EVIDENCE ON DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF METHYL TERTIARY-BUTYL ETHER

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DRAFT

September, 1998

PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of reproductive toxicity are identified as the members of the Developmental and Reproductive Toxicant (DART) Identification Committee of OEHHA's Science Advisory Board (22 CCR 12301).

The Local Drinking Water Protection Act of 1997 (SB1189 and AB592) requires that the OEHHA Science Advisory Board make recommendations on or before January 1, 1999 as to whether methyl tertiary butyl ether (MTBE) should be listed under Proposition 65. A public request for information relevant to the reproductive toxicity assessment was announced in the *California Regulatory Notice Register* on December 5, 1997.

This draft document *Evidence on Developmental and Reproductive Toxicity of Methyl Tertiary-Butyl Ether* was developed to provide the DART Identification Committee with relevant information for use in its deliberations. The document reviews the available scientific evidence on the reproductive toxicity potential of methyl tertiary-butyl ether. A public meeting of the Committee to discuss this evidence is scheduled for December 1998. The exact meeting date will be published in the *California Regulatory Notice Register*. Written public comment on the document should be submitted to OEHHA by November 24, 1998, in order to be considered by the Committee in advance of the meeting. During the December meeting, the public will have an opportunity to present verbal comments to the Committee.

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A. ABSTRACT

Methyl tertiary-butyl ether (MTBE) is a volatile compound used as a gasoline additive. Risk assessment activities for MTBE are currently being conducted in California due principally to concerns about MTBE contamination of drinking water sources. The acute toxic effects of MTBE include CNS depression and chronic effects include increased incidence of tumors in animal studies.

This document addresses potential developmental and reproductive hazards of MTBE. There are no human studies directly relevant to MTBE developmental and reproductive toxicity. There are 2 sets of animal reproductive and developmental toxicity studies conducted by the inhalation route of exposure using standard regulatory testing guidelines. The maximum inhalation concentration was 2500 ppm for the first set of studies and 8000 ppm for the second set of studies.

The first set of studies, using the lower concentration range, did not report MTBE effects on development. A possible exception to this lack of findings was the report of increased early postnatal death in a rat one-generation study. The second set of studies, using the higher concentration range, found dose-dependent effects on fetal weight and incidence of delayed ossification in mice after exposure at 4000 and 8000 ppm from gestation day 6 to 15. Other effects (intra-uterine death, cleft palate) occurred only at 8000 ppm. Developmental toxicity was not reported in a similar rabbit study; maternal clinical observations indicated that rabbits were less sensitive to MTBE than mice. A rat two-generation reproductive toxicity study described MTBE effects on postnatal growth and survival of offspring at 3000 and 8000 ppm exposures.

No effects on male or female fertility were reported in 2 available rat reproductive toxicity studies, a one-generation study with a maximum exposure of 2500 ppm and a two-generation study with a maximum exposure of 8000 ppm. In addition to these reproductive toxicity studies, there are a few recently published and ongoing studies of potential endocrine effects of MTBE. These studies reported decreased uterine and ovarian weights, altered uterine histology and longer estrous cycles in mice exposed by inhalation to 8000 ppm MTBE. Lower doses were not evaluated. Several possible mechanisms of action of MTBE in producing these effects have been considered, but none has been confirmed experimentally. Reduction in circulating testosterone after MTBE exposure was recently reported in an abstract. Subchronic and chronic toxicity studies in rats using other routes, doses and durations of exposure have not reported effects on ovary or testis weights or histopathology.

MTBE is well absorbed and rapidly eliminated. Animal studies describe wide distribution to tissues. A primary metabolite of MTBE is tertiary butyl alcohol, which is more slowly eliminated than MTBE. It is not known whether the parent compound and/or the metabolites are responsible for MTBE toxic effects. Tertiary butyl alcohol has been reported to produce developmental retardation when administered by inhalation during gestation.

B. INTRODUCTION

B.1 CHEMICAL STRUCTURE AND PHYSICAL PROPERTIES

Methyl tertiary-butyl ether (MTBE) (CAS No. 1634-04-4) is an alkyl ether with the formula $C_5H_{12}O$ and a molecular weight of 88.15. The structure is shown in Figure 1. It is a clear, colorless liquid with high volatility (vapor pressure 245 mm Hg at 25 ^oC). Explosion limits of 1.65 to 8.4% in air indicate a high explosiveness, and it is also highly flammable. It has a pungent, terpene-like odor (ATSDR 1996). The conversion factor for MTBE in air is 1 ppm = 3.57 mg/m^3. MTBE is moderately soluble in water (4.8 g MTBE/100 g water), highly soluble in alcohols and ethers, and is blended into gasoline.

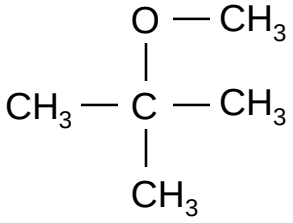

Figure 1. Structure of MTBE.

B.2 REGULATORY HISTORY[i]

The U.S. EPA has not established drinking water standards for MTBE, but included MTBE on the Drinking Water Contaminant Candidate List published in the Federal Register on March 2, 1998 (U.S. EPA 1998). An advisory released by U.S. EPA in December 1997 recommended that MTBE concentrations in the range of 20 to 40 ppb or below would assure both consumer acceptance of the water and a large margin of exposure safety from any toxic effects (U.S. EPA 1997a). Recently, Cal/EPA recommended a drinking water Public Health Goal of 14 ppb for MTBE based on carcinogenicity (OEHHA 1998).

