Interim: 06/2009

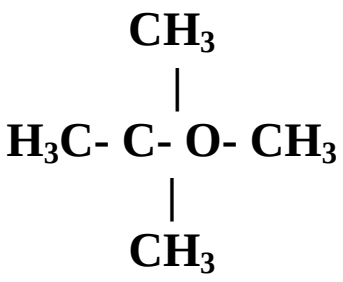
ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

FOR

METHYL *tertiary*-BUTYL ETHER

(CAS Reg. No. 1634-04-4)

$C_5H_{12}O$



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Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels — AEGL-1, AEGL-2 and AEGL-3 — are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

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SUMMARY

Methyl *tertiary*-butyl ether (MTBE) is a volatile synthetic chemical currently used as an additive to gasoline for its octane-enhancing and pollution-reducing properties. MTBE is a colorless liquid that dissolves easily in water. Exposure to MTBE occurs primarily by inhalation during production, blending, transportation or distribution and sale of gasoline. Ingestion of contaminated drinking water due to leaking MTBE storage tanks into the groundwater is becoming more of a concern (IARC 1999). Both human and animal data were utilized to develop AEGL values.

A concentration of 50 ppm across all time-points was adopted for AEGL-1. Male volunteers were exposed to 50 ppm MTBE by inhalation under light exercise for 2 hours with no effects observed of notable discomfort or irritation (Nihlén et al., 1998). On initial entry into the chamber, volunteers noted the odor but this diminished with time. The 50 ppm concentration was the no-effect level in humans. A six-hour inhalation study (Daughtrey et al., 1997) exposed rats to 800 ppm resulting in no-effects and supports the 50 ppm point-of-departure. An uncertainty factor of 1 was applied as this was a human study and a higher concentration in rats resulted in no effects. Extrapolation to other time-points was not performed as no effects were observed at 50 ppm and sensory effects are usually concentration, rather than time, dependent.

AEGL-2 values were derived from an acute six hour inhalation study in rats demonstrating clinical effects during the one hour post-exposure functional observation battery (FOB) (Daughtrey et al., 1997). No overt clinical signs were observed during exposure. Rats exposed to 4000 ppm demonstrated altered gait (ataxia, duckwalk), piloerection**,** and decreased hind-limb strength (females). These signs were more pronounced at the 8000 ppm concentration. The 4000 ppm level was chosen as some clinical signs were observed but were under the threshold that would impair mobilization; even at the 8000 ppm level, the rats were not immobilized. All clinical signs were transient and none were seen at the six hour post-exposure FOB. Supporting documents show that humans administered MTBE directly into the gallbladder to dissolve gallstones in sufficient doses to exhale MTBE on their breath with peak blood levels of 40,000 μg/L (11,200 ppm) (Leuschner et al., 1991) were seen to display only mild nausea, discomfort or vomiting in 37/113 (33%) patients. The interspecies uncertainty factor of 3 was chosen as effects observed were similar between all species and Amberg et al. (1999) found similar metabolism and excretion after inhalation of MTBE in both human and rat subjects. An intraspecies uncertainty factor value of 3 was chosen based on MTBE acting as a CNS depressant and several papers on anesthetics (de Jong and Eger, 1975; Gregory et al., 1969) as well as the NRC AEGL SOP (NRC, 2001) describing the CNS depression variability in the human population being no greater than 3 fold. Time-scaling was performed using n=2 for extrapolating to the 10 min., 30 min., and 1 hr. The value was flat-lined at 4 and 8 hours. A metabolism inhalation rat study (Miller et al., 1997) resulted in rats achieving steady state in 2 hours at both 40 and 400 ppm. Also, PBPK modeling data, while not used in the AEGL derivations, also showed steady state of MTBE being achieved in rats in 2 hours at both 500 and 5000 ppm and humans reaching steady state at 4 hours. Both of these data-points thus justify the using 2 hours as the point of departure and holding the value constant at 4 and 8 hrs. The n=2 was derived from ten Berge et al. (1986) in his study on the time mortality response relationship of irritant gases based on an LC_{50} study by Snamprogetti, 1980.

AEGL-3 values were derived from the acute LC_{50} study exposing rats to MTBE vapor for 4 hours (ARCO, 1978). Clinical signs ranging from prostration, hypo-activity, and labored breathing followed by death were recorded. The calculated LC_{50} in this study was 33,427 ppm. From these data, a 4-hour $BMCL_{05}$ value was calculated by a log-probit analysis using U.S. EPA Benchmark Dose Software version 1.3.2. The resulting 4-hour $BMCL_{05}$ of 26,690 ppm was used to derive the AEGL-3 values. Data from a mouse study, Snamprogetti, 1980, used by ten Berge to derive the n = 2 value had very similar values when compared to the ARCO data thus supporting the point-of-departure number. An uncertainty factor of 10 was used based on an inter- and intraspecies factors of 3 each. The interspecies uncertainty factor of 3 was chosen based on the similar data results seen between rats and mice. An intraspecies uncertainty factor value of 3 was chosen based on the variability in CNS depression being no greater than 3 fold in the human population as explained under AEGL-2. Time-scaling was utilized in this derivation including the 10- min. value because of the availability of the Snamprogetti and ARCO data that ranged in time from 3 minutes to 4 hours. The formulation of $C^n \times t = k$ with n=2 was used based on the studies of ten Berge (ten Berge, 1986).

PBPK models for MTBE have been published. These models, however, were not used to develop AEGL values because of limitations in the data available to evaluate the models.

The AEGL values are listed in Table 1.

TABLE 1. Summary of AEGL Values for Methyl t-butyl ether in ppm (mg/m^3)

Classification	10-min	30-min	1-h	4-h	8-h	Endpoint (Reference)
AEGL-1 (Nondisabling)	50 (180)	50 (180)	50 (180)	50 (180)	50 (180)	NOAEL in human exposure at 50 ppm (Nihlén et al., 1998)
AEGL-2 (Disabling)	1400 (5000)	800 (3000)	570 (2000)	400 (1400)	400 (1400)	Ataxia, piloerection and decreased hindlimb strength with no loss of consciousness; NOEL for inability to escape at 4,000 ppm (Daughtrey et al., 1997)
AEGL-3 (Lethality)	**	7500* (27000)	5300* (19000)	2700* (9700)	1900* (6800)	Calculated $BMCL_{05}$ from LC_{50} data (ARCO, 1978)

Lower Explosive Limit (LEL) = 16,000 ppm

* = ≥10% LEL; the 30-min through 8 hr AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of MTBE in air (LEL = 16,000 ppm). Therefore, safety consideration on the hazard of explosion must be taken into account.

** = ≥ 50% LEL; the 10-min AEGL-3 value of 13,000 ppm is higher than 50% of the LEL of MTBE in air (LEL = 16,000 ppm). Therefore, extreme safety considerations on the hazard of explosion must be taken into account.

References

ARCO Chemical Co. 1978. Acute inhalation toxicity study in rats tert-butyl methyl ether (TBME) final report. Hazleton Laboratories, Inc. Vienna, Va. Project No. 2024-127.

Daughtrey W.C., M.W. Gill, I.M. Pritts, J.F. Douglas, J.J. Kneiss and L.S. Andrews. 1997. Neurotoxicological evaluation of methyl tertiary-butyl ether in rats. Journal of Applied Toxicology, Vol. 17 (S1), p. S57-S64.

IARC. 1999. IARC monographs on the evaluation of carcinogenic risks to humans: some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. Volume 73. pp. 339-383.

Leuschner, U., A. Hellstern, K. Schmidt, H. Fischer, S. Guldutuna, K Hubner and M. Leuschner. 1991. Gallstone dissolution with Methyl *tert*-butyl ether in 120 Patients- efficacy and safety. Digestive Diseases and Sciences. Vol. 36, No 2, pp. 193-199.

National Research Council. 2001. Standard operating procedures for developing acute exposure guideline levels for hazardous chemicals. National Academy Press. Washington, D.C.

Nihlén A., A. Löf, G. Johanson and R. Walinder. 1998. Experimental exposure to methyl *tertiary*-butyl ether. Part I. Toxicokinetics in humans and Part II. Acute effects in humans. Toxicology and Applied Pharmacology 148, p. 274 - 287.

Snamprogetti. 1986. MTBE toxicological data book with cover letter. EPA Microfiche No. OTS0000518-0. Document No. FYI-OTS-1086-0518. (from Snamprogetti study in 1980).

ten Berge, W.F., A. Zwart and L.M. Appelman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. Journal of Hazardous Materials, 13. Elsevier Science Publishers. pp. 301-309.

1. INTRODUCTION

Methyl *tertiary*-butyl ether (MTBE) is a volatile synthetic chemical that has been used since the 1980's as a component in gasoline**.** MTBE has octane-enhancing and air pollution-reducing properties. Under ambient conditions, MTBE is a colorless liquid with a characteristic terpene-like odor. MTBE is classified as a flammable (Class 3) liquid under current Department of Transportation regulations.

MTBE is produced by a chemical reaction of methanol and isobutylene. The United States was the largest consumer until 2006 when the Renewable Fuels Standard was enacted. Prior to this, the Clean Air Act amendments of 1990 mandated reformulated gasoline be used to help address air pollution problems. MTBE added to gasoline increased the oxygen level, thus helping to lower the harmful emissions in vehicle exhaust. Typically, MTBE concentrations in gasoline ranged from 2-8% volume with the maximum allowable amount of 15% (Borghoff et al., 1996). Since 2006, most US-based MTBE production has ceased with only a few units remaining on line. MTBE is sold into the export market where it is blended into gasoline in Mexico, South America, Europe and Africa. MTBE has also been used in the medical treatment of gallstones. MTBE can be injected directly into the gallbladder to allow stone dissolution instead of resorting to surgery (Karas and Piel, 2004).

The contamination of drinking water with MTBE has become a concern. MTBE is water-soluble, and binds poorly to soil, thus readily allowing water contamination. The most common source of groundwater contamination is from leaking underground storage tanks. Primary surface water contamination is thought to be primarily from personal water crafts and/or boats (California EPA, 1999).

Properties of MTBE are listed in the Table 2.

TABLE 2. Chemical and Physical Data

Characteristic/Property	Data	Reference
Common Name	Methyl *tertiary*-butyl ether	O' Neil, 2001
Synonyms	MTBE, *tert*-butyl methyl ether	O' Neil, 2001
Cas registry No.	1634-04-4	O' Neil, 2001
Chemical formula	$C_5H_{12}O$	O' Neil, 2001
Molecular weight	88.2	O' Neil, 2001
Physical state	Colorless liquid with characteristic terpene-like odor	Bingham et el., 2001
Vapor pressure	245 mm Hg @ 25°C	O' Neil, 2001
Density (water = 1)	0.7	O' Neil, 2001
Specific gravity	0.74	Bingham et al., 2001
Solubility (in water)	4.8 g/100 ml at 20°C	O' Neil, 2001
Melting point	-109 °C	O' Neil, 2001
Boiling point	55°C	O' Neil, 2001
Flash point	-28°C	O' Neil, 2001
Explosive limits (air, vol%)	LEL - 1.6% UEL - 8.4%	Bingham et al., 2001
Conversion factors	1 mg/m^3 = 0.28 ppm 1ppm= 3.61 mg/m^3	

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

There are no reported episodes of acute lethality in humans from exposure to MTBE. Reviews of MTBE have been published (Borak et al., 1998) about the acute human health effects of MTBE. The signs typically associated with MTBE exposure include headache, eye/nose/throat irritation, coughing, and dizziness.

2.2. Nonlethal Toxicity

Human exposure to MTBE comes primarily through inhalation routes. Multiple investigators have measured the concentration of MTBE in air at areas such as gasoline stations, garages, and trucking areas where MTBE is loaded/unloaded. Estimates of occupational exposure cited in IARC 1999 show geometric means of 2.4 mg/m^3 in short-term (less than 30 minutes) in manufacturing of MTBE to 43 mg/m^3 in transport of undiluted MTBE. In one occupational study, exposures to fuel with MTBE added to a concentration of 12% resulted in MTBE concentrations in the personal breathing zone of attendants of 0.54 ppm (Hartle et al., 1993). Another study had levels of MTBE from15 µg/L for service station attendants to 1.73 µg/L for car-repair shop workers (White et al., 1995). Common symptoms reported after low exposure levels include headaches, coughing, eye irritation, and throat burning (State of Alaska Epidemiology Bulletin, 1993).

2.2.1. Odor Threshold

MTBE is often recognized by its pungent, “terpene-like” odor (Bingham et al, 2001). The odor threshold for MTBE averages 0.89 to 0.13 ppm (0.32 to 0.47 mg/m^3) (ACGIH, 1996). Information is not available to derive a LOA.

2.2.2. Epidemiologic Studies/Occupational Exposures

No epidemiologic studies showing the long-term health effects of MTBE exposure have been conducted; however, several studies reported MTBE occupational exposure levels.

The State of Alaska Epidemiology section collaborated with the National Center of Environmental Health to determine any potential for illness due to exposure to oxygenated fuels in Anchorage, Alaska (State of Alaska Epidemiology Bulletin, 1993). Motor vehicle travelers were given a questionnaire, including 25 taxi drivers, 29 employees at the Anchorage Neighborhood Health Center and 108 employees of a hospital. These surveys took place during the time when gas stations were using the oxyfuel. A similar study had taken place earlier in Fairbanks. In Anchorage, the taxi drivers had a higher proportion of symptoms than the other two groups. Symptoms were mild, of short duration and were primarily headaches, cough, nose/throat burning and eye irritation. These results were similar to those found in the survey in Fairbanks. Concentration levels of MTBE were not reported.

NIOSH conducted a health hazard evaluation (HHE) study for the American Petroleum Institute (API) on gasoline-related exposures to service station attendants and operators

(Hartle et al., 1993). NIOSH chose three different locations to reflect the different uses and concentrations of MTBE in gasoline. Two locations chosen used MTBE as an octane enhancer (blended in at less than 1% of the fuel-blend), two used MTBE as an oxygenate (12-15% of the fuel-blend) and two locations incorporated a vapor recovery system as an engineering control. These were designated as low MTBE, high MTBE and vapor recovery, respectively. Sampling media were attached to the lapels of the attendees to collect samples in the personal breathing zone (PBZ). Only 1/16 samples in the low MTBE group was above the lowest detectable concentration (LDC) at 0.16 ppm. In the high MTBE group, 41 PBZ samples had a mean of 0.54 ppm and the vapor recovery group had 15/48 samples above the lowest detectable concentration with a mean of 0.18 ppm. With the MTBE exposures averaging less than 1 ppm even at stations using the higher MTBE blends, the only acute health hazard appeared to be transient irritative symptoms. The vapor recovery system in place did not diminish MTBE inhalation significantly.

