified
intercept = 0.95046
slope = 0.000538515

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept	slope
intercept	1	-0.51
slope	-0.51	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-0.560447	0.626213	-1.7878	0.666908
slope	0.00573261	0.00243007	0.000969767	0.0104955

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-10.5613	4			
Fitted model	-10.5621	2	0.00170557	2	0.9991
Reduced model	-20.8621	1	20.6017	3	0.0001274

AIC: 25.1243

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3634	3.998	4	11	0.001
500.0000	0.9094	10.003	10	11	-0.003
1,750.0000	0.9999	10.999	11	11	0.029
5000.0000	1.0000	11.000	11	11	0.000

Chi^2 = 0.00 d.f. = 2 P-value = 0.9996

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 46.5325

BMDL = 23.9564

Organ: Adrenal Gland

Endpoint: Adrenal Gland Weight (absolute)

Species/Gender: F344 Male Rats

(Medinsky et al., 1999)

Table B-56. Mean absolute adrenal gland weight (and SD) in F344 male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean adrenal gland weight, g (SD)
Control (n = 11)	0.035 (0.005)
500 (n = 11)	0.039 (0.009)
1,750 (n = 11)	0.038 (0.007)
5,000 (n = 11)	0.047[a] (0.007)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

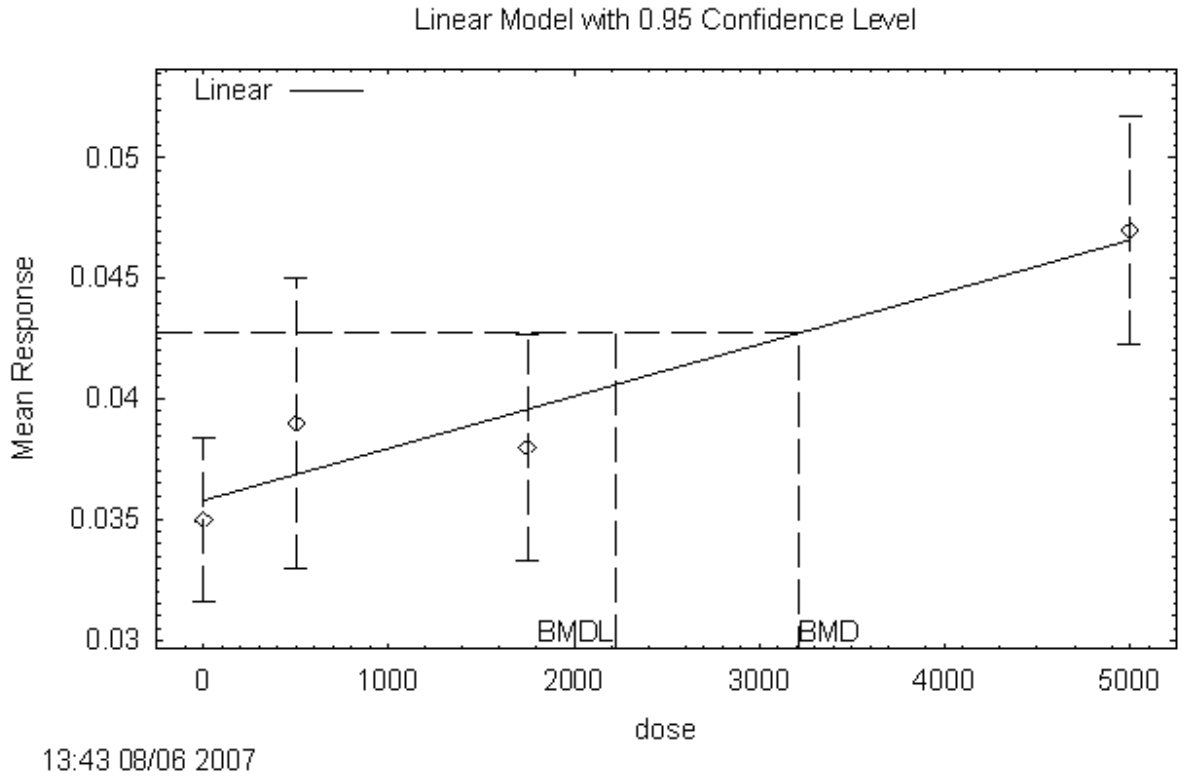
Source: Medinsky et al. (1999).

Table B-57. A summary of BMDS (version 1.4.1) modeling results based on mean absolute adrenal gland weight in F344 male rats exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.40**	**-387.3**	**3,214**	**2,223**
Power	0.19	-385.4	3,759	2,234
Hill	NA	-383.3	3,814	1,408