The federal Occupational Health and Safety Administration (OSHA) established a TLV-TWA for workplace exposure of 40 ppm (144 mg/m^3) in 1994. U.S. Environmental Protection Agency (EPA) has established a reference concentration (RfC) for air contaminant levels of 3 mg/m^3 (U.S. EPA 1997c). MTBE is included in the federal Superfund Amendments and Reauthorization Act (SARA) Title III Extremely Hazardous Substances list. It is also included in the Hazardous Air Pollutant list of the federal Clean Air Act Amendments (CAAA) and is thus a Toxic Air Contaminant in California.

Regulatory concern about MTBE has led to several recent reviews of potential MTBE health effects (NSTC 1996, 1997; HEI 1996; ATSDR 1996, NRC 1996).

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[i] Information in this section is adapted from *Public Health Goal for Methyl Tertiary-Butyl Ether (MTBE) in Drinking Water* (OEHHA, 1998)

B.3 CALIFORNIA USE AND EXPOSURE INFORMATION[ii]

MTBE is a synthetic solvent used almost exclusively as an oxygenate in unleaded gasoline to improve combustion efficiency. Reformulated gasoline with MTBE has been used nationwide to meet the 1990 Federal Clean Air Act Amendments requirements for reducing carbon monoxide and ozone. About 40% of the U.S. population live in areas where MTBE is included in oxyfuel or reformulated gasoline. Federal and State law required the exclusive sale of reformulated gasoline in California beginning in 1996. Currently, MTBE is added at 11% volume to virtually all of the gasoline used in California.

Due to its use as a fuel additive, MTBE is a high volume production chemical. It was the second most-produced chemical in the U.S. in 1997. California produced 181 million of the 2.9 billion gallons of MTBE estimated to be produced in the U.S. in 1997. In addition, MTBE is imported for use in California. MTBE can be produced in connection with oil refining.

MTBE is present in ambient air in California. Potential sources of MTBE in ambient air are manufacture and distribution of oxyfuel and reformulated gasoline, vehicle refueling, and evaporative and tailpipe emissions from motor vehicles. Monitoring for MTBE was initiated by the California Air Resources Board (CARB) in 1996. Preliminary data suggest a statewide 24-h average of approximately 2 parts per billion volume (ppbv) with higher concentration in the South Coast of about 4 ppbv. These values are similar to limited data available from other states.

MTBE has become a drinking water contaminant in California because of its high water solubility and persistence in groundwater. Potential sources of drinking water contamination are leaking underground storage tanks, recreational power-boating and refinery wastewater. MTBE has been detected in groundwater in connection with leaking underground storage tanks by water quality management authorities in Santa Clara, Orange County, Solano County and San Francisco County among others. MTBE can reach concentrations of 20 ppm in groundwater near the source of the fuel release. MTBE has been detected in lakes and reservoirs, with concentrations higher in reservoirs that allow use of gasoline-powered boats. Affected lakes include Lakes Tahoe, Shasta, Merced, and Havasu, and Clear, Donner, and Canyon Lakes. Beginning in 1997, monitoring of drinking water sources for MTBE was instituted by the California Department of Health Services. As of December 1997, MTBE was detected in 33 of 2500 sources monitored. This included sources for 18 of 516 water systems monitored. Three of these sources reported concentration in excess of the U.S. EPA Hazard Advisory level of 20-40 ppb. Wells have been shut down in Santa Monica, Santa Clara County and the Lake Tahoe Public Utilities District due to MTBE contamination.

[ii] Information in this section is adapted from *Public Health Goal for Methyl Tertiary-Butyl Ether (MTBE) in Drinking Water* (OEHHA, 1998)

B.4 PHARMACOKINETICS

B.4.1 Overview

The pharmacokinetics of MTBE have been recently reviewed (ATSDR 1996; NSTC 1997; US EPA 1997a; OEHHA 1998). Available information is mainly limited to humans and rats, and indicates that the pharmacokinetics and metabolism of MTBE are reasonable similar in these two species. Very little information was located for mice. MTBE is readily absorbed by inhalation (in humans and rats) and orally (rats), and absorbed to a modest extent dermally (rats). MTBE is probably widely distributed in the body. MTBE is metabolized *in vivo* by cytochromes P450 to TBA, and, probably, formaldehyde. The data suggest that MTBE induces its own metabolism to a limited extent, and that some degree of saturation occurs. TBA is also probably widely distributed. Information on the distribution and fate of formaldehyde produced from MTBE is lacking. TBA is further metabolized to several compounds. The primary metabolites of TBA are 2-methyl-1,2-propanediol and a-hydroxyisobutyric acid. Additional metabolites are the glucuronide and sulfate conjugates of TBA, and acetone. Both MTBE and TBA are eliminated by exhalation in rats and humans, but the unchanged compounds do not appear appreciably in urine. TBA is eliminated more slowly than MTBE, especially in humans. Metabolites of TBA are eliminated in the urine in rats and humans, but elimination in feces is negligible. Physiologically based pharmacokinetic (PBPK) models have been developed for rats and extended to humans.

B.4.2 Absorption

Studies indicate that MTBE is readily absorbed by inhalation in humans. In 10 male volunteers exposed to MTBE at 5, 25, or 50 ppm during 2 h of light exercise, net respiratory uptake ranged from 32% to 42% (Johanson *et al.* 1995). Blood levels of MTBE increased rapidly at the beginning of the exposure, and leveled off at the end. See Figure 2. At 50 ppm MTBE in air, the peak blood level was 13 µmol/L (1.14 mg/L). Average blood levels were proportional to air concentrations (Johanson *et al.* 1995; Nihlen *et al.* 1998). In another