Another NIOSH study was conducted for Exxon at two New Jersey service stations to examine MTBE exposure levels in service station attendants (Cook and Kovein, 1995). The stations chosen contained formulated gasoline with an MTBE content of 15%. These stations also were equipped with vapor recovery systems. Conventional air sampling and video exposure monitoring were utilized for obtaining the results. The video was utilized to record attendants' activities. The combined data were superimposed to show the relationship between the activity performed and the chemical concentration numbers associated with that activity. Twenty-one air samples from the service station attendants' personal breathing zone (PBZ) were evaluated. These samples were used to record total hydrocarbons (THC) and MTBE concentration. At the first station, the mean concentration of MTBE and THC were 0.51 and 2.11 ppm, respectively. Concentrations for the second station were 0.49 and 2.52 ppm, respectively. MTBE concentration was measured by gas chromatography of samples. All were well below the recommended American College of Government Industrial Hygienists (ACGIH) threshold limit value (TLV) of 40 ppm for MTBE and 300 ppm for total hydrocarbons. Brief exposures (1-2 seconds duration) to greater than 300 ppm total hydrocarbons did occur. Video recordings showed these peak exposures occurred primarily during manual refueling. This task took 25% of the total activity time yet accounted for 73.2% of THC exposures greater than 50 ppm. From this, estimations of MTBE levels during the peak exposures were calculated and may have been as high as 70 ppm. As with the other HHE study, vapor recovery systems in place did not diminish the vapors effectively. Suggestions from the study included the simple work practice of engaging the gas pump's automatic refueling lock allowing the attendant to move away from the immediate area during the refueling stage.

2.2.3. Experimental Studies

Results from human experimental studies are summarized below in Table 3.

TABLE 3. Human MTBE Exposure Data

Exposure Concentration (ppm)	No.	Time of Exposure	Parameters Measured	Clinical Signs Observed	Reference
1.39 ppm	19 M 18 F	1 h	- symptoms questionnaire - ocular parameters - neurobehavioral evaluation - nasal lavage	No clinical signs	Prah et al., 1994
1.7 ppm	22 M 21 F	1 h	- symptoms questionnaire - ocular parameters - neurobehavioral evaluation - nasal lavage	No clinical signs	Cain et al., 1996
5, 25 or 50 ppm	10 M	2 h	5 and 25 ppm - symptoms questionnaire and nasal lavage 50 ppm - symptoms questionnaire, nasal lavage and ocular measurements	No clinical signs noted**;** however, initial strong rating to odor upon entering chamber that decreased with time	Nihlén et al., 1998

Nineteen male and eighteen female participants were exposed to 1.39 ppm (5.0 mg/m^3) MTBE and clean air (CA) in a repeated-measures design by inhalation for one hour (Prah et al., 1994). Exposures were separated by at least one week. Mean ages of the participants were 24.7 and 25.4 years for males and females, respectively. In addition, two subjects (one male and one female) participated in a pharmacokinetic study after a 1 hour exposure to MTBE. All subjects were given a thorough physical exam. Prior to exposures, all participants chosen were tested to ensure they could detect the chemical. For the main study, four to six subjects of the same sex were exposed. Exposures took place in a 3000 ft^3 chamber with a temperature of 24°C and humidity of 40%. MTBE concentration was controlled within ± 5% although the method of analysis was not stated. When oxygenated fuel additive use was initiated in Alaska (see study above), many residents started complaining about vague clinical signs such as headache, eye/nose irritation and dizziness. Based on these symptoms, participants in this study were given a questionnaire to fill out asking about the presence and severity of these symptoms pre-exposure, immediately on entry into the chamber, after 30 minutes of exposure and in the last 5 minutes of exposure. A neurobehavioral evaluation system including symbol-digit substitution, switching attention and mood scales were also given to the participants prior to entering the chamber and 45 minutes into the exposure. Some ocular parameters were measured to determine if any ocular irritation was occurring. Tear film breakup time was measured pre- and post-exposure using a keratoscope that projects a white-light pattern of concentric rings onto the cornea and videotapes it. Breakup time is measured as time from a blink to the appearance of discontinuities of the pattern. Hyperemia or eye redness was measured pre- and post-exposure by the use of color slides based on a ratio of the degree of foreground redness to background whiteness. The final ocular parameter measured was impression cytology done on about ½ of the participants. Those participating in cytology were not included in the ocular hyperemia and tear film breakup tests due to the invasive quality of the test. In the cytology test, the number of inflammatory cells (PMNs) were counted as well as assessing the level of mRNA coding for

inflammatory mediators present. This test was conducted pre- and 20 hours post-exposure. Nasal lavage was also utilized to examine for nasal irritation. Lavages were examined for the number of neutrophils (inflammatory cells) and albumin (which acts as a marker of edema based on increased vascular permeability) by an ELISA test and mast-cell degranulation, by a radioimmunoassay (RIA) kit.

In the PK portion of the study, blood samples were obtained pre-exposure and 2, 5, 10, 20, 30, 60, 62, 65, 70, 80, 100, 140, 220, 380, 480 and 580 minutes after the start of the exposure. MTBE and one of its metabolites, tertiary-butyl alcohol (TBA), were analyzed for by the CDC in Atlanta. Based on a previous VOC experiment, Prah selected four questions to be used for confirmatory analysis as a valid test for the main hypothesis. These dealt with headache, nasal irritation, air quality and odor intensity. An analysis of variance (ANOVA) was used to evaluate the data with an alpha level of 0.05 used to indicate statistical significance. ANOVA results of the four questions showed there was no overall effect of MTBE on air quality but other questions indicated a gender effect on reporting air quality. Females reported that the air quality was worse in the MTBE chamber. Females reported a better clean air quality than the males thus providing a bigger difference between the clean air and MTBE concentrations even though the rating of MTBE air quality was about the same between the genders. No statistically significant effect was seen on headache, nasal irritation, or odor intensity with MTBE. The analysis of the neurobehavioral testing also showed no significant effects with MTBE exposure. Neither tear film breakup time nor hyperemia were altered by MTBE exposure and no evidence of ocular inflammation was found. Nasal lavages indicated little nasal inflammation. PK results showed a rapid rise in MTBE in blood to 8.2 and 14.7 ppb in the male and female, respectively. Clearance half-times were 36 minutes in the male and 37 minutes in the female. At the last timepoint, 7 hours post-exposure, blood levels were 0.2 and 0.6 ppb, respectively. TBA concentrations increased more slowly, peaked at 7-10 ppb, and were maintained at this level for up to 7 hours post-exposure. The odor threshold for MTBE was determined to be 0.24 µl/L (0.18 ppm) . In both physical recordings and subjective reporting, there were no effects from MTBE exposure in humans on ocular, nasal, irritative or neurobehavioral parameters.

A similar study exposed healthy adults to MTBE at a concentration of 1.7 ppm (6.1 mg/m^3) in a two-part one hour inhalation study (Cain et al., 1996). In the first part of the study, four individuals participated in a pharmacokinetic study of blood levels. In the second part, forty-three individuals participated in a double-blind study of reactions to exposure to MTBE (1.7 ppm), a mixture of seventeen volatile organic compounds (VOCs)(7.1ppm) and air. All participants in the study were given a full physical exam and were excluded if they did not meet the established criteria. Two males and two females were chosen for the pharmacokinetic study. Forty three individuals were chosen for the second part of the study: twenty-two males (18-32 years) and twenty-one females (18-34). Exposures took place in a 650 ft^3 (18.5 m^3) chamber. Temperature was maintained at 24 ± 0.2 ºC, relative humidity of 40 ± 3% and fresh air rates of 60 ± 2 ft^3/min (28 L/s). Air was also filtered prior to entering the chamber. MTBE was delivered into the chamber by a compressed air cylinder at a concentration of 0.20 mol% (2000 ppm) in nitrogen. A mixture of 16 VOCs was also created by a cylinder containing 3.7% (37,000 ppm)**.** This mixture resembled the mix of components in air samples taken at service stations and included butene-1, isobutylene, and isopentane. Another component of isopropyl mercaptan (IPM) was added to this mixture to allow odor amplification to match the odor of the MTBE used. Concentrations of the MTBE and VOCs were monitored inside and outside the

chamber continually during the exposure by an online HP gas chromatograph with a photoionization detector and hydrocarbon analyzer. Calibrations using standards were performed daily on this equipment, and the average concentration during the experiment was 1.74 ppm ± 3.5% for MTBE and 7.14 ppm ± 5.7% for VOCs.

For the pharmacokinetic study, blood samples of at least 7 ml were obtained. A base-line blood sample was taken 5 minutes prior to entering the chamber. During the 1-hour exposure, blood was obtained at 2, 5, 10, 20, 30 and 60 minutes. Post exposure samples were taken at 2, 5, 10, 20, 40, 60, and 90 minutes. Blood samples were measured for MTBE and its metabolite, tertiary-butyl alcohol (TBA), by gas chromatography/mass spectroscopy.

In the second study, individuals were divided into four groups and either exposed to MTBE-air- VOCs or VOCs-air- MTBE. To study the possibilities of irritants during this study, ocular parameters were measured and nasal lavages performed. Ocular parameters included: duration of tear-film breakup, epithelial damage in the conjunctiva, eye redness and presence of inflammatory cells in the tear fluid. Participants had eye redness (hyperemia), tear-film breakup time and epithelial cell damage evaluated in the left eye only before and after each exposure. Pictures were taken and then judged blindly by five trained judges. Scans of the cornea were performed after fluorescein stain had been applied to look for any spot or lines indicating breaks in the tear film. Epithelial cell turnover was measured after application of sterile 1% lissamine green B dye and the number of blue-green dots on the cornea were counted with a slit-lamp apparatus. Finally, 5 µl samples of tear fluid were collected for cytological assessment. Polymorphonuclear neutrophilic leukocytes (PMNs) indicating inflammation were the parameter being examined. Nasal lavages occurred pre-exposure, immediately post-exposure and 18-24 hours post-exposure. Again, the number of PMNs was counted.

A series of neurobehavioral tests given in the hour before and during the last 15 minutes of exposure was utilized to determine any effects on the participants' motor performance, perception or cognitive function. A series of questionnaires regarding the environmental attributes (odor, temperature, eye irritation, air quality, light etc.) and symptoms experienced (headache, nasal/eye irritation, wheezing, etc) was also given to participants at 10-minute intervals during the exposure.

Results in the PK study showed MTBE concentrations rising steeply from 0.83 ± 0.50 µg/L pre-exposure to 17.1 ± 2.01 µg/L 60 minutes post-exposure. Upon exposure cessation, concentrations dropped immediately, reaching ½ peak at about 40 minutes post-exposure. TBA quantification was more difficult but results showed similar concentrations as MTBE with a slower rate of decline**.** Ocular parameters, in general, showed a tendency for eyes to become more irritated with increased time in the chamber in all exposure groups but this was a nonsignificant tendency. Multivariate analysis of variance (MANOVA) was run on all of these parameters to determine statistical significance. The only parameter statistically significant in the nasal lavages was an increase in the number of PMNs in the delayed nasal lavage when compared to the pre-exposure in the group exposed to VOCs. This did not occur in the MTBE or air exposed groups. CNS function based on the neurobehavioral tests performed showed a latency difference between pre-exposure and the last 15 minutes of exposure in the digit-symbol substitution but it was not based on the component they were exposed to (-25 ± 30, -3 ± 40 and -17 ± 20 ms for air, MTBE and VOCs, respectively). Symptomatic and environmental

questionnaires did not reveal many effects experienced by the participants. Females found odor intensity greater, odor pleasantness worse and overall air quality worse as well as the thermal temperature cooler although there was no increase in symptoms in response to MTBE or VOCs versus air. Results in the PK portion of the study were similar to rat studies as both species exhibited a rapid uptake and elimination of MTBE after inhalation exposure. Overall, the effects of MTBE at these concentrations caused minimal clinical signs.

Another controlled human study exposed ten healthy white male volunteers to 5, 25 or 50 ppm MTBE by inhalation for 2 hours to assess the toxicokinetic and acute effects (Nihlén et al., 1998). During the exposure, light physical exercise (50 W) was performed by the participants on a computer-controlled bicycle ergometer. Each participant was exposed three times with at least two weeks between exposures. Participants were exposed two at a time in a 20 m^3 exposure chamber with a temperature of 20 °C, a relative humidity of 40% and 18-20 air exchanges per hour. The concentration in the chamber was analyzed approximately every five minutes during the exposure using a fourier transform infrared spectrophotometer (FTIR). Average chamber air concentrations of MTBE were 4.8, 24 and 49 ppm.

The following parameters were measured: symptom ratings (by questionnaire); ocular measurements including blinking frequency, eye redness, tear film break-up and conjunctival epithelial damage; and nasal measurements of peak expiratory flow (PEF) and acoustic rhinometry. These parameters were measured in the 50 ppm dose group and since they were all negative for any adverse effects, only the symptom questionnaire and some nasal measurements were performed in the lower dose groups.

There was a dramatic increase in the ratings of solvent smell ($p = 0.0001$) upon entering the chamber and this increased ($p = 0.001$) with higher exposure levels. However, there were not any significant effects on the other questions that included ratings on discomfort in eyes, throat or nose, headaches, difficulty in breathing, nausea, dizziness and fatigue. These questionnaires were administered before exposure, at 4 time-points during exposure and 3 time-points after exposure. No statistically significant effects were noted in any of the ocular measurements, nasal measurements or nasal lavages.

Exhaled air and blood/urine samples were taken. Exhaled air was collected in a mouthpiece attached to an electronic spirometer to measure pulmonary ventilation and to a tube going to a mixing chamber. The air in the mixing chamber then went to either the FTIR spectrophotometer or was absorbed onto a sorbent sample tube. The FTIR measured MTBE in expired air during the exposure and the sample tubes were used after exposure. Pulmonary ventilation was measured before, during and after exposure. Exhaled air samples were collected before, four times during and six times after exposure. Capillary blood samples (200 µL) were taken through finger pricks before, several times during and up to 24 hours post-exposure (48 hours in 50 ppm group). Urine samples were also collected pre-exposure and at time intervals up to 24 hours post-exposure (48 hours in 50 ppm group).