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-25. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute adrenal gland weight in F344 male rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_ADRENAL_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_ADRENAL_WT_MEDINSKY99.plt
                                        Mon Aug 06 13:43:50 2007
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 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
```

Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 5.1e-005
rho = 0 Specified
beta_0 = 0.0358301
beta_1 = 2.16272e-006

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	6.9e-010	-1.1e-012
beta_0	6.9e-010	1	-0.68
beta_1	-1.1e-012	-0.68	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	4.83101e-005	1.02997e-005	2.8123e-005	6.84972e-005
beta_0	0.0358301	0.0014314	0.0330246	0.0386356
beta_1	2.16272e-006	5.38026e-007	1.10821e-006	3.21723e-006

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	0.035	0.0358	0.005	0.00695	-0.396
500	11	0.039	0.0369	0.009	0.00695	0.997
1,750	11	0.038	0.0396	0.007	0.00695	-0.771
5000	11	0.047	0.0466	0.007	0.00695	0.17

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	197.537892	5	-385.075785
A2	199.354746	8	-382.709491
A3	197.537892	5	-385.075785
fitted	196.633146	3	-387.266291
R	189.751789	2	-375.503578

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	19.2059	6	0.00383
Test 2	3.63371	3	0.3038
Test 3	3.63371	3	0.3038
Test 4	1.80949	2	0.4046

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3213.8

BMDL = 2222.59

Organ: Adrenal Gland

Endpoint: Adrenal Gland Weight (absolute)

Species/Gender: F344 Female Rats

(Medinsky et al., 1999)

Table B-58. Mean absolute adrenal gland weight (and SD) in F344 female rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean adrenal gland weight, g (SD)
Control (n = 10)	0.045 (0.004)
500 (n = 11)	0.048 (0.004)
1,750 (n = 11)	0.048 (0.007)
5,000 (n = 11)	0.053[a] (0.005)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

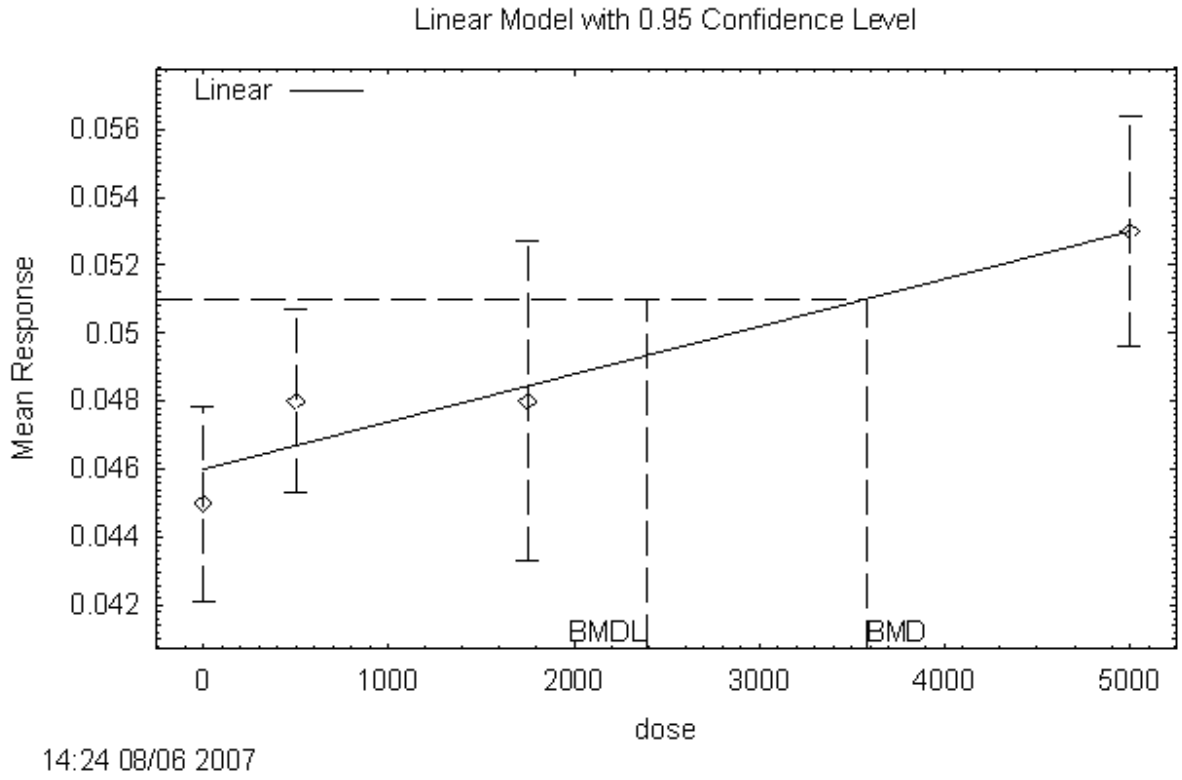
Source: Medinsky et al. (1999).

Table B-59. A summary of BMDS (version 1.4.1) modeling results based on mean absolute adrenal gland weight in F344 female rats exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.54**	**-406.7**	**3,576**	**2,394**
Power	0.54	-406.7	3,576	2,394
Hill	0.26	-404.7	3,437	750

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-26. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean absolute adrenal gland weight in F344 female rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_ADRENAL_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_ADRENAL_WT_MEDINSKY99.plt
                                        Mon Aug 06 14:24:18 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 2.67692e-005
rho = 0 Specified
beta_0 = 0.0459464
beta_1 = 1.40886e-006

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	beta_0	beta_1
alpha	1	-2e-008	2.8e-008
beta_0	-2e-008	1	-0.69
beta_1	2.8e-008	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	2.49955e-005	5.39067e-006	1.443e-005	3.5561e-005
beta_0	0.0459884	0.00105217	0.0439262	0.0480506
beta_1	1.39812e-006	3.90961e-007	6.31853e-007	2.16439e-006

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	0.045	0.046	0.004	0.005	-0.625
500	11	0.048	0.0467	0.004	0.005	0.871
1,750	11	0.048	0.0484	0.007	0.005	-0.289
5000	11	0.053	0.053	0.005	0.005	0.0139

```
Model Descriptions for likelihoods calculated


Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                    Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1          206.956762            5    -403.913524
            A2          209.411887            8    -402.823775
            A3          206.956762            5    -403.913524
         fitted         206.331505            3    -406.663010
             R          200.733556            2    -397.467113


                Explanation of Tests

Test 1:  Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
Test 2:  Are Variances Homogeneous? (A1 vs A2)
Test 3:  Are variances adequately modeled? (A2 vs. A3)
Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
(Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                   Tests of Interest

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

  Test 1             17.3567          6        0.008058
  Test 2             4.91025          3          0.1785
  Test 3             4.91025          3          0.1785
  Test 4             1.25051          2          0.5351