MTBE blood concentrations rose rapidly in all groups and final concentrations at the end of the study were approximately 1.4, 6.5 and 13 µmol/L in the 5, 25 and 50 ppm groups, respectively**.** Toxicokinetic calculations showed the clearance by exhalation is nearly as high as the clearance of metabolism. In the blood, four decay phases were identified with half- lives of: 1

minute, 10 minutes, 1.5 hours and 19 hours. In urine, two half-lives of 20 minutes and 3 hours were found. Levels of TBA, an MTBE metabolite, continued to increase during the exposure and then started to decrease about 6 hours post-exposure. The post-exposure half-life was ten hours in blood and 7-9 hours in urine. Overall, the results were consistent with the Prah et al., (1994) and Cain et al. (1996) studies above and MTBE had little to no effect as measured by sensory irritation.

2.3. Neurotoxicity

Two human exposures by Prah et al., (1994) and Cain et al., (1996) mentioned above administered neurobehavioral tests during exposures to 1.39 and 1.7 ppm MTBE, respectively with no effects observed.

2.4. Developmental/Reproductive Toxicity

No data are available on the developmental and reproductive toxicity of MTBE in humans.

2.5. Genotoxicity

No genotoxicity data for MTBE are available in humans.

2.6. Carcinogenicity

No current data are available on the carcinogenicity of MTBE in humans.

2.7. Summary

Some experimental data are available on MTBE and exposures ranged from 1.39 ppm to 50 ppm (Cain et al., 1996; Prah et al., 1994 and Nihlén et al., 1998). Clinical effects observed in these studies were minimal and were primarily from MTBE's odor. No effects were noted in nasal irritation, ocular irritation or in neurobehavioral changes. Occupational effects were noted in those working most closely with MTBE and some vague side effects of headaches, nasal and eye irritation were observed. All of these signs were observed, however, with MTBE enriched gasoline and not the pure chemical, suggesting either a synergistic effect of MTBE mixed with gasoline, or another component of gasoline creating the effect. Data are not available on genotoxicity, neurotoxicity, developmental/reproductive toxicity and carcinogenicity in humans.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Rats

MTBE (99.1 and 96.2% purity, a.i.) was used in an acute inhalation lethality study exposing ten male Sprague-Dawley rats per concentration for four hours (ARCO Chemical Company, 1978). For the purposes of this document, the results using the purest form of MTBE shall be utilized. Five exposures were chosen: 230.57 mg/L (63,870 ppm), 150.92 mg/L (41,806ppm), 139.37 mg/L (38,607 ppm), 123.04 mg/L (34,083 ppm) and 68.11 mg/L (18,867

ppm). Rats were exposed for four hours, and then observed for 14 days post-exposure prior to sacrifice. Exposures were conducted in a 38 liter glass inhalation chamber and the MTBE was pumped into a needle atomizer. Air was then passed through the atomizer to produce the test vapor. A supplemental airflow was added to the chamber separately to produce a total airflow of 10 L/min. Nominal chamber concentrations were determined from the total weight of the test compound disseminated into the chamber divided by the total airflow through the chamber during generation. Mortality data are provided below in Table 4. Clinical signs noted during exposures included: eye, nose and muzzle irritation, irregular respiration, reduced coordination and prostration. The LC_{50} was calculated to be 120.309 mg/L (33,427 ppm), with lower and upper 95% confidence limits of 104.394 and 138.650 mg/L, respectively.

TABLE 4. Acute Inhalation Data in Rats Exposed to MTBE [1]

Exposure	Dose mg/L (ppm)	No. Dead/ No. Tested	Results
A	230.57[2] (63,870)	10/10	3 min- uncoordinated/barely able to walk 5 min- tachypnea, prostrate 60 min -1st rat died 153 min- all rats dead
B	150.92 (41,806)	9/10	Same results as Exposure A but longer period of time before onset 150 min- 1st rat died 25 min post-exposure- 9th rat died Only survivor had nasal discharge/inactivity 1st 2 days post-exposure
C	139.37 (38,607)	9/10	Same results as Exposure A but longer period of time before onset 150 min- 1st rat died One survivor- no clinical signs reported
D	123.04 (34, 083)	2/10	Upon observation of wet fur- inactivity, reduced coordination, eye irritation and shallow, labored breath 208 min - 1st rat died 43 min post-exposure- 2nd rat died Survivors normal post-exposure
E	68.11 (18,867)	0/10	Slower onset of clinical signs. By end of exposure, rats were prostrate or reduced coordination, labored/heavy respiration Post exposure- Several had nasal area crust for 2 days

[1]Source: ARCO Chemical Co. 1978.
[2]Concentrations are nominal.

3.1.2. Mice

An acute inhalation LC_{50} study with mice was conducted by Snamprogetti (1986). An EPA document dated 1986 was used for the information. In the EPA document, methods were described but no data were included. As this was the study that ten Berge (1986) used for his derivation of n in the time-scaling study, he was contacted, and the following data in Table 5 on mice was received. The EPA document stated that male adult Swiss mice were used in the study with a mean weight of 26 g. Animals were exposed in a cylindrical gas chamber of 20 liters

capacity. The apparatus used for exposure consisted of: (1) an inhalatory chamber consisting of a cylindrical glass container (20 L) provided with an inlet and outlet, (2) a mixing chamber adjoined the inhalation chamber inlet through a glass connector into which atmospheric air mixed with the test substance, (3) a vapor generator of a cylindrical glass container where atmospheric air pumped through an air inlet and bubbled in the liquid and (4) a thermostatic bath in which the vapor generator was placed to attain different temperatures. Concentrations were adjusted by adjusting flow and calculating the amount of substance evaporated inside the vapor generator. Two sets of experiments were run with the first maintaining the MTBE concentration in the inhaled air constant while the exposure time was varied and in the second, varying the MTBE concentration while the exposure time was held constant. In each case, the total flow value was corrected according to the temperature and pressure measured. The dose-percentage lethality was calculated by probit analysis and the Litchfield and Wilcoxon method. No data were presented as to the clinical signs noted during the study.

TABLE 5. Acute Inhalation Data on Mice Exposed to MTBE [1]

Sequence No.	Concentration (mg/m^3)	Min	No. exposed	No. responded
1	738000	3	40	0
2	738000	4	40	8
3	768000	5	40	19
4	811000	6	40	23
5	810000	9	40	32
6	740000	12	40	40
7	303000	10	20	0
8	447000	10	20	3
9	613000	10	20	10
10	735000	10	20	13
11	803000	10	20	15
12	961000	10	20	20

[1]Data received from ten Berg (4-2005) based on study by Snamprogetti, 1986 that was used to derive the n=2 value.

One acute study tested the LC_{50} of mice (only states 'white mice') in an inhalation study (Marsh and Leake, 1950). This study identified the LC_{50} to be approximately 39,000 ppm for a 15 minute study. Twenty- liter, wide-mouth jars were filled with oxygen and plugged. A measured amount of MTBE was then placed in the jar and 4 mice were placed in each jar. The jar was rotated every 30 seconds for 15 minutes. Any mouse that developed respiratory collapse and did not revive was considered dead. Tests were repeated and the amount of vapor that was needed to kill 9 to 11 out 20 mice was considered the LC_{50}.

3.2. Nonlethal Toxicity/Neurotoxicity

Data for all non-lethal toxicity studies are included in Table 6.

TABLE 6. Nonlethal Animal Data for MTBE in Inhalation Studies				
Species	**Exposure Time**	**Conc. (ppm)**	**Effects[1]**	**Reference**
Rat	6 hrs	0, 800, 4000 or 8000	4000 - ataxia, ↑ piloerection, ↓ body temperature and hind grip strength (F) 8000 - ataxia, labored respiration, ↑ leg splay (M), ↓ muscle tone (M), ↓ body temperature (M), ↓ mean motor activity (ALL EFFECTS NOTED AT 1-HR POST-EXPOSURE FOB)	Daughtrey et al., 1997
Rat	6 hrs/d, 5 d/wk for 13 wks	0, 800, 4000 or 8000	8000 - ataxia immediately following exposure for first 4 weeks.	Daughtrey et al., 1997 (same study as Lington et al. below)
Rat	6 hr/d/5 d/wk for 13 wks	0, 800, 4000 or 8000	4000 - hypoactivity (during exposure), increased organ weight in liver, kidneys and adrenals 8000- ataxia (during- entire study and post-exposure- 1st 25 days), ↑ corticosterone levels, SGPT, SGOT,increased organ weight in liver, kidneys and adrenals	Lington et al., 1997
Rat	6 hrs/d for 10 wks	0, 400, 3000 or 8000	3000 - hypoactivity, ataxia, blepharospasms, lack of startle reflex 8000 - hypoactivity, ataxia, blepharospasms, lack of startle reflex (effects seen during exposure)	Bevan et al., 1997b
Rat	6 hrs/d, 5 d/wk for 24 months	0, 400, 3000 or 8000	3000 - blepharospasm, eye twitching, hypoactivity, ataxia, lack of startle reflex, sl ↑ mortality in females from chronic progressive neuropathy. ↑ mortality in males from chronic progressive neuropathy, ↑ renal changes and renal tubular cell tumors (M) 8000 - reversible CNS depression, ↓ body weight and body weight gain, blepharospasm, eye twitching, hypoactivity, ataxia, lack of startle reflex, sl ↑ mortality in females from chronic progressive neuropathy. ↑ mortality in males from chronic progressive neuropathy, ↑ renal changes and renal tubular cell tumors (M)	Bird et al., 1997
Mouse	6 hrs/d for GD 6-15	0, 1000, 4000 or 8000	4000 - hypo-activity, ataxia, 4/26 color change in lungs, fetotoxicity 8000 - hypo-activity; ataxia; prostration; labored respiration; lacrimation; ↓ food consumption, body weight and weight gain; 5/30 color change in lungs; ↓ uterine weight; altered gestational parameters	Bevan et al., 1997a

TABLE 6. Nonlethal Animal Data for MTBE in Inhalation Studies				
Species	**Exposure Time**	**Conc. (ppm)**	**Effects[1]**	**Reference**
Mouse	6 hrs/d, 5 days/wk for 18 months	0, 400, 3000 or 8000	3000 - hypo-activity, blepharospasm, lack of startle reflex, ataxia 8000 - reversible CNS depression, hypo-activity, blepharospasm, lack of startle reflex, ataxia, prostration, ↑ hepatocellular adenoma (F)	Bird et al., 1997
Rabbit	6 hrs/day for GD 6-18	0, 1000, 4000 or 8000	4000 - ↓ food consumption 8000 - ↓ food consumption, hypo-activity, ataxia, ↓ liver weights	Bevan et al., 1997a

[1]If concentration is not listed, this indicates no effects observed

3.2.1. Rats

An acute six hour exposure of 22 male and 22 female Fischer 344 rats to MTBE vapor concentrations of 0 (controls), 800, 4000 or 8000 ppm (0, 2889, 14400 or 28800 mg/m^3, respectively) was conducted (Daughtrey et al., 1997). Exposures took place in four 4 m^3 chambers with approximately 14 air changes per hour. MTBE chamber concentrations were measured every 20 minutes during the exposures. Mean concentrations (± SD) were 8043 ± 194, 3920 ± 129 and 797 ± 23 ppm. Groups of eight male and eight female rats at each concentration were used for behavioral evaluation using a Functional Observational Battery (FOB) at pre-exposure, 1, 6 and 24 hours post-exposure. The remaining 14 of each sex/concentration level were observed for motor activity changes prior to and immediately post-exposure. The FOB was conducted according to EPA guidelines. The motor activity was recorded with an automated photocell recording apparatus. No mortalities were noted in any exposure groups. At one hour post-exposure in the 4000 ppm exposure group, the following clinical signs were noted in the FOB: increased incidence/intensity of ataxia (both sexes), increased piloerection (both sexes), decreased mean rectal temperature (females), and decreased hind grip strength (females). FOB effects seen in the 8000 ppm at 1 hour post-exposure were: altered gait (ataxia) (both sexes), labored respiration (both sexes), increased mean latency to rotate on an inclined screen (females), decreased mean rectal temperature (males), increased hind leg splay (males), decreased muscle tone (males) and decreased mean treadmill duration (males). None of these effects were recorded in the 6 or 24 hour post-exposure time-points. Mean motor activity decreased during the first 10 minutes of the 90-minute test session post-exposure in the 8000 ppm exposed males and females compared to controls. Lower exposure levels showed increased motor activity in the males with no change in the females. Results indicate a transient reversible CNS depression following exposure to MTBE. In this acute study, the NOAEL for CNS effects was 800 ppm.

Daughtrey et al. also conducted a subchronic study by exposing rats to 0 (controls), 800, 4000 or 8000 ppm MTBE for 6 hours/day, 5 days/week for 13 weeks. The same parameters were measured as in the acute study with the addition of necropsy. Ataxia was noted in the high dose rats immediately after exposure for the first 4 weeks. However, no persistent or cumulative effects in CNS function were noted. At termination, body weight and absolute brain weight were decreased in the 8000 ppm group, but there were no differences between groups when brain

weight was expressed relative to body weight. Histopathological changes in the brain were not observed. This study is described in more detail below under Lington et al., 1997.

Twenty five male and 25 female Fischer 344 CDF rats per dose were exposed by inhalation to MTBE vapor at concentrations of 0 (controls), 800, 4000 or 8000 ppm (0, 2888, 14440 or 28880 mg/m^3) 6 hours/day, 5 days/week for 13 weeks (Lington et al., 1997). Rats were exposed in four 4,300 L stainless-steel chambers. Chamber concentrations of MTBE vapor were analyzed approximately every 20 minutes by gas chromatography and measurements were within 5% of the target doses. Animals were monitored individually daily for clinical signs with group observations occurring during exposures. Eyes were examined, body weight monitored, food consumption measured and blood collected for hematology/clinical chemistry. After 13 weeks of exposure, necropsy took place and 10 animals per dose had nervous system evaluations and the remaining 15 had complete necropsies. Statistical methods used were ANOVA, Barlett's homogeneity of variance and Duncan's multiple range tests.