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here
```

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3575.9

BMDL = 2394.08

Organ: Adrenal Gland

Endpoint: Adrenal Gland Weight (absolute)

Species/Gender: Sprague-Dawley Male Rats

(White et al., 1995)

Table B-60. Mean absolute adrenal gland weight (and SD) in Sprague-Dawley male rats exposed to four different concentrations of ETBE via inhalation for 4 weeks

Administered dose (ppm)	Mean adrenal gland weight, g (SD)
Control (n = 9)	0.051 (0.004)
500 (n = 10)	0.047 (0.006)
2,000 (n = 10)	0.051 (0.006)
4,000 (n = 10)	0.058[a] (0.008)

[a]Statistically significantly different from control at $p < 0.05$ as reported by White et al. (1995).

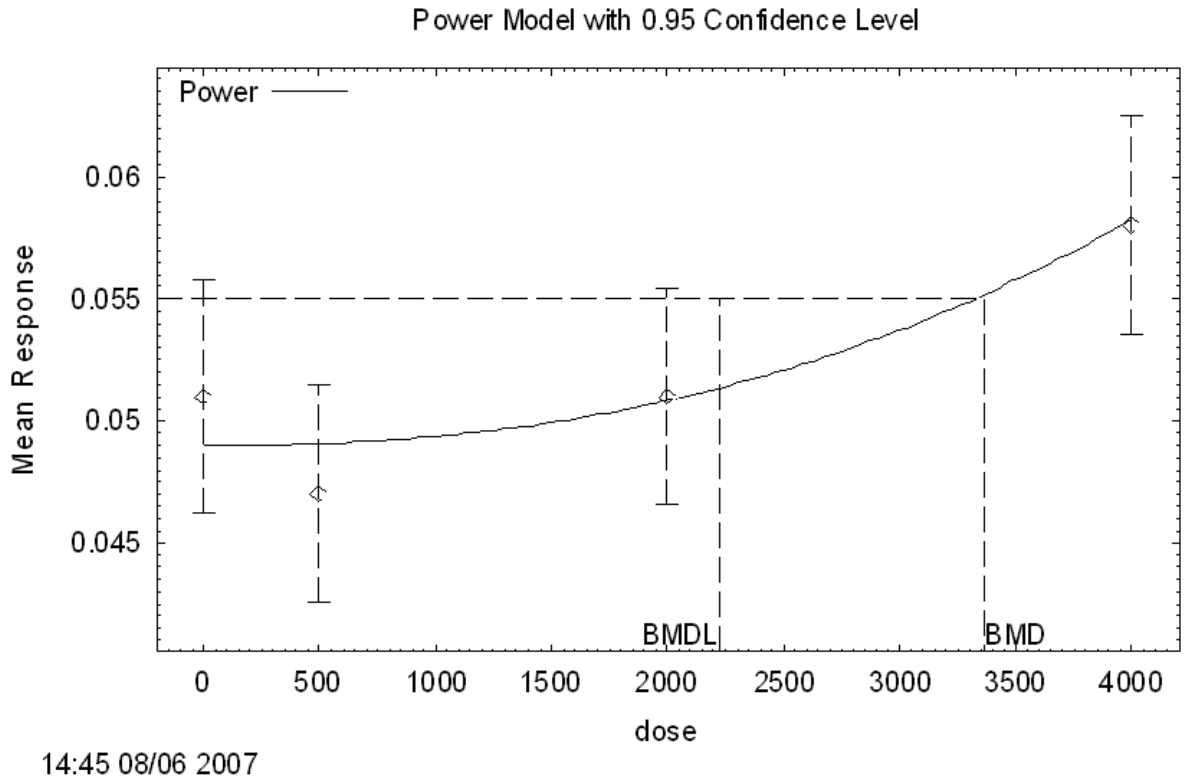
Source: White et al. (1995).

Table B-61. A summary of BMDS (version 1.4.1) modeling results based on mean absolute adrenal gland weight in Sprague-Dawley male rats exposed to ETBE via inhalation for 4 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (2°)	0.17	-351.6	3,522	2,341
Power	**0.14**	**-351.3**	**3,367**	**2,220**
Hill	NA	-348.6	3,912	1,898

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: White et al. (1995).



Source: White et al. (1995).

Figure B-27. BMDS (version 1.4.1) model output for the best-fit model (i.e., power) based on mean absolute adrenal gland weight in Sprague-Dawley male rats exposed to ETBE via inhalation for 4 weeks.

```
 ================================================================
        Power Model. (Version: 2.14;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_ADRENAL_WT_WHITE95.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\SD_RATS_MALES_ADRENAL_WT_WHITE95.plt
                                    Mon Aug 06 14:45:20 2007
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 3.86286e-005
rho = 0 Specified
control = 0.047
slope = 6.08741e-008
power = 1.45943

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	control	slope	power
alpha	1	1.8e-009	-3.9e-009	3.9e-009
control	1.8e-009	1	-0.51	0.5
slope	-3.9e-009	-0.51	1	-1
power	3.9e-009	0.5	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	3.66996e-005	8.31082e-006	2.04107e-005	5.29885e-005
control	0.0489671	0.00140151	0.0462202	0.051714
slope	3.45133e-011	4.59598e-010	-8.66282e-010	9.35308e-010
power	2.33737	1.59943	-0.79745	5.47219

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	9	0.051	0.049	0.004	0.00606	1.01
500	10	0.047	0.049	0.006	0.00606	-1.06
2000	10	0.051	0.0508	0.006	0.00606	0.125
4000	10	0.058	0.058	0.008	0.00606	-0.0165

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2


                     Likelihoods of Interest

           Model      Log(likelihood)   # Param's      AIC
            A1          180.759773           5    -351.519546
            A2          182.906633           8    -349.813266
            A3          180.759773           5    -351.519546
         fitted         179.648531           4    -351.297063
             R          173.330901           2    -342.661801


                  Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

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

   Test 1             19.1515          6        0.003915
   Test 2             4.29372          3          0.2314
   Test 3             4.29372          3          0.2314
   Test 4             2.22248          1           0.136

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears to
be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
```

to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 3366.55

BMDL = 2219.59

Organ: Bone Marrow

Endpoint: Bone Marrow Congestion

Species/Gender: F344 Female Rats

(Medinsky et al., 1999)

Table B-62. Incidence of bone marrow congestion in F344 female rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Incidence
Control	0/10
500	0/11
1,750	5/11[a]
5,000	11/11[a]

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

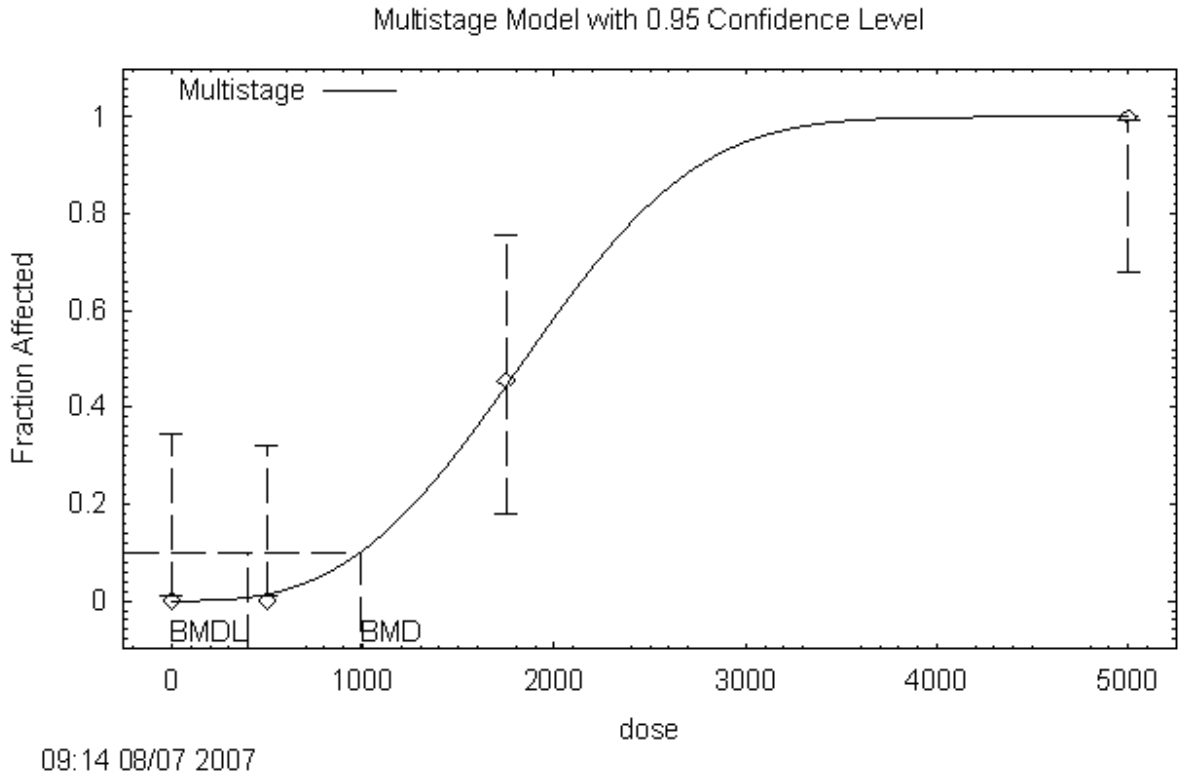
Source: Medinsky et al. (1999).

Table B-63. A summary of BMDS (version 1.4.1) modeling results based on incidence of bone marrow congestion in F344 female rats exposed to ETBE via inhalation for 13 weeks

Model	χ^2 *p*-value	AIC	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
Gamma	1.00	17.2	1,305	568
Logistic	1.00	19.2	1,615	753
Log-logistic	1.00	17.2	1,565	678
Multistage (3°)	**0.98**	**17.5**	**987**	**401**
Probit	1.00	19.2	1,488	684
Log-probit	1.00	19.2	1,418	642
Quantal-linear	0.23	24.8	253	162
Weibull	1.00	19.2	1,456	526

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{10} = Benchmark concentration corresponding to a 10% change relative to controls.
$BMCL_{10}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 10% change relative to controls.

Source: Medinsky et al. (1999).



Source: Medinsky et al., (1999).

Figure B-28. BMDS (version 1.4.1) model output for the best-fit model (i.e., 3° multistage) based on incidence of bone marrow congestion in F344 female rats exposed to ETBE via inhalation for 13 weeks.

```
 ======================================================================
        Multistage Model. (Version: 2.8;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_BONE_MARROW_CONGEST_INCIDENCE_MEDINSKY9
9.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_BONE_MARROW_CONGEST_INCIDENCE_MEDINSKY9
9.plt
                                        Tue Aug 07 09:14:48 2007
 ======================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

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

The parameter betas are restricted to be positive

Dependent variable = Response
Independent variable = Dose

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

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

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 0
Beta(3) = 8.10541e+008

Asymptotic Correlation Matrix of Parameter Estimates

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

	Beta(3)
Beta(3)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	1.09591e-010	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-7.5791	4			
Fitted model	-7.73218	1	0.306165	3	0.9589
Reduced model	-28.3826	1	41.607	3	<.0001

AIC: 17.4644

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	10	0.000
500.0000	0.0136	0.150	0	11	-0.390
1,750.0000	0.4442	4.886	5	11	0.069
5000.0000	1.0000	11.000	11	11	0.004

Chi^2 = 0.16 d.f. = 3 P-value = 0.9843

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 986.964

BMDL = 400.522

BMDU = 1304.05

Taken together, (400.522, 1304.05) is a 90 % two-sided confidence interval for the BMD

Organ: Testes

Endpoint: Degenerated Spermatocytes

Species/Gender: F344 Male Rats

(Medinsky et al., 1999)