No animals died during the study. High-dose (8000 ppm) males and females exhibited ataxia immediately following daily exposure during the first 25 days. Ataxia and hypoactivity were also noted during exposure in the 8000 ppm group throughout the study. Hypoactivity occurred in the 4000 ppm group during exposure only and was not observed after exposure. No treatment-related ocular effects were recorded. High-dose males and females showed a slight decrease in body weight compared to controls, down 6% and 3% respectively. Hematological findings showed mild effects but no more than 5% change from control. Effects on clinical chemistry results were observed in weeks 5 and 13. The most notable effect was an increased level ($p \leq 0.05$) of corticosterone in high-dose males and females. SGOT and SGPT were decreased in the 8000 ppm dose groups and to a lesser extent in the 4000 ppm group. No treatment-related gross lesions were identified on necropsy; however, organ weights in the liver, kidneys and adrenals were significantly ($p < 0.05$) increased in the rats in the 4000 and 8000 ppm dose groups. This trend was seen more in males. Mild histopathological findings were seen in the male rats but not the females. These included: 11/15 high-dose males having lymphoid hyperplasia in the submandibular lymph node when compared to 0/14 of the controls. Splenic hemosiderosis was also seen in 15/15 of the high-dose males compared to 11/15 of the controls. Finally, an increased size in hyaline droplets was noted in the high-dose males when compared to controls. Based on the findings, the NOAEL for this study was determined to be 800 ppm.

3.3. Developmental/Reproductive Toxicity

Pregnant CD-1 mice (30 per group) and New Zealand White rabbits (15 per group) were exposed in a developmental study to 0 (controls), 1000, 4000 or 8000 ppm MTBE vapor for 6 hours/day during gestation day (GD) 6-15 and 6-18, respectively (Bevan et al., 1997a). Scheduled sacrifices took place on GD 18 for the mice and 29 for the rabbits. Exposures took place in four 4.3 m^3 stainless-steel and glass chambers. Airflow allowed 14 air changes per hour. Liquid MTBE flowed from a piston pump into a heated glass evaporator with temperature maintained at the lowest level creating vaporization. Chamber concentrations were monitored every 20 minutes during the 6-hour exposure and were analyzed by gas chromatography with a flame ionization detector. Nominal concentrations were also calculated daily for each chamber. No pregnant animals died during exposure; however, results in both mice and rabbits showed

maternal toxicity in the 4000 and 8000 ppm groups. In both mice and rabbits, only findings that were significantly different from controls at $p < 0.05$ were reported in the results.

Three mice in the control group and two in the 4000 ppm group delivered early and were removed from the study. Clinical signs in individual dams exposed to 8000 ppm included: hypoactivity, ataxia, prostration, labored respiration, periocular encrustation and lacrimation. Group observations of mice in the 4000 and 8000 ppm groups revealed hypoactivity and ataxia. Mice in the 8000 dose group also had decreased maternal body weight, food consumption and weight gain. Decreased maternal body weight and weight gain were seen in the 4000 ppm concentration dose group but were not statistically significant. The only treatment-related gross pathological changes in dams were color changes in the lungs in 1/27 of controls, 4/26 in the 4000 ppm group and 5/30 in the 8000 ppm group. Uterine weights from the 8000 ppm treated mice were significantly reduced compared to controls, 12.4 ± 5.8 vs. 19.0 ± 5.2 grams, respectively. Fetuses showed reduction in both body weight and skeletal ossification. In the 8000 ppm dose group, gestational parameters affected included: post-implantation loss, altered sex ratio (decreased males) and an increased number of fetal cleft palates. The authors suggested that one possible cause for the increased cleft palate could be an exacerbation of the maternal toxicity manifested as maternal stress. Stress is known to create an increase in endogenous cortisone production which in turn can cause a cleft palate deformity in mice.

In rabbits, maternal body weight reduction during GD 6-18 associated with reduced maternal food consumption was significant ($p < 0.01$) in the 8000 ppm treated rabbits compared to controls, 132.7 ± 103.9 vs. $- 48.2 \pm 120.7$ g, respectively. Food consumption was also significantly reduced in the 4000 ppm treated does but only until GD 10. The only treatment-related clinical signs were hypo-activity and ataxia observed in the 8000 ppm group on six of the thirteen exposures. Post-mortem gross examination showed no significant changes except increased liver weight relative to body weight in the 8000 ppm does. All gestational parameters were equivalent in the does, and no significant fetotoxicity in rabbits was noted. Hence, the NOEL in mice and rabbits for maternal toxicity in this study is 1000 ppm. The developmental toxicity NOEL's are 1000 and 8000 ppm for mouse and rabbit, respectively.

A two-generation reproductive study exposed twenty-five/sex Sprague-Dawley rats to 0 (controls), 400, 3000 or 8000 ppm MTBE vapor by inhalation 6 hours/day for 10 weeks prior to mating (Bevan et al., 1997b). Parental females were exposed during mating, gestation and lactation (from day 5). Parental males were exposed during mating through delivery of their last litter sired. Offspring from these matings were designated as the F_1 generation**.** At weaning, post-natal day (PND) 28, at least one pup of each sex per litter was selected to be included in a pool from which the F_1 adults would be chosen for treatment. The selected 25 neonates per sex per group began exposures on PND 28. The same protocol was followed as with the parental animals. Clinical signs noted in both the parental and F_1 8000 ppm treated animals included hypoactivity, ataxia, blepharospasms and lack of startle reflex. In the 3000 ppm group, hypoactivity**,** blepharospasms and lack of startle reflex were observed. In the pre-mating period, both high-dose males and females exhibited decreased body weight compared to controls. In the F_1 generation, both males and females in the 8000 ppm group had increased liver weight at necropsy but no histopathological findings were identified. No treatment-related effects were noted on reproductive parameters in the study. Both the 3000 and 8000 ppm treated F_1 and F_2 litters exhibited some lower body weights up to PND 28. One possible cause was the maternal

toxicity (CNS depression, low body weight) exhibited by the parental females. Hence, the NOEL for both parental and developmental toxicity is 400 ppm with the reproductive effects' NOEL at least 8000 ppm.

3.4. Genotoxicity

Genotoxicity data for MTBE were primarily negative with only one study showing a weak positive response. Therefore, MTBE is not considered to be genotoxic.

In a bone marrow cytogenetics test, five/sex/dose F-344 rats were exposed by inhalation to MTBE vapor at concentrations of 800, 4000 or 8000 ppm for 6 hours/day for 5 consecutive days (McKee et al., 1997). Target concentrations were measured analytically and were 776 (± 7.5), 4098 (± 34) and 8086 (± 43) ppm. Animals showed clinical signs of reduced weight gain in the 4000 and 8000 ppm groups and ataxia in the 8000 ppm group only. Colchicine was administered prior to sacrifice to produce mitotic arrest and a positive control was also utilized. No statistically significant increases in chromosomal aberrations were recorded in males or females at any dose group. The positive control produced significant numbers of changes. The same authors exposed CD-1 mice to MTBE vapor at 400, 3000 or 8000 ppm for 6 hour/day for 2 days for a bone marrow micronucleus test. No significant increases in micronucleus frequency were found at any concentration. Both studies show MTBE to be non-genotoxic.

While most genotoxicity studies with MTBE have been negative, Williams-Hill et al. (1999) reported that MTBE and TBA were weakly mutagenic when tested with *Salmonella typhimurium* TA102 at 750 μg/plate. A later study ((McGregor et al., 2005), found MTBE and TBA to be non-mutagenic in *Salmonella typhimurium* TA102 and five other strains when tested up to 5000 μg/plate.

3.5. Carcinogenicity

Results from carcinogenicity studies in animals are presented in Table 7. A weak tumorigenic response was reported in one tumor type (kidney) in male rats, for another tumor type (testicular) in male rats and for one tumor type (liver) in female mice. Cruzan et al. (2007) have discussed these tumor types in terms of potential genotoxic and non-genotoxic modes of action. The US EPA has not dervied either a Cancer Slope Factor or Inhalation Risk for MTBE from these data.

TABLE 7. Carcinogenicity Studies with MTBE

Animal Species/Strain	Exposure Route	Concentration	Tumor Type and Incidince Rate	Reference
CD-1 mice	Inhalation	0, 400, 3000 or 8000 ppm	Females- hepatocellular adenomas 2/50- controls 1/50- 400 ppm 2/50- 3000 ppm 10/50- 8000 ppm	Bird et al., 1997
Fischer 344 rats	Inhalation	0, 400, 3000 or 8000 ppm	Males- renal tubular cell tumors 1/50-controls 0/50- 400 ppm 8/50- 3000 ppm 3/50- 8000 ppm	Bird et al., 1997
Sprague-Dawley rats	oral (gavage)	0, 250 or 1000 mg/kg	Males- testicular tumors 34.4% of rats in 1000 7.7% of rats in controls females-leukemias/lymphomas 25.5% rats in 1000 11.8% of rats in 250 3.4% - controls.	Belpoggi et al., 1995

CD-1 mice and Fischer 344 rats (50/species/sex/group) were exposed in an oncogenicity study to 0 (controls), 400, 3000 or 8000 ppm MTBE for 6 hrs/day, 5 days/week for 18 and 24 months, respectively (Bird et al., 1997). Both species exhibited reversible central nervous system depression at the 8000 ppm dose level. Rats displayed this for the first week only and mice throughout the study.

In the mouse study, clinical signs exhibited in both genders at the 3000 or 8000 ppm dose group were prostration (8000 only), blepharospasm or eyelid twitching, hypo-activity, lack of startle reflex, stereotypy (3000 only), and ataxia. Body weight and body weight gain were also decreased in the 8000 dose group. There was an increased mortality rate and decreased mean survival time in the 8000 male mouse dose group and a slight increased frequency of obstructive uropathy. This has been recorded in other studies as a cause of death in this mouse species and urinary bladder tumors were not found at necropsy. Female mice in the 8000 dose group exhibited a statistically significant increase in the number of hepatocellular adenomas compared to the controls. The incidence rate was 10/50 for the 8000 dose group and 2/50 for the controls. An increase in hepatocellular carcinomas were seen in the male mice in the 8000 dose group; however, the increase was not statistically significant, 8/49 compared to 2/49 for controls.

Rats also demonstrated clinical signs in the 3000 and 8000 dose groups including blepharospasm or eye twitching, hypoactivity, ataxia and lack of startle reflex. Swollen periocular tissue was noted also especially in the males. Body weight and weight gain decreased in both sexes in the 8000 ppm group. The 8000 ppm male rat group terminated at week 82 because of high mortality and at that time, absolute body weight and weight gain were decreased. Males also showed an increased mortality in the 3000 ppm dose groups and the study was terminated at week 97. The cause of death was chronic progressive nephropathy. All male

rats showed an exposure-related increase in incidence and severity of renal changes on microscopic examination with a lesser extent noted in the 3000 and 8000 ppm female rats. Renal tubular cell tumors were also noted in male rats. The incidence of these tumors was 1/50 in control group, 0/50 in the 400 ppm group, 8/50 in the 3000 ppm group and 3/50 in the 8000 ppm group. An increased incidence of interstitial cell adenomas was found in male rats in the 3000 and 8000 dose groups. Incidence rates were 41/40 and 47/50 for 3000 and 8000 ppm, respectively compared to 32/50 for the controls. Historical data on aged F-344 rats suggest that this is a common tumor, and the incidence rate found in this study was within the reported frequency.

In an oral lifetime carcinogenicity study, sixty Sprague-Dawley rats/sex/dose were administered 0 (controls), 250 or 1,000 mg/kg body weight MTBE by stomach tube daily for 4 days/week for 104 weeks (Belpoggi et al., 1995). The MTBE was administered mixed in olive oil. The animals were maintained until natural deaths. No treatment-related clinical signs or differences in body weight were reported in any dose group. At necropsy, MTBE treated rats showed an increase ($p = 0.05$) in Leydig interstitial cell tumors of the testes in the high-dose males and a dose-related increase in lymphomas and leukemias in the females. In females, this increase was highly significant ($p < 0.01$) in the high-dose group and less significant in the mid-dose group. In males, 34.4% of the high-dose group had leydig-cell tumors compared to 7.7% of the controls and 25.5% of the females had lymphomas/leukemias compared to 11.8% of mid-dose and 3.4 % of the controls. This determination was based on a large historical database maintained on the labs' colony of rats.

An acute lethality study in rats with MTBE gave an LC_{50} of 120.309 mg/L or 33,427 ppm in a 4 hour inhalation study (ARCO, 1978). Clinical signs noted in the study included: prostration, lacrimation, labored breathing and ocular/nasal discharges. An acute LC_{50} inhalation study in mice was also conducted by Snamprogetti (performed 1980, EPA document 1986). The LC_{50} was 613,000 mg/m^3 (170,000 ppm) for 10 minutes. While both studies did not have analytical concentrations taken in the chamber, the numbers had very similar values. In an acute 6- hour inhalation study exposing rats to 0, 800, 4000 or 8000 ppm MTBE, evidence of transient CNS depression were observed at the 1-hour post-exposure FOB in the rats in the 4000 or 8000 ppm group (Daughtery et al., 1997). Rats had ataxia, piloerection at 4000 ppm and included labored respiration, decreased muscle tone, and decreased motor activity at the 8000 ppm concentration.

On repeat-dose studies, transient CNS sedation was observed in rats, mice and rabbits. Rats displayed the same types of clinical effects noted in the acute studies and included: ataxia, hypo-activity, and lack of startle reflex. Most of the clinical signs were observed at 3000 and 8000 ppm (Bevan et al., 1997b and Bird et al., 1997).

For developmental studies, mice and rabbits were exposed to 0, 1000, 4000 or 8000 ppm MTBE (Bevan et al., 1997a). Mice exhibited some fetotoxicity and altered gestational parameters at 4000 and 8000 ppm as well as the transient CNS effects. Rabbits also exhibited the transient CNS effects at these same concentrations but no fetal effects were observed.

MTBE did not display any genotoxic effects but did result in some carcinogenicity in mice and rats. Mice were observed to have more hepatocellular adenomas in females when

exposed to 8000 ppm MTBE via inhalation (Bird et al., 1997). Male rats exposed to 3000 and 8000 ppm MTBE by inhalation were also observed to have an increased incidence of renal tubular cell tumors (Bird et al., 1997). Finally, rats exposed by oral route to 1000 mg/kg had an increased incidence of testicular tumors in males and leukemias/lymphomas in females (Belpoggi et al., 1995).

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Many studies exist on the metabolism and deposition of MTBE. After inhalation exposure to MTBE, it can be exhaled or initially oxidized to TBA and formaldehyde by human liver microsomal enzymes; the most important thought to be CYP2A6 (McGregor, 2006). TBA can be further metabolized to 2-methyl-1,2 propanediol and 2-hydroxyisobutyrate. A small conglomeration of studies is included with emphasis on those studies involving humans.