Table B-64. Mean degenerated spermatocytes (and SD) in the testes of F344 male rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean degenerated spermatocytes (SD)
Control (n = 11)	2.09 (0.944)
500 (n = 11)	2.36 (1.80)
1,750 (n = 11)	7.82[a] (3.71)
5,000 (n = 11)	12.70[a] (10.8)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

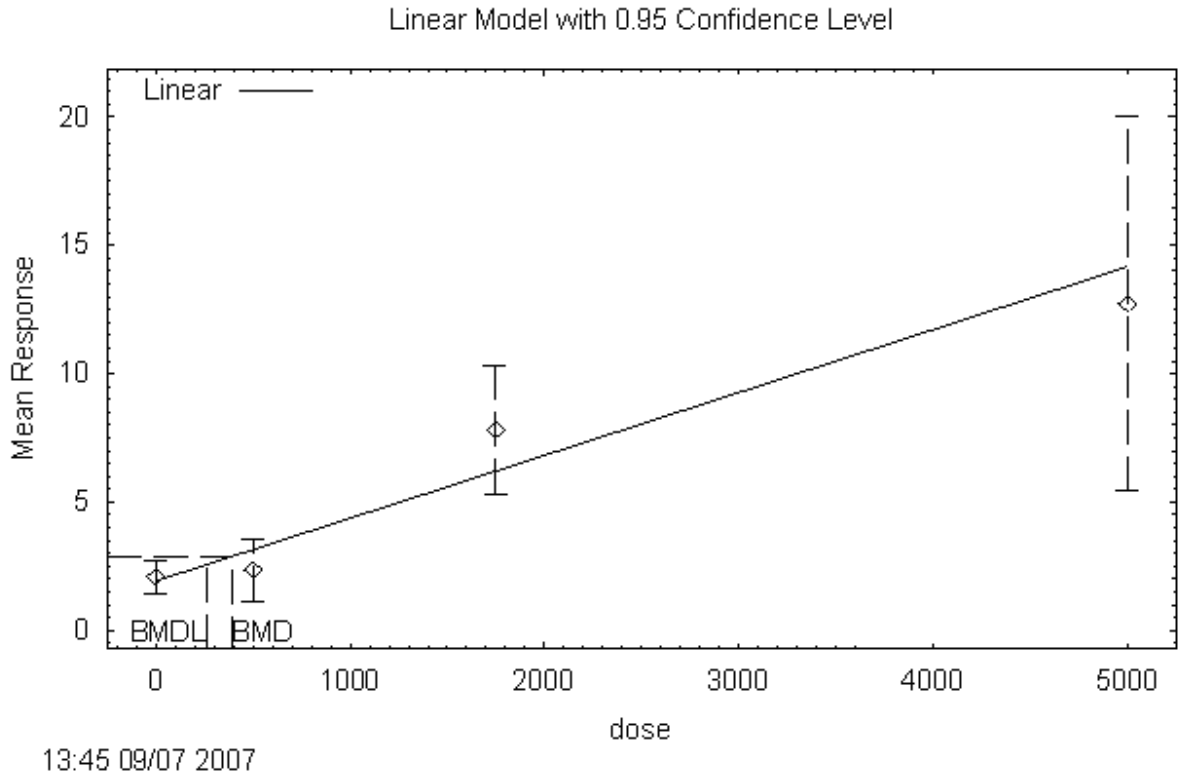
Sources: Medinsky et al (1999).

Table B-65. A summary of BMDS (version 1.4.1) modeling results based on mean degenerated spermatocytes in the testes of F344 male rats exposed to ETBE via inhalation for 13 weeks

Model (non-constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	**0.41**	**145.7**	**397**	**268**
Power	0.19	147.7	425	268
Hill	NA	147.9	598	307

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.
NA = Degrees of freedom for the chi-square test for goodness-of-fit are less than or equal to 0; therefore, this test is not valid for evaluating lack of fit.

Source: Medinsky et al. (1999).



Source: Medinsky et al., 1999.

Figure B-29. BMDS (version 1.4.1) model output for the best-fit model (i.e., 1° polynomial or linear) based on mean regenerative spermatocytes in the testes of F344 male rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Polynomial Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_TESTES_DEGEN_SPERMATOCYTES_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_MALES_TESTES_DEGEN_SPERMATOCYTES_MEDINSKY99.plt
                                          Fri Sep 07 13:45:21 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
```

Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

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

Default Initial Parameter Values

lalpha =	3.51992
rho =	0
beta_0 =	2.28527
beta_1 =	0.00218743

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.91	-0.091	0.1
rho	-0.91	1	0.088	-0.097
beta_0	-0.091	0.088	1	-0.36
beta_1	0.1	-0.097	-0.36	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-1.61488	0.506123	-2.60686	-0.622894
rho	2.38634	0.291921	1.81419	2.9585
beta_0	1.9212	0.266417	1.39903	2.44336
beta_1	0.00245095	0.000456859	0.00155553	0.00334638

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	2.09	1.92	0.944	0.972	0.579
500	11	2.36	3.15	1.8	1.75	-1.48
1,750	11	7.82	6.21	3.71	3.94	1.35
5000	11	12.7	14.2	10.8	10.6	-0.455

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-97.341461	5	204.682921
A2	-66.381513	8	148.763026
A3	-67.972913	6	147.945826
fitted	-68.858357	4	145.716715
R	-108.026956	2	220.053912

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	83.2909	6	<.0001
Test 2	61.9199	3	<.0001
Test 3	3.1828	2	0.2036
Test 4	1.77089	2	0.4125

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