Male and female human volunteers (3 of each) and male and female F344 NH rats (5 of each) were exposed by inhalation to 4 or 40 ppm MTBE for four hours in the same chamber (Amberg et al., 1999). All human subjects were required to refrain from fueling their vehicles for two days prior to and during the sample collection period to avoid any incidental MTBE exposure. The inhalation chamber had a 8 m^3 capacity and 28 m^3/hr air flow rate. MTBE chamber concentrations were taken at different sampling ports every 15 minutes to ensure steady concentration rates were maintained. At the end of the exposures, all urine excreted was collected for 72 hours at 6-hour intervals to quantify MTBE and MTBE metabolites including TBA, 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate. Blood samples of 10 mL in humans and 100 µL for rats were also obtained from subjects pre-exposure and every two hours for 12 hours and at 24 hours post-exposure to quantify MTBE and TBA. Pre-exposure urine samples in rats and humans contained small levels of TBA, 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate, but these levels greatly increased after the inhalation exposure with 2-hydroxyisobutyrate being the most prevalent. Results for metabolism and excretion of MTBE in the rats followed a course similar to that of humans, but MTBE was cleared more rapidly from rat blood compared to human blood. MTBE blood concentrations after the 4 hour exposure were 5.9 ± 1.8 µM in rats and 6.7 ± 1.6 µM in humans for the 40 ppm dose group, and 2.3 ± 1.0 µM in rats and 1.9 ± 0.4 µM in humans for the 4 ppm dose group. MTBE was rapidly eliminated from the blood with a half-life of 2.6 ± 0.3 hours and 0.5 ± 0.2 hours in humans and rats, respectively. MTBE was rapidly absorbed by inhalation in humans and cleared from the blood by exhalation of the parent compound or urinary excretion of its metabolites with 35-69% of the inhaled dose recovered as urine metabolites. Elimination half-lives for the urinary metabolites of MTBE were between 7.8- 17.0 hours in humans and 2.9-5.0 hours in rats. No gender differences were seen. A similar oral study was performed by the same investigators and dosed six human volunteers (3 male and 3 female) with 5 or 15 mg ^{13}C-MTBE in tap water (Amberg et al., 2001). Data obtained in this study revealed MTBE metabolism and excretion during ingestion similar to that demonstrated in inhalation. Evidence also indicated a lack of first-pass metabolism of MTBE in the liver. This study supports the conclusion that rats are a good model for MTBE toxicity because metabolism is similar in humans and rats.

A study using human liver cells determined that MTBE is metabolized to *tert*-butyl alcohol (TBA) (Hong et al., 2001). Correlation between ether-metabolizing activities and

cytochrome P450 (CYP) enzymes was found especially with CYP2A6. Liver samples from liver cancer patients (n=8) and a HepatoScreen kit derived from liver organ donors (n=15) were utilized in this study. The liver cells from the cancer patients and from the kit all exhibited metabolism of MTBE into TBA. All of these also identified CYP2A6 as one of the enzymes involved. The other prominent isoenzyme was CYP2E1. Further evidence that CYP enzymes are involved was proven when carbon monoxide was added to the liver cells and the MTBE was not metabolized to TBA. Carbon monoxide is a known inhibitor of CYP enzymes.

In another inhalation pharmacokinetic study, fourteen male volunteers were exposed to 3.1 ppm MTBE for one hour (Prah et al., 2004). Upon acceptance into the study, all volunteers were given a medical exam including urine and blood sampling. The subjects were asked to avoid any contact with gasoline prior to and during the study. Inhalation exposure took place in a body plethysmograph with a volume of 1.75 m^3. The chamber's air supply was HEPA-filtered room air flowing at a rate of 0.566 m^3/min. MTBE concentration in the chamber was continuously monitored to achieve a concentration of 3.0 ppm with the mean MTBE chamber level during the exposure being 3.1 ppm. Ten milliliter blood samples were obtained at the following schedule: baseline, 5, 15, 30, 45, 60, 65, 75, 90, 120, 180, 240, 360 and 1440 minutes after the start of the exposure. Exhaled breath samples were also obtained at these same time-points. Little to no MTBE was identified in the pre-exposure blood sample; however, during the study, MTBE blood levels increased rapidly and declined to baseline within 24 hours. MTBE levels peaked at 0.28 μmol/L at the end of the exposure. In contrast, most subjects had measurable tert-butyl alcohol (TBA) in their baseline blood sample (0.0 to 3.0 ppb). TBA increased more slowly to a plateau (240 minutes) and maintained at this level for about six hours. At the twenty-four hour blood sample, TBA levels were still above baseline. Exhaled breath was obtained on seven of the 14 volunteers. Little or no MTBE was found in the exhaled air baseline levels but a slightly elevated amount of TBA was identified. The study showed 47.2% of the total inhaled dose of MTBE was exhaled by the participants. The same participants were also given MTBE through oral and dermal routes. The oral route demonstrated a significantly greater amount of MTBE metabolized into TBA than other routes showing a first-pass metabolism. MTBE levels peaked at 65 minutes and 15 minutes in the dermal and oral exposures, respectively.

Pharmacokinetics and disposition of MTBE and ^{14}C-MTBE in Fisher-344 rats were investigated in a nose-only inhalation study (Miller et al., 1997). Additional routes of exposure examined included intravenous, oral, and dermal. In the study, 52 rats/sex/dose group were exposed nose-only for six hours to 400 ppm (1400 mg/m^3) or 8000 ppm (29000 mg/m^3) MTBE. The MTBE concentration in the dose solutions was evaluated analytically by a gas chromatograph fitted with an automatic liquid sampler and flame ionization detector. Mean chamber concentrations in the single exposure studies were 408 ± 26.8 and 8038 ± 460.6 ppm for male rats, and 407 ± 38.1 and 8250 ± 525.1 ppm for females. Repeat exposures (6 hrs/day x 15 days) at 400 ppm (mean air concentrations of 416 ± 20.4 ppm) were also conducted in 40 rats of each sex. Control rats were exposed to dry compressed chamber air. Sacrifices occurred at 10, 20, and 40 minutes or 1, 2, 3, 4, 6, 6.5, 7, 9, 13 and 25 hours after the start of inhalation in the single exposure. In the repeat exposure, sacrifices were on day 15 at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 18 hours after the last 6 hour exposure. Blood, expired air, urine and feces were collected and quantified for content for up to seven days post-exposure. Results showed that the MTBE was rapidly absorbed and metabolized after inhalation. Elimination from the blood occurred

quickly by exhalation and/or metabolism to *tert*-butyl alcohol (TBA). ^{14}C-MTBE disposition studies exposed six rats of each sex/dose group to 400 or 8000 ppm six hours for one day with mean chamber concentrations of 402 ± 19.7 or 7901 ± 206.3 ppm. Repeat daily exposures for 6 hrs/day to 400 ppm ^{14}C-MTBE for 15 days had a mean concentration of 407 ± 12.0 ppm. All rats were killed 48 hours post-exposure.

In both studies, the pharmacokinetics and disposition of MTBE were similar in males and females in all routes of administration so only the data on the males were reported. Plasma samples of MTBE and TBA were analyzed by gas chromatography. Plasma concentrations increased rapidly after inhalation to a steady-state within two hours with both the low and high concentrations. MTBE was metabolized to TBA within six hours after inhalation. This metabolism occurred faster (1-4 hrs) in the other administered routes. A greater than proportional rise in MTBE concentration occurred in the high concentration (8000 ppm) dose group with a less than expected TBA increase suggesting a saturation of MTBE metabolism at higher doses. MTBE plasma elimination half-life was 0.5 hrs with the half-life of TBA being 1.8 hrs in the repeat inhalation exposure, 3.3 hrs in the low concentration and 3.4 hrs in the high concentration study. Disposition of ^{14}C in the urine was similar in both the low concentration dose group and the repeated exposure dose group, 64.7% and 71.6 % , respectively. Four metabolites were identified in the urine. The most prevalent, α- hydroxyisobutyric accounted for 70% of the total radioactivity excreted in urine. The second, accounting for 14%, was methylpropane-1,2-diol. The other two metabolites were unidentified. In comparison, the high dose showed a statistically significant increase in radioactivity found in exhaled air instead of urine. MTBE is rapidly cleared the from the blood through exhalation or by metabolism to TBA; almost all of the ^{14}C-MTBE was recovered in expired air and urine within 48 hours after exposure (86-98%). In expired air, 78-83% of the radioactivity was eliminated during the first 0-3 hours

An inhalation study in rats exposed male F344/N and F344/Crl BN rats by nose-only for 4 hours to 4, 40 or 400 ppm ^{14}C- MTBE. Single and repeat exposures to 20 or 200 ppm of light fraction gasoline (LFG) were also performed (Benson et al., 2001). In the single LFG exposure, rats were exposed for four hours to 20 or 200 ppm LFG containing 4 or 40 ppm ^{14}C-MTBE, respectively. Repeat exposures were for 4 hrs/day to 20 or 200 ppm LFG containing 4 or 40 ppm MTBE, respectively for seven consecutive days. On the eighth day, the LFG mixture contained ^{14}C-MTBE. Respiratory movements were measured on some of the rats (n=5) including frequency and tidal volume to estimate the amount of vapors inhaled. Five rats were used after a four hour exposure to collect urine and feces. These rats also were used in measuring radioactive VOCs, MTBE, TBA and $^{14}CO_2$ in exhaled air. Samples were collected for 72 hours post-exposure prior to euthanasia. Blood and tissues were collected at sacrifice. Tissues examined to look for the radiolabled MTBE were liver, kidney, lungs, heart, brain, perirenal fat and gonads. An additional thirty-three rats were sacrificed at the following time-points (3 at each time): 0.5, 1, 2 and 4 hrs of exposure; 2, 4, 8, 12, 27, 48 and 72 hr after exposure. The same tissues listed above were examined to look for the radiolabled MTBE.

In the MTBE exposure, the group mean minute volume was significantly greater in rats exposed to the 400 ppm MTBE compared to those receiving 4 or 40 ppm. The addition of LFG did not change the minute volume in the one time or repeat exposures. Immediately and 72 hours post exposure, the liver was identified as the organ containing the most MTBE. In all cases,

MTBE and/or its metabolites were excreted primarily in urine with smaller amounts excreted in exhaled air and feces. Most elimination of MTBE through urine occurred 36 to 48 hours after exposure. Exhaled air showed most of the MTBE excreted within the first 12 hours after exposure. The study demonstrated that the uptake of MTBE between 4 and 400 ppm was not linear suggesting a saturation in uptake may occur as demonstrated by an increase in MTBE in the exhaled air rather than the urine excretion in the 400 ppm MTBE dose group. The study showed that uptake after co-exposure with LFG was similar in comparison between 4 ppm MTBE and 20 ppm LFG but the MTBE uptake was much less in the 200 ppm LFG compared to the 40 ppm MTBE alone. This study did not examine individual MTBE metabolites.

Several PBPK models for MTBE have been published (Blancato et al., 2007; Borghoff et al., 1996; Rao and Gingsberg, 1997). These models, however, were not used to develop AEGL values primarily due to uncertainty in the blood:air partition coefficient for MTBE in humans, a key parameter for estimating retained dose and the lack of data for model validation in humans at exposures greater than 50 ppm.

4.2. Mechanism of Toxicity

Toxicity is most evident as transient CNS depression in animals. MTBE is metabolized by oxidative demethylation to tert-butyl alcohol (TBA); however, the underlying mechanisms that initiate cellular alterations by MTBE and its metabolites are unknown (Williams-Hill et al., 1999).

4.3. Other Relevant Information

4.3.1. Species Variability

Several inhalation studies have demonstrated that absorption and metabolism of MTBE in rats and humans are similar. Amberg 1999, found that uptake of MTBE was very similar in both rats and humans but rats cleared the chemical slightly faster. PBPK modeling data reported humans exercising at 50 W will have 1.5 to 2.5 more MTBE concentration in their blood than the rats. This was seen at both 500 and 5000 ppm. There were no significant differences in MTBE toxicity between animal species. Rats, mice and rabbits all displayed some transient effects of CNS depression such as hypo-activity and ataxia at similar concentrations.

4.3.2. Susceptible Populations

Little information is available on toxicity of MTBE in children or susceptible populations. Most reported age-dependent susceptibilities on effects of solvents or vapors are less than threefold on the order of magnitude in human population (Bruckner and Warren 2001). One study was conducted with persons self-reported as sensitive to MTBE. Subjects were exposed to either 15% MTBE or 11% MTBE but it was mixed with gasoline and was not administered alone (Fiedler et al., 2000) making this study unsuitable for interpretation of reactions to only MTBE. Therefore, there is still speculation as to effects to MTBE in sensitive populations being psychological or definitive.

4.3.3. Concentration-Exposure Duration Relationship

The concentration-exposure time relationship for many irritant and systemically-acting vapors and gases can be described by the relationship $c^n \times t = k$, where the exponent, n, ranges from 0.8 to 3.5 (ten Berge et al., 1986). MTBE inhalation data were subjected to probit analysis in ten Berge's studies and the exponent, n, for the endpoint of lethality for MTBE was 2.

However, for nonlethal effects, i.e. CNS depression, the extent of the effect is dependent on the concentration of the parent compound in the brain.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Nihlén et al. (1998) conducted a study in humans exposing male volunteers to 50 ppm MTBE vapor during light exercise. Questionnaires were administered during the study about chamber conditions and any clinical signs noted. Physical parameters, including ocular and nasal changes, were monitored. All parameters exhibited few to no effects from the MTBE vapor. There was an increased rating to the initial odor on entering the chamber but this diminished with time. Based on the lack of notable discomfort or irritation at this concentration, 50 ppm will be utilized for AEGL-1 value determination.

5.2. Summary of Animal Data Relevant to AEGL-1

In animal studies, both 400 ppm in subchronic studies (Daughtery et al., 1997 and Bevan et al., 1997 b) and 800 ppm in an acute study (Daughtery et al., 1997) were NOAELs for CNS depression in rats indicating higher concentrations than the 50 ppm chosen from human exposure studies with no effects.

5.3 Derivation of AEGL-1

The 50 ppm concentration was the highest dose tested in humans resulting in no notable discomfort or irritation. An uncertainty factor of 1 was applied as this was a human study and no effects were noted besides the odor. Also, other animal studies with concentrations of 400 ppm resulted in no signs of toxicity. Extrapolation to other time-points was not performed as no effects were observed at 50 ppm and sensory effects are usually concentration, rather than time, dependent. Values are listed in Table 8.

TABLE 8. AEGL-1 Values for MTBE

10-min	30-min	1-h	4-h	8-h
50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)

Both 400 and 800 ppm were NOAELs in rat studies, subchronic and acute, respectively. Using the 800 ppm concentration and dividing by a uncertainty factor of 10 (3 each for inter- and intra-species) results in a value of 80 ppm making the 50 ppm concentration a conservative number.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Adequate human data were not available for the determination of AEGL-2; however, supporting data were available. The value chosen for AEGL-1, 50 ppm, is the highest concentration tested in humans by inhalation. Medical dissolution of gallstones in humans with MTBE has been used and studied. One publication followed 113 patients that were administered MTBE directly into the gallbladder and were found to have blood concentrations up to 11,000 ppm (Leuschner et al., 1991) with minimal side effects of nausea, discomfort and vomiting. MTBE blood concentrations were tested by gas chromatography.

6.2. Summary of Animal Data Relevant to AEGL-2

The neurotoxicity study exposing twenty-two M/F rats to 4000 ppm MTBE vapor for 6 hours will be utilized for AEGL-2 derivation (Daughtrey et al., 1997). No mortalities occurred in this study, and no clinical signs were observed during exposure. At the 1 hour post-exposure functional observational battery (FOB), clinical signs noted included ataxia, increased piloerection, and decreased hind- limb grip strength (F only). Males also exhibited decreased motor activity at this dose. All of these clinical signs recorded were absent during the 6 hour and 24 hour post-exposure FOB. These results support a transient reversible CNS depression following MTBE exposure in an acute study.

A developmental study in mice exposing them to 4000 ppm MTBE for approximately 9 days also recorded clinical signs of hypoactivity and ataxia with no mortalities (Bevan et al., 1997 a). Rabbits exposed to this concentration did not exhibit any clinical signs. A genotoxicity study (McKee et al., 1997) exposed rats to by inhalation to 4000 or 8000 ppm MTBE for 6 hrs/day for five consecutive days and confirmed concentrations with analytical results. Rats exhibited decreased body weight in both the 4000 and 8000 ppm group and ataxia in the 8000 ppm group only.

6.3. Derivation of AEGL-2

The 4000 ppm concentration shall be utilized in AEGL-2 value derivation. This concentration showed reversible, transient CNS depression in an acute inhalation rat study. An uncertainty factor of 10 will be used. The interspecies uncertainty factor of 3 was chosen as effects observed were similar between all species and Amberg et al. (1999) found similar metabolism and excretion after inhalation of MTBE in both human and rat subjects. An intraspecies uncertainty factor of 3 was chosen based on MTBE acting as a CNS depressant and several papers on anesthesia (de Jong and Eger, 1975; Gregory et al., 1969) as well as the NRC AEGL SOP (NRC, 2001) describing the CNS depression variability in the human population being no greater than 3 fold. Time-scaling was performed using n=2 for extrapolating to the 10 min, 30 minutes, and 1 hour. The value was held constant for 4 and 8 hours. Miller et al. (1997) reported a steady state of 2 hours in a rat inhalation study with 40 and 400 ppm. PBPK modeling data, while not used in the AEGL derivations, also showed steady state of MTBE being achieved in 2 hours at 500 and 5000 ppm and 4 hours in humans. The n=2 was derived from ten Berge et al. (1986) in his study on the time mortality response relationship of irritant gases. Values are listed in Table 9.

TABLE 9. AEGL-2 Values for MTBE				
10-min	**30-min**	**1-h**	**4-h**	**8-h**
1400 ppm (5000 mg/m^3)	800 ppm (3000 mg/m^3)	570 ppm (2000 mg/m^3)	400 ppm (1400 mg/m^3)	400 ppm (1400 mg/m^3)

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Adequate human data were not available for the determination of AEGL-3. The value derived for AEGL-1, 50 ppm, is the highest concentration tested in humans.

7.2. Summary of Animal Data Relevant to AEGL-3

Bench-mark calculation by a log-probit analysis was performed on the data from an acute inhalation LC_{50} study in rats (ARCO, 1978) to derive AEGL-3 values. Clinical signs observed included prostration, labored/heavy breathing and some nasal crusting in some rats post-exposure. The calculated LC_{50} from this study was 33, 427 ppm. Data from an acute inhalation lethality mouse study by Snamprogetti provided for by Dr. ten Berge resulted in very similar values to the ARCO data.

7.3. Derivation of AEGL-3

The ARCO study (1978) presented the LC_{50} data for the rat in an acute, four-hour inhalation study. From these data, a 4-hour $BMCL_{05}$ value was calculated by a log-probit analysis using U.S. EPA Benchmark Dose Software version 1.3.2. The resulting 4-hour-$BMCL_{05}$ of 26,690 ppm was used to derive the AEGL-3 values. A total uncertainty factor of 10 was applied. Data from a mouse study, Snamprogetti, 1986, used by ten Berge to derive the n = 2 value had very similar values when compared to the ARCO data, thus supporting the point-of-departure number. An uncertainty factor of 10 was used based on an inter- and intraspecies factors of 3. The interspecies uncertainty factor of 3 was chosen based on the similar data results seen between rats and mice when the data sets for the ARCO study and Snamporgetti study were compared. An intraspecies uncertainty factor value of 3 was chosen based on the variability of effect seen with CNS depression being no greater than 3 fold in the human population as explained under AEGL-2. Time-scaling was utilized in this derivation since exposure duration data from the two studies ranged from 3 minutes to 4 hours. The formulation of $C^n \times t = k$ with n=2 was used based on the studies of ten Berge (ten Berge, 1986). Values calculated for AEGL-3 are listed in Table 10.

TABLE 10. AEGL-3 Values for MTBE				
10-min	**30-min**	**1-h**	**4-h**	**8-h**
**	7500* ppm (27000 mg/m^3)	5300* ppm (19000 mg/m^3)	2700* ppm (9700 mg/m^3)	1900* ppm (6800 mg/m^3)

Lower Explosive Limit (LEL) = 16,000 ppm
* = ≥10% LEL; the 30-min through 8 hr AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of MTBE in air (LEL = 16,000 ppm). Therefore, safety considerations on the hazard of explosion must be taken into account.
** = ≥50% LEL; the 10-min AEGL-3 value of 13,000 ppm is higher than 50% of the LEL of MTBE in air (LEL = 16,000 ppm). Therefore, extreme safety considerations on the hazard of explosion must be taken into account.

8 SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity Endpoints

AEGL-1 is based on the highest concentration tested in humans that did not result in any clinical signs, 50 ppm. AEGL-2 is based on the endpoint of transient CNS depression that was reversible in rats and AEGL-3 is based on a $BMCL_{05}$ calculation from an acute lethality study in rats. All derived AEGL values are listed in Table 11.

TABLE 11. Summary of AEGL Values for MTBE					
Classification	**10-min**	**30-min**	**1-h**	**4-h**	**8-h**
AEGL-1 (Nondisabling)	50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)	50 ppm (180 mg/m^3)
AEGL-2 (Disabling)	1400 ppm (5000 mg/m^3)	800 ppm (3000 mg/m^3)	570 ppm (2000 mg/m^3)	400 ppm (1400 mg/m^3)	400 ppm (1400 mg/m^3)
AEGL-3 (Lethality)	**	7500* ppm (27000 mg/m^3)	5300* ppm (19000 mg/m^3)	2700* ppm (9700 mg/m^3)	1900* ppm (6800 mg/m^3)

Lower Explosive Limit (LEL) = 16,000 ppm
*= ≥10% LEL; the 30-min through 8 hr AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of MTBE in air (LEL = 16,000 ppm). Therefore, safety considerations on the hazard of explosion must be taken into account.
** = ≥50% LEL; the 10-min AEGL-3 value of 13,000 ppm is higher than 50% of the LEL of MTBE in air (LEL = 16,000 ppm). Therefore, extreme safety considerations on the hazard of explosion must be taken into account.

8.2. Comparisons with Other Standards and Guidelines

Standards and guidance levels for the workplace are summarized in Table 12.

TABLE 12. Extant Standards and Guidelines for MTBE

Guideline	Exposure Duration				
	10 min	30 min	1 h	4 h	8 h
AEGL-1	50 ppm	50 ppm	50 ppm	50 ppm	50 ppm
AEGL-2	1400 ppm	800 ppm	570 ppm	400 ppm	400 ppm
AEGL-3	**	7500* ppm	5300* ppm	2700* ppm	1900* ppm
ERPG[a]	MTBE is currently under consideration/review by the AIHA/ERP committee				
TLV-TWA (ACGIH)[b]					50 ppm (reproductive/ kidney)
LLV (Dutch)[c]					50 ppm
MAK (German)[d]					50 ppm
STV (United Kingdom)[e] (15-min)	60 ppm				
LLV (Sweden)[f]					30 ppm

Lower Explosive Limit (LEL) = 16,000 ppm
* = ≥ 10% LEL; the 30-min through 8 hr AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of MTBE in air (LEL = 16,000 ppm). Therefore, safety considerations on the hazard of explosion must be taken into account.
** = ≥ 50% LEL; the 10-min AEGL-3 value of 13,000 ppm is higher than 50% of the LEL of MTBE in air (LEL = 16,000 ppm). Therefore, extreme safety considerations on the hazard of explosion must be taken into account.

[a] **ERPG (Emergency Response Planning Guide) AIHA Handbook.** (2007). Establishes the emergency response planning guidelines and workplace environmental exposure levels.

[b] **ACGIH (American Conference of Governmental Industrial Hygienists)** (ACGIH 2007) **Threshold Limit Value - Time Weighted Average** is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[c] **National MAC list (Dutch).** 2000. The Hague. SDU Uitgevers (under the auspices of the Ministry of Social Affairs and Employment.) The Netherlands. p. 35

[d] **MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration])** (Deutsche Forschungsgemeinschaft [German Research Association] 2007) List of MAK and BAT values. Is defined analogous to the ACGIH-TLV-TWA.

[e] **STV (Short-Term Value)** Swedish Occupational Exposure Limits. 2000. By Ordinance of the Swedish National Board of Occupational Safety and Health, Adopted 28th August, 1996. Defined as a recommended value consisting of a time-weighted average for exposure during a reference period of 15 minutes.

[f]**LLV (Level Limit Value)** Swedish Occupational Exposure Limits. 2005. By Ordinance of the Swedish National Board of Occupational Safety and Health, Adopted October, 2005. Defined analogous to the ACGIH-TLV-TWA.

8.3. Data Adequacy and Research Needs

The available data for MTBE appear to be adequate and complete. Occupational data available at this time are mostly based on MTBE enhanced gasoline; however, adequate inhalation studies to pure MTBE exists. No obvious data deficiencies were identified.

9. REFERENCES

ACGIH (American College of Governmental Industrial Hygienists). 2007. TLVs and BEIs- Threshold limit values for chemical substances and physical agents & biological exposure indices. p. 40.

ACGIH. (American College of Governmental Industrial Hygienists). 1996. Supplements to the 6th edition- Documentation of the threshold limit values and biological exposure indices. Cincinnati, Ohio. p. 1

AIHA (American Industrial Hygiene Association) 2007. Emergency response planning guidelines and workplace environmental exposure level guides. Fairfax, Va. p. 26.

Amberg A., E. Rosner and W. Dekant. 1999. Biotransformation and kinetics of excretion of methyl-*tert*-butyl ether in rats and humans. Toxicological Sciences 51, p. 1-8.

Amberg A., E. Rosner and W. Dekant. 2001. Toxicokinetics of methyl *tert*- butyl ether and its metabolites in humans after oral exposure. Toxicological Sciences 61, p. 62-67.

ARCO Chemical Co. 1978. Acute inhalation toxicity study in rats tert-butyl methyl ether (TBME) final report. Hazleton Laboratories, Inc. Vienna, Va. Project No. 2024-127.

Belpoggi F., M. Soffritti and C. Maltoni. 1995. Methyl-tertiary- butyl ether (MTBE)- a gasoline additive-causes testicular and lympho-haematopoietic cancers in rats. Toxicology and Industrial Health, Vol. 11, No. 2, p. 119-149.

Benson J., E. Barr and J. Krone. 2001. MTBE inhaled alone and in combination with gasoline vapor: uptake, distribution, metabolism and excretion in rats. Health Effects Institute Research Report 102. p. 73-94.

Bevan C., R.W. Tyl, T.L. Neeper-Bradley, L.C. Fisher, R.D. Panson, J.F. Douglas, L.S. Andrews. 1997(a). Developmental toxicity evaluation of methyl *tertiary*-butyl ether (MTBE) by inhalation in mice and rabbits. Journal of Applied Toxicology, Vol. 17(S1), p. S21-S29.

Bevan C., T.L. Neeper-Bradley, R.W. Tyl, L.C. Fisher, R.D. Panson, J.J. Kneiss and L.S. Andrews. 1997(b). Two-generation reproductive toxicity study of methyl tertiary-butyl (MTBE) in rats. Journal of Applied Toxicology, Vol. 17 (S1), p. S13- S19.

Bingham E., B. Cohrssen and C. Powell. 2001. Patty's Toxicology. 5th Edition. John Wiley and Sons, N.Y. p. 885-895.

Bird M.G., H.D. Burleigh-Flayer, J.S. Chun, J.F. Douglas, J.J. Kneiss and L.S. Andrews. 1997. Oncogenicity studies of inhaled methyl *tertiary*-butyl ether (MTBE) in CD-1 mice and F-344 rats. Journal of Applied Toxicology, Vol. 17(7), p. S45-S55.

Blancato, J.N., M.V. Evans, F.W. Power and J.C. Caldwell. 2007. Development and use of PBPK modeling and the impact of metabolism on variability in dose metrics for the risk assessment of methyl tertiary butyl ether (MTBE). J Environ Protect Sci 1:29-51.

Borak, J. H. Pastides and M. Van Ert. 1998. Exposure to MTBE and acute human health effects. Article quoted in Casarett and Doull's Toxicolgy. 6th Edition.McGraw-Hill Co, Inc. New York, New York. p. 901.

Borghoff, S.J., J.E. Murphy and M.A. Medinsky. 1996. Development of a physiologically based pharmacokinetic model for methyl tertiary-butyl ether and tertiary butanol in male Fisher 344 rats. Fundam Appl Toxicol 30:264-275.

Borghoff S.J., J.S. Prescott-Matthews and T.S. Poet. 1996. The mechanism of male rat kidney tumors induced by methyl *tert*-butyl ether and its relevance in assessing human risk. CIIT Activitites: Chemical Industry Institute of Toxicology. Vol. 16, No. 10, p 1-8.

Bruckner, J.V. and D.A. Warren. 2001. Toxic effects of solvents and vapors. Casarett & Doull's Toxicology. 6th Edition. McGraw-Hill Co., Inc. New York, New York. pp. 869-916.

Cain W., B. Leaderer, G. Ginsberg, L. Andrews, J.E. Cometto-Muniz, J.F. Gent, M. Buck, L.G. Berglund, V. Mohsenin, E. Monahan and S. Kjaergaard. 1996. Acute exposure to low-level methyl tertiary-butyl ether (MTBE): human reactions and pharmacokinetic response. Inhalation Toxicology, 8:21-48.

California Environmental Protection Agency. 1999. Office of Environmental Health Hazard Assessment. Public health goal for methyl tertiary butyl ether (MTBE) in drinking water.

Cook C. and R. Kovein. 1995. NIOSH: Health hazard evaluation report- 94-0220-2526. Exxon Company, Houston, Texas.

Cruzan, G., S. J. Borghoff, A. de Peyster, G.C. Hard, M. McClain, D. B. McGregor and M. G. Thomas. 2007. Methyl *tertiary*-butyl ether mode of action for cancer endpoints in rodents. Regulatory Toxicology and Pharmacology 47:156-165.

Daughtrey W.C., M.W. Gill, I.M. Pritts, J.F. Douglas, J.J. Kneiss and L.S. Andrews. (1997). Neurotoxicological evaluation of methyl tertiary-butyl ether in rats. Journal of Applied Toxicology, Vol. 17 (S1), p. S57-S64.

De Jong, R. H. and E.I. Eger. 1975. AD_{50} and AD_{95} values of common inhalation anesthetics in man. Anesthesiology 42: 384-389.

Deutsche Forschungsgemeinschaft. 2002. List of MAK and BAT values. Report No. 38. Wiley-VCH. p. 33.

Fiedler, Nancy, Kathie Kelly-McNeil, Sandra Mohr, Paul Lehrer, Richard Opiekun, ChiaWei Lee, Tom Wainman, Robert Hamer, Clifford Weisel, Robert Edelberg and Paul J. Lioy. 2000. Controlled human exposure to Methyl Tertiary Butyl Ether in gasoline: symptoms, psychophysiologic and neurobehavioral responses of self-reported sensitive persons. Environmental Health Perspectives. Vol. 108, No. 8. p. 753-763.

Gregory, G.A., E.I. Eger, and E.S. Munson. 1969. The relationship between age and halothane requirement in man. Anesthesiology 30:488-491.

Hartle R.W., J. Kelly, N.C. Burton and C.Cook.**.** 1993. NIOSH: Health hazard evaluation report 88-304-2326. American Petroleum Institute, Washington, D.C.

Hong, J., Y. Wang, S. Mohr, F. Bondoc and C. Deng. 2001. Human cytochrome P450 isozymes in metabolism and health effects of gasoline ethers. Health Effects Institute Research Report No. 102. pp. 95-109.

IARC. 1999. IARC monographs on the evaluation of carcinogenic risks to humans: some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. Volume 73. pp. 339-383.

Karas, L. and W.J. Piel. 2004. Ethers. *Kirk-Othmer Encyclopedia of Chemical Toxicology.* John Wiley & Sons, Inc. Article on-line posting date: March 29, 2004.

Le Gal, A., Y. Dréano, P.G. Gervasi and F. Berthou. 2001. Human cytochrome P450 2A6 is the major enzyme involved in the metabolism of three alkoxyethers used as oxyfuels. Toxicology Letters, 124. pp. 47-58.

Leuschner, U.**,** A. Hellstern, K. Schmidt, H. Fischer, S. Guldutuna, K Hubner and M. Leuschner. 1991. Gallstone dissolution with Methyl *tert*-butyl ether in 120 Patients- efficacy and safety. Digestive Diseases and Sciences. Vol. 36, No 2, pp. 193-199.

Lington A. W., D.E. Dodd, S.A. Ridlon, J.F. Douglas, J.J. Kneiss and L.S. Andrews. 1997. Evaluation of 13-week inhalation toxicity study on methyl *t*-butyl ether (MTBE) in Fischer 344 rats. Journal of Applied Toxicology, Volume 17 (S1), p. S37-S44.

Marsh, D.F. and C.D. Leake. 1950. The comparative anesthetic activity of the aliphatic ethers. Anaesthesiology 11. pp. 455- 463.

McGregor, D. 2006. Methyl *tertiary*-butyl ether: studies for potential human health hazards. Critical Review in Toxicology 36:319-358.

McGregor, D.B., G. Cruzan, R.D. Callander, K. May and M. Banton. 2005. The mutagenicity testing of tertiary-butyl alcohol, tertiary-butyl acetate and methyl tertiary-butyl ether in *Salmonella typhimurium.* Mutat. Res 565: 181-189.

McKee R.H., J.S. Vergnes, J.B. Galvin, J.F. Douglas, J.J. Kneiss and L.S. Andrews. 1997. Assessment of the *in vivo* mutagenic potential of methyl tertiary-butyl ether. Journal of Applied Toxicology, Vol. 17 (S1), p. S31-S36.

Miller M.J., E.S. Ferdinandi, M. Klan, L.S. Andrews, J.F. Douglas and J.J. Kneiss. 1997. Pharmacokinetics and disposition of methyl *t*-butyl ether in Fischer-344 rats. Journal of Applied Toxicology, Vol. 17 (S1). p. S3-S12.

National MAC list**.** 2000. The Hague. SDU Uitgevers (under the auspices of the Ministry of Social Affairs and Employment.) The Netherlands. p. 35

Nihlén A., A. Löf, G. Johanson and R. Walinder. 1998. Experimental exposure to methyl *tertiary*-butyl ether. Part I. Toxicokinetics in humans and Part II. Acute effects in humans. Toxicology and Applied Pharmacology 148, p. 274 - 287.

O' Neil M., A. Smith and P. Heckelman. 2001 Merck Index. 13th edition. Merck and Co., N.J. p. 1078.

NRC (National Research Council). 2001. Standard operating procedures for developing acute exposure guideline levels for hazardous chemicals. National Academy Press. Washington, D.C.

Prah J., D. Ashley, B. Blount, M. Case, T. Leavens, J. Pleil and Frederick Cardinali. 2004. Dermal, oral, and inhalation pharmacokinetics of methyl tertiary butyl ether (MTBE) in human volunteers. Toxicological Sciences 77, p. 195-205.

Prah J., G.M. Goldstein, R. Devlin, D. Otto, D. Ashley, D. House, K.L. Cohen and T. Gerrity. 1994. Sensory, symptomatic, inflammatory, and ocular responses to and the metabolism of methyl tertiary butyl ether in a controlled human exposure experiment. Inhalation Toxicology. Vol. 6: pp. 521-538.

Rao, H.V. and G.L. Ginsberg. 1997. A physiologically based pharmacokinetic model assessment of methyl t-butyl ether in groundwater for a bathing and showering determination. Risk Anal 17:583-598.

State of Alaska Epidemiology Bulletin. 1993. Potential illness due to exposure to oxygenated fuel. Bulletin No. 1. Anchorage, Alaska.

Swedish National Board of Occupational Safety and Health. 2005. Occupational exposure limit values and measures against air contaminants.

Snamprogetti. 1986. MTBE toxicological data book with cover letter. EPA Microfiche No. OTS0000518-0. Document No. FYI-OTS-1086-0518. (from Snamprogetti study in 1980).

ten Berge, W.F., A. Zwart and L.M. Appelman. 1986. Concentration-time mortality response relationship of irritant and systemically acting vapours and gases. Journal of Hazardous Materials, 13. Elsevier Science Publishers. pp. 301-309.

U.S. EPA. 1988. Fuels and fuel additives; waiver decision. Notice. Federal Register, 44:33846-33847. Washington D.C.: U.S. Environmental Protection Agency.

White, M.C., C.A. Johnson, D.L. Ashley, T.M. Buchta and D.J. Pelletier. 1995. Exposure to Methyl *tertiary*- butyl ether from oxygenated gasoline in Stamford, Connecticut. Archives of Environmental Health. May/June 1995 [Vol. 50 (No. 3)].

Williams-Hill, D., C.P. Spears, S. Prakash, G.A. Olah, T. Shamma, T. Moin, L.Y. Kim and C.K. Hill. 1999. Mutagenicity studies of methyl-*tert*-butyl ether using the Ames tester strain TA102. Mutation Research 446, p. 15-21.

APPENDIX A: Derivation of AEGL Values for MTBE

DERVIATION OF AEGL-1 VALUES

Key Study:	Nihlén et al., 1998
Toxicity Endpoint:	No significant clinical signs noted
Scaling:	No extrapolation performed as no effects noted and sensory effects usually concentration dependent, not time.
Uncertainty factors:	Intraspecies =1
10-min. AEGL-1:	10- min AEGL-1 = 50/1 = 50 ppm
30-min. AEGL-1:	30-min AEGL-1 = 50/1 = 50 ppm
1-h AEGL-1:	1 h AEGL-1 = 50/1 = 50 ppm
4-h AEGL-1:	4 h AEGL-1 = 50/1 = 50 ppm
8-h AEGL-1:	8 h AEGL-1 = 50/1 = 50 ppm

DERIVATION OF AEGL-2 VALUES

Key Study:	Daughtrey et al., 1997
Toxicity Endpoint:	Ataxia, decreased hind limb strength, piloerection (all transient)
Scaling:	$C^n \times t = k$ n = 2 (based on ten Berge et al., 1986) $(4000)^2 \times 2 = 3.2 \times 10^7$ ppm • h
Uncertainty Factors:	Intraspecies: 3 Interspecies: 3 Total UF = 10
10-min AEGL-2:	$C^2 \times 0.167$ h = 3.2×10^7 ppm • h $C^2 = 1.92 \times 10^8$ C = 13842 10- min AEGL-2 = 14000/10 = 1400 ppm
30-min AEGL-2:	$C^2 \times 0.5$ h = 3.2×10^7 ppm • h $C^2 = 6.40 \times 10^7$ C = 8000 30- min AEGL-2 = 8000/10 = 800 ppm
1-h AEGL-2:	$C^2 \times 1.0$ hr = 3.2×10^7 ppm • h $C^2 = 3.2 \times 10^7$ C = 5657 1 h AEGL-2 = 5700/10 = 570 ppm
4-h AEGL-2:	4 h AEGL-2 = 4000/10 = 400 ppm
8-h AEGL-2:	8 h AEGL-2 = 4000/10 = 400 ppm

DERIVATION OF AEGL-3 VALUES

Key Study: ARCO 1978

Toxicity Endpoints: A 4-h acute rat study provided data for lethality. From these data, a 4-hour $BMCL_{05}$ was calculated by log-probit analysis. The 4-h $BMCL_{05}$ of 26, 690 ppm was used.

Scaling: $C^n \times t = k$, $n = 2$ (based on ten Berge et al., 1986)
$(26{,}690 \text{ ppm})^2 \times 4 \text{ h} = 2.85 \times 10^9 \text{ ppm h}$

Uncertainty factors: 3 = interspecies variability; 3 = intraspecies variablity
Total UF = 10

10-min AEGL-3: $C^2 \times 0.167 \text{ h} = 2.85 \times 10^9 \text{ ppm} \cdot \text{h}$
$C^2 = 1.7 \times 10^{10}$ ppm
C = 130600 ppp
10 min AEGL-3 = 130600/10 = 13,000** ppm

30-min AEGL-3: $C^2 \times 0.5 \text{ h} = 2.85 \times 10^9 \text{ ppm} \cdot \text{h}$
$C^2 = 5.7 \times 10^9$ ppm
C = 75500 ppm
30-min AEGL-3 = 75500/10 = 7500* ppm

1-hr AEGL-3: $C^2 \times 1 \text{ h} = 2.85 \times 10^9 \text{ ppm} \cdot \text{h}$
$C^2 = 2.85 \times 10^9$ ppm
C = 53400 ppm
1 h. AEGL-3 = 53400/10 = 5300* ppm

4-hr AEGL-3: $C^2 \times 4 \text{ h} = 2.85 \times 10^9 \text{ ppm} \cdot \text{h}$
$C^2 = 7.13 \times 10^8$ ppm
C = 26700 ppm
4 h. AEGL-3 = 26700/10 = 2700* ppm

8-hr AEGL-3: $C^2 \times 8 \text{ h} = 2.85 \times 10^9 \text{ ppm} \cdot \text{h}$
$C^2 = 3.56 \times 10^8$ ppm
C = 18900 ppm
8 h. AEGL-3 = 18900/10 = 1900* ppm

*= ≥ 10% LEL; the 30-min through 8 h AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of MTBE in air (LEL = 16,000 ppm). Therefore, safety considerations on the hazard of explosion must be taken into account.

** = ≥50% LEL; the 10-min AEGL-3 value of 13,000 ppm is higher than 50% of the LEL of MTBE in air (LEL = 16,000 ppm). Therefore, extreme safety considerations on the hazard of explosion must be taken into account.

APPENDIX B: Derivation Summary for MTBE AEGLs

ACUTE EXPOSURE GUIDELINE LEVELS FOR METHYL *tertiary*-BUTYL ETHER (CAS Reg. No. 1634-04-4) DERIVATION SUMMARY

AEGL-1 VALUES				
10-min	**30-min**	**1-h**	**4-h**	**8-h**
50 ppm	**50 ppm**	**50 ppm**	**50 ppm**	**50 ppm**

Key Reference: Nihlén A., A. Löf, G. Johanson and R. Walinder. 1998. Experimental exposure to methyl *tertiary*-butyl ether. Part I. Toxicokinetics in humans and Part II. Acute effects in humans. Toxicology and Applied Pharmacology 148, p. 274 - 287.

Test Species/Strain/Number/Sex: 10 male humans

Exposure Route/Concentrations/Durations: Inhalation/5, 25 or 50 ppm/2 hours

Effects: None noted

Endpoint/Concentration/Rationale: NOAEL for sensory irritation- 50 ppm

Uncertainty Factors/Rationale: Intraspecies of 1 since much higher concentrations in rats showed no effects and this was a human study**.**

Modifying Factor: N/A

Animal to Human Dosimetric Adjustment: N/A

Time Scaling: No extrapolation performed as no effects noted and sensory effects are usually concentration, rather than time dependent.

Data Adequacy: Inhalation studies with MTBE are adequate to use in deriving AEGL values.

1

AEGL-2 VALUES				
10-min	**30-min**	**1-h**	**4-h**	**8-h**
1400 ppm	**800 ppm**	**570 ppm**	**400 ppm**	**400 ppm**
Key Reference: Daughtrey W.C., M.W. Gill, I.M. Pritts, J.F. Douglas, J.J. Kneiss and L.S. Andrews. (1997). Neurotoxicological evaluation of methyl tertiary-butyl ether in rats. Journal of Applied Toxicology, Vol. 17 (S1), p. S57-S64.				
Te**st Species/Strain/Number/Sex:** Rat/ Fischer 344/ 22/M/F				
Exposure Route/Concentrations/Durations: Inhalation/ 0, 800, 4000 or 8000 ppm/ 6 hours				
Effects: 0 and 800 ppm- No effects observed 4000 ppm- ataxia, ↑piloerection, ↓ body temperature, ↓hind grip strength (f) 8000 ppm- ataxia, labored respiration, ↑leg splay (M), ↓ muscle tone (M), ↓ body temperature (M), ↓ mean motor activity (ALL EFFECTS NOTED AT 1-H POST-EXPOSURE FOB)				
Endpoint/Concentration/Rationale: Transient and reversible CNS signs at 4000 ppm				
Uncertainty Factors/Rationale: interspecies: 3- The interspecies uncertainty factor of 3 was chosen as effects observed were similar between all species and Amberg et al. (1999) found similar metabolism and excretion after inhalation of MTBE in both human and rat subjects. intraspecies: 3- The uncertainty factor of 3 was chosen based on MTBE acting as a CNS depressant and several papers on anesthesia (de Jong and Eger, 1975; Gregory et al., 1969) as well as the NRC AEGL SOP (NRC, 2001) describing the CNS depression variability in the human population being no greater than 3 fold. Total UF- 10				
Modif**ying Factor:** N/A				
Animal to Human Dosimetric Adjustment: N/A				
Time Scaling: Extrapolation over time was performed on the 10-min, 30-min, and 1 hr values. Extrapolation was utilized for time-points using the equation $C^n \times t = k$, with n = 2 based on ten Berge et al., 1986. Values were held constant for the 4 and 8 hour time-points due to the steady-state achieved by 2 hours in the rat.				
Data Adequacy: Data is adequate in this 6 hour inhalation study to derive AEGL-2 values. No mortalities occurred. Other subchronic studies also showed some of these similar effects at the 4000 ppm level.				

2

1

AEGL-3 VALUES				
10-min	**30-min**	**1-h**	**4-h**	**8-h**
**	7500* ppm	5300* ppm	2700* ppm	1900* ppm

Key Reference: ARCO Chemical Co. 1978. Acute inhalation toxicity study in rats tert-butyl methyl ether (TBME) final report. Hazleton Laboratories, Inc. Vienna, Va. Project No. 2024-127.

Test Species/Strain/Number/Sex: Rat/ Sprague-Dawley/ 10/ M

Exposure Route/Concentrations/Durations: Inhalation/ 18867, 34083, 38607, 41806 or 63870 ppm/ 4 hours

Endpoint/Concentration/Rationale: A four-hour $BMCL_{05}$ value was calculated by a log-probit analysis. The resulting $BMCL_{05}$ of 26,690 was used to derive the AEGL-3 values.

Effects: 33,427 ppm: 4-hour LC_{50}

Uncertainty Factors/Rationale:

interspecies: 3- The interspecies uncertainty factor of 3 was chosen based on the similar data results seen between rats and mice when the data sets for the ARCO study and Snamporgetti study were compared.

intraspecies: 3- The uncertainty factor of 3 was chosen based on MTBE acting as a CNS depressant and several papers on anesthesia (de Jong and Eger, 1975; Gregory et al., 1969) as well as the NRC AEGL SOP (NRC, 2001) describing the CNS depression variability in the human population being no greater than 3 fold.

Total UF- 10

Modifying Factor: N/A

Animal to Human Dosimetric Adjustment: N/A

Time Scaling: Extrapolation was utilized for time-points using the equation $C^n \times t = k$, with n = 2 based on ten Berge et al., 1986. A 10-minute value was also time-scaled due to the availability of data ranging from 3 minutes to 4 hours.

Data Adequacy: This was the acute lethality study in rats (ARCO, 1978). The concentration of 50 ppm was the highest concentration tested in humans.

Lower Explosive Limit (LEL) = 16,000 ppm

* = ≥10% LEL; the 30-min through 8 hr AEGL-3 values are higher than 1/10 of the lower explosive limit (LEL) of MTBE in air (LEL = 16,000 ppm). Therefore, safety considerations on the hazard of explosion must be taken into account.

** = ≥50% LEL; the 10-min AEGL-3 value of 13,000 ppm is higher than 50% of the LEL of MTBE in air (LEL = 16,000 ppm). Therefore, extreme safety considerations on the hazard of explosion must be taken into account.

2

APPENDIX C: Benchmark Calculations

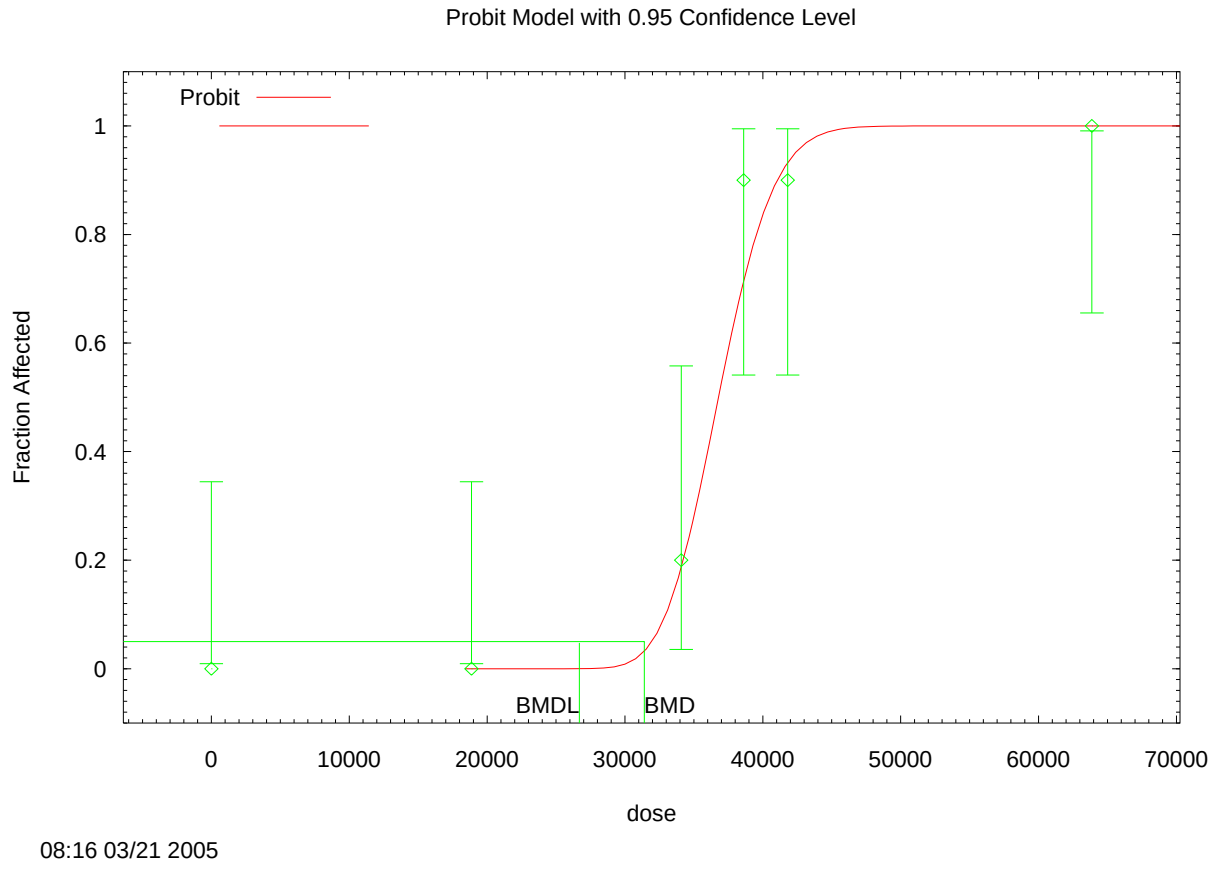
Benchmark Calculations

The benchmark calculations are based on the study by ARCO, 1978 using a series of six concentrations in rats to determine a 4-hour LC_{50}. For the derivation of AEGL-3, a $BMCL_{05}$ of 26,690 ppm, derived with the Log-Probit model, was used.

$BMCL_{05}$ = 26,690 ppm- value used in calculations
BMC_{05} = 31,400 ppm
BMC_{01} = 29,627 ppm

Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
Input Data File: C:\BMDS\DATA\MTBE-4HR.(d)

Figure 1. Probit Model with 0.95 Confidence Level



Mon Mar 21 08:16:56 2005

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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 = Mortality
Independent variable = Conc.
Slope parameter is not restricted

Total number of observations = 6
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 = -31.6337
slope = 3.04272

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.
Background	0	NA
Intercept	-123.024	37.2862
Slope	11.7223	3.54278

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)**	**Deviance**	**Test DF**	**P-value**
full	-11.5057			
fitted	-12.3403	1.66918	4	0.7963
reduced	-41.5888	60.1663	5	<.001

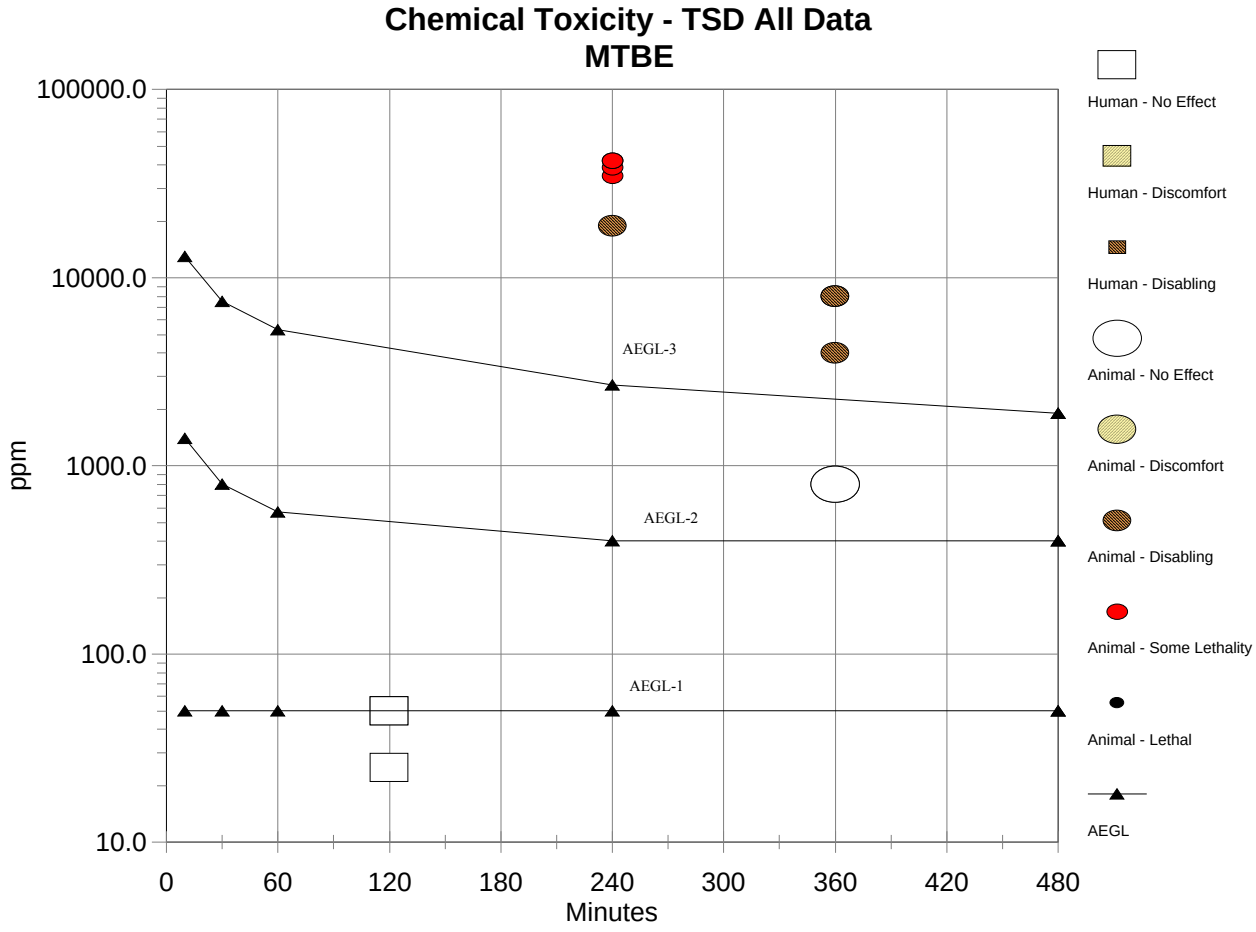
AIC: 28.6805

Goodness of Fit					
Dose	**Est. Prob.**	**Expected**	**Observed**	**Size**	**Scaled Residual**
0.0000	0.0000	0.000	0	10	0
18867.0000	0.0000	0.000	0	10	-3.613e-007
34083.0000	0.2470	2.470	2	10	-0.3447
38607.0000	0.7814	7.814	9	10	0.9072
41806.0000	0.9564	9.564	9	10	-0.8732
63870.0000	1.0000	10.000	10	10	1.1e-005
Chi-square = 1.70		DF = 4		P-value = 0.7900	

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk

Confidence level = 0.95
BMD = 31400.5
BMDL = 26690.3

APPENDIX D: Time-Scaling Category Plot for MTBE



No effect= No effect or mild discomfort
Discomfort= Notable transient discomfort/irritation
Disabling= Irreversible/long lasting effects or impaired ability to escape
Some lethality= Some, but not all, exposed animals died
Lethal= All exposed animals died