```
The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


             Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =        396.596


            BMDL =        267.653
```

Organ: Heart

Endpoint: Heart Weight (absolute)

Species/Gender: F344 Female Rats

(Medinsky et al., 1999)

Table B-66. Mean absolute heart weight (and SD) in F344 female rats exposed to four different concentrations of ETBE via inhalation for 13 weeks

Administered dose (ppm)	Mean heart weight, g (SD)
Control (n = 10)	0.495 (0.034)
500 (n = 11)	0.545[a] (0.037)
1,750 (n = 11)	0.532 (0.040)
5,000 (n = 11)	0.556[a] (0.032)

[a]Statistically significantly different from control at $p < 0.05$ as reported by Medinsky et al. (1999).

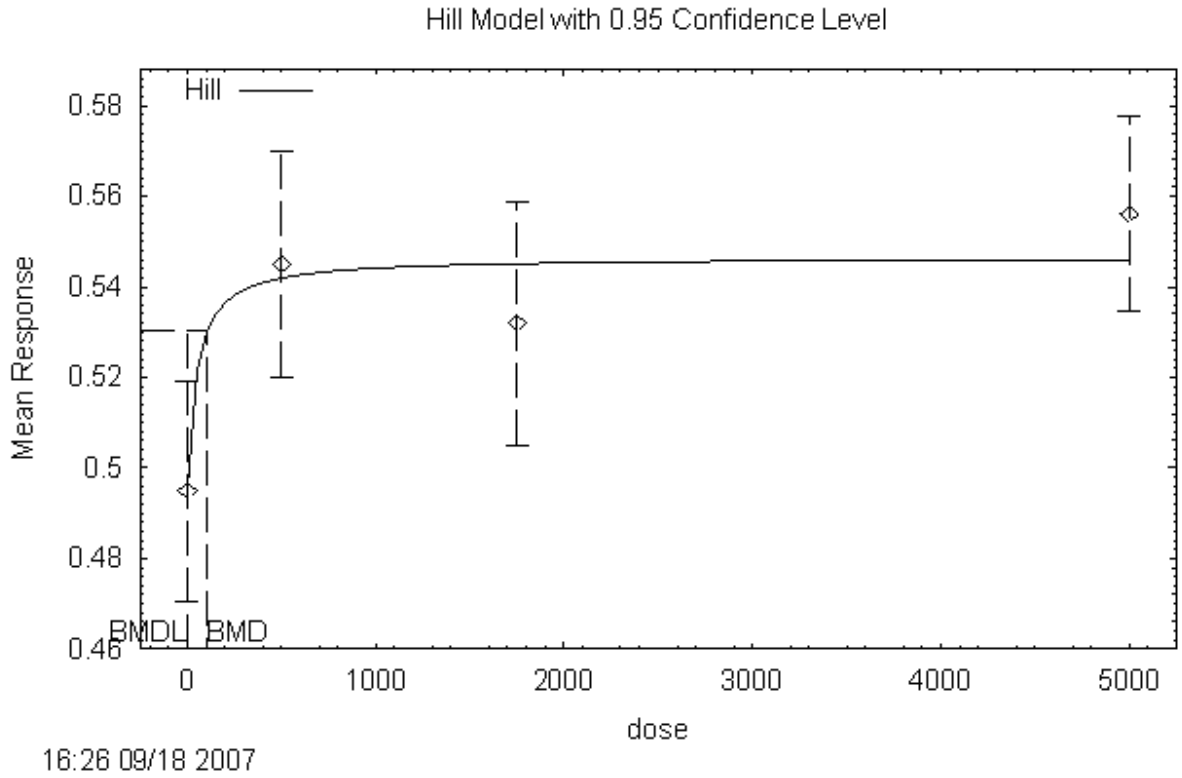
Source: Medinsky et al. (1999).

Table B-67. A summary of BMDS (version 1.4.1) modeling results based on mean absolute heart weight in F344 female rats exposed to ETBE via inhalation for 13 weeks

Model (constant variance)	χ^2 *p*-value	AIC	BMC_{1SD} (ppm)	$BMCL_{1SD}$ (ppm)
Polynomial (1°)	0.01	-232.7	4,674	2,873
Power	0.01	-232.7	4,674	2,873
Hill	0.11	-236.7	105	Computation of the lower bound failed

Chi-square *p*-value = *p*-value from the chi-square test for goodness-of-fit (*p*-values < 0.1 indicate that the model exhibits significant lack-of-fit).
BMC_{1SD} = Benchmark concentration corresponding to a 1 SD change relative to the control mean.
$BMCL_{1SD}$ = 95% lower confidence limit on the benchmark concentration corresponding to a 1 SD change relative to the control mean.

Source: Medinsky et al. (1999).



Source: Medinsky et al. (1999).

Figure B-30. BMDS (version 1.4.1) model output for the best-fit model (i.e., Hill) based on mean absolute heart weight in F344 female rats exposed to ETBE via inhalation for 13 weeks.

```
 ================================================================
        Hill Model. (Version: 2.12;  Date: 02/20/2007)
        Input Data File: G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_HEART_WT_MEDINSKY99.(d)
        Gnuplot Plotting File:  G:\ETBE DOSE-RESPONSE
MODELING\INHALATION\F344_RATS_FEMALES_HEART_WT_MEDINSKY99.plt
                                        Tue Sep 18 16:26:16 2007
 ================================================================

 BMDS MODEL RUN
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   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
```

rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

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

Default Initial Parameter Values

alpha =	0.00129062	
rho =	0	Specified
intercept =	0.495	
v =	0.061	
n =	0.234371	
k =	695	

Asymptotic Correlation Matrix of Parameter Estimates

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

	alpha	intercept	v	k
alpha	1	-8.8e-010	2.7e-008	4.4e-008
intercept	-8.8e-010	1	-0.72	0.07
v	2.7e-008	-0.72	1	0.5
k	4.4e-008	0.07	0.5	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
alpha	0.00124247	0.000267959	0.000717284	0.00176766
intercept	0.495017	0.0111515	0.47316	0.516873
v	0.0514534	0.0152581	0.021548	0.0813587
n	1	NA		
k	48.4598	201.386	-346.25	443.169

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	0.495	0.495	0.034	0.0352	-0.00152

500	11	0.545	0.542	0.037	0.0352	0.289
1,750	11	0.532	0.545	0.04	0.0352	-1.23
5000	11	0.556	0.546	0.032	0.0352	0.943

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	123.630904	5	-237.261808
A2	123.948426	8	-231.896853
A3	123.630904	5	-237.261808
fitted	122.349007	4	-236.698014
R	115.878403	2	-227.756806

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	16.14	6	0.01302
Test 2	0.635045	3	0.8884
Test 3	0.635045	3	0.8884
Test 4	2.56379	1	0.1093

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance

model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 105.411

BMDL = 5e-012

APPENDIX C. DERIVATION OF THE ORAL MINIMAL DATA VALUE

Considering the uncertainties in the ETBE database described below, the total composite UF is for the derivation of an RfD is 10,000, consisting of four areas of maximum uncertainty. In the report, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the RfD/RfC technical panel concluded that, in cases where maximum uncertainty exists in four or more areas of uncertainty, or when the total UF is 10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Because of this uncertainty, an RfD is not derived and instead an oral minimal data value is presented. The use of the minimal data value for ETBE is not recommended except in limited circumstances, for example, in screening level risk assessments or to rank relative risks. Any use of this value should include a discussion of the uncertainty associated with its derivation.

The minimal data value is based on increased relative kidney weight in F0 generation male rats exposed to ETBE by gavage as part of a two-generation reproduction and fertility study (CIT 2004b, unpublished report). This study was chosen as the principal study because it provides the most sensitive measure of effects of oral exposure to ETBE. Increased relative kidney weight in F0 generation males was selected as the critical effect because represents the most sensitive effect and resulted in the lowest BMDL. BMD modeling revealed that the BMDL associated with the increased relative kidney weight is 143 mg/kg-day. The BMDL provides the POD for the minimal data value.

A total UF of 10,000 was applied to the POD of 143 mg/kg-day: 10 for interspecies extrapolation from animals to humans (UF_A); 10 for human intraspecies variability (UF_H); 10 for extrapolation from a subchronic to a chronic study (UF_S); and 10 to account for database deficiencies (UF_D).

A 10-fold UF was used to account for uncertainties in extrapolating from rats to humans. The available data do not provide evidence that rats, or any other species, are more sensitive to ETBE than humans. Consequently, the default UF value of 10 for extrapolating from laboratory animals to humans was applied.

A 10-fold UF was used to account for variation in susceptibility among members of the human population (i.e., interindividual variability). Insufficient information is available to predict potential variability in human susceptibility.

The duration of exposure in the principal study (CIT, 2004b, unpublished report) is 120 days. This length of exposure is greater than the 90 day exposure period commonly utilized in subchronic studies but falls short of a chronic exposure duration. The only chronic exposure study in the database for ETBE is a carcinogenicity bioassay that did not report any noncancer effects other than mortality (Maltoni et al., 1999). Therefore, no data are available to inform the nature and extent of effects that would be observed with a longer duration of exposure to ETBE.

For these reasons, a 10-fold UF was used to account for the extrapolation from subchronic to chronic exposure duration.

A 10-fold UF was used to account for deficiencies in the toxicity database on oral exposure to ETBE. There are no available human occupational or epidemiological studies of oral exposure to ETBE. There are no standard subchronic or chronic toxicity animal studies available for oral exposure to ETBE. The toxicity data on oral exposure to ETBE is limited and largely restricted to a series of unpublished prenatal developmental toxicity and two-generation reproduction and fertility studies (CIT, 2004a, b, 2003, unpublished reports). Due to the limited scope and design of the reproductive and development studies, these studies cannot be considered an adequate assessment of general toxicity from oral exposure to ETBE. In particular, the lack of systematic histopathological data on the liver and kidney that would be part of a standard subchronic or chronic toxicity study represents a limitation on the available data. Note that the database UF is not applied because of a lack of a chronic study per se, but because of a lack of studies that examined multiple systemic endpoints.

A UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a change of one SD from the control mean was selected under the assumption that it represents a minimal biologically significant change.

The oral minimal data value for ETBE was calculated as follows:

Minimal data value = BMDL ÷ UF
= 143 mg/kg-day ÷ 10,000
= 0.0143 or 1×10^{-2} mg/kg-day

The overall confidence in this chronic oral minimal data value is low. Confidence in the principal study (CIT, 2004b, unpublished report) is medium. Confidence in the database is low due to the limited scope and design of the reproductive and development studies that comprise the available data. These studies cannot be considered an adequate assessment of general toxicity from oral exposure to ETBE, particularly because the studies lack histopathological data on the liver and kidney. Reflecting medium confidence in the principal study and low confidence in the database, confidence in the minimal data value is low.

Figure C-1 presents the POD, applied UFs, and derived potential chronic RfVs for additional endpoints that were modeled using EPA BMDS (version 1.4.1), or for endpoints where the data were not amenable to BMD modeling as indicated by NOAELs and LOAELs. This comparison is intended to provide information on additional effects associated with ETBE exposure.

PODs and potential chronic RfVs that could be derived from the additional effects identified in Table 5-1 are presented in Figure C-1 to allow a comparison with the chosen critical

effect and resulting minimal data value. For reduced body weight gain, and increased kidney weights in males, the total UF factor applied was 10,000-fold: 10-fold to account for uncertainty in extrapolating from laboratory animals to humans, 10-fold UF to account for variation in susceptibility among members of the human population, 10-fold UF to account for subchronic-to-chronic extrapolation, and 10-fold UF for database deficiencies. For increased liver weight in male rats of the F1 generation and increased kidney weights in female rats of the F1 generation, the total UF applied was 1000-fold: 10-fold to account for uncertainty in extrapolating from laboratory animals to humans, 10-fold UF to account for variation in susceptibility among members of the human population, and 10-fold UF for database deficiencies. A UF was not used to account for extrapolating from less than chronic exposure for these endpoints because these endpoints represent developmental toxicity resulting from a narrow period of exposure.

The increased kidney weight is the effect with the lowest BMDL and the lowest potential reference value from the data amenable to BMD modeling. Increased kidney weights were observed in both males and females with effects at lower doses in males. Given the data suggesting an effect of ETBE on body weight gain (e.g., in males of the F0 generation), relative kidney weights were considered a more appropriate measure than absolute kidney weights because of the potential effect that body weight changes may have on absolute organ weights. The dose-response relationship for increased liver weight for oral exposure to ETBE and reduced body weight gain are also suitable for deriving a chronic reference value, but are associated with higher BMDLs. An increase in mortality was also considered for the critical effect. Given the frank effect, and lack of quantitative mortality data, and a consistent dose-response trend, this endpoint was not considered ideal for the derivation of a chronic reference value and was not amenable to BMD modeling. Consideration of the available dose-response data to determine an estimate of oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime has led to the selection of the two-generation reproduction and fertility study in Sprague-Dawley rats (CIT 2004b, unpublished report) as the principal study and increased relative kidney weight in F0 generation male rats as the critical effect for deriving the oral minimal data value for ETBE. As discussed above, data suggesting kidney toxicity associated with oral exposure to ETBE is limited to increased kidney weights in males and females (at higher doses), with limited histopathological support. Additional evidence of kidney toxicity is provided by the ETBE inhalation exposure database, which includes effects such as increased cellular proliferation in the kidney, histological evidence of cellular necrosis (increased incidence of regenerative foci) in the kidneys, and increased kidney weights (Medinsky et al., 1999; White et al., 1995).

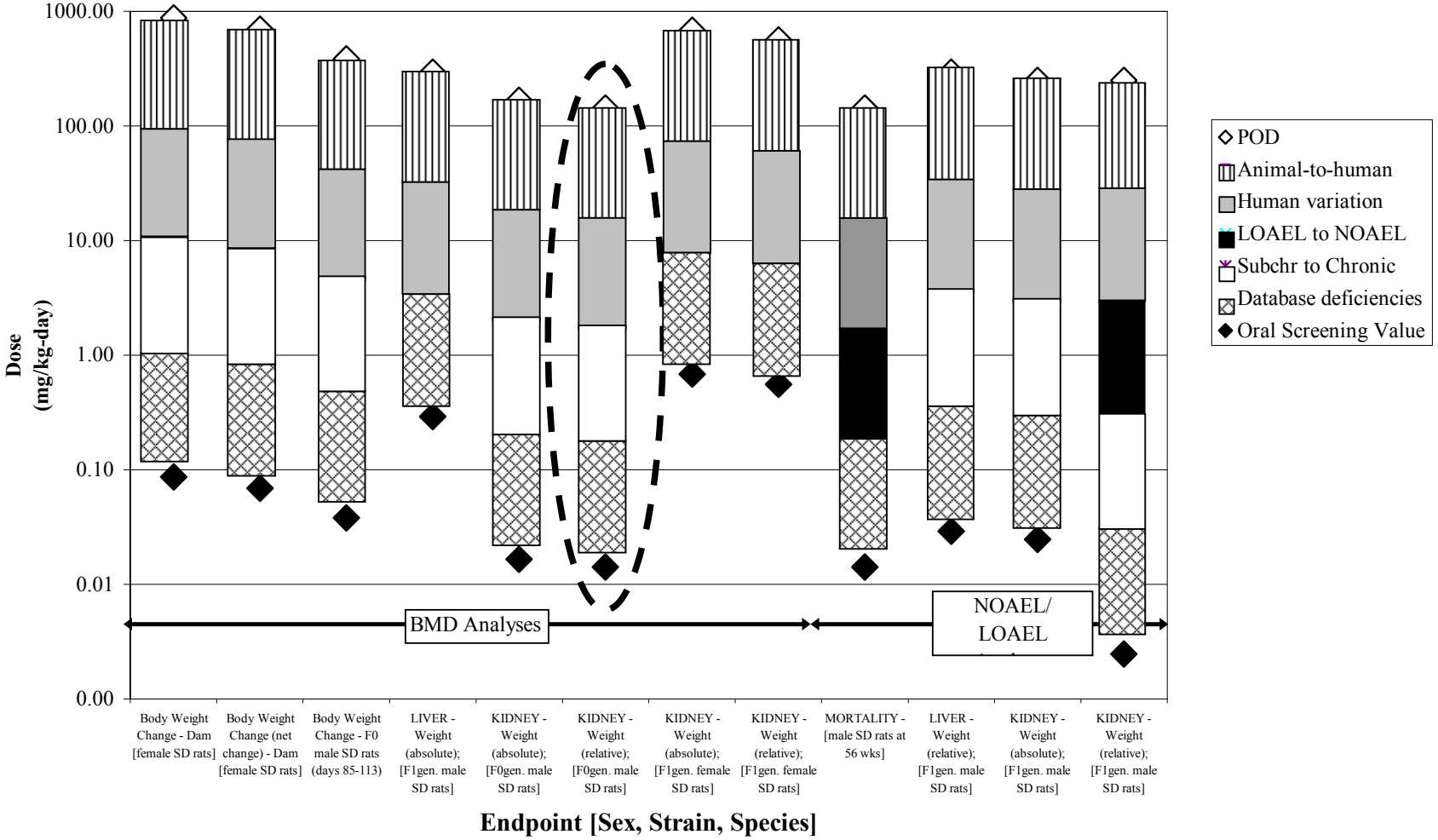

Figure C-1. Potential RfV comparison array for alternative PODs for oral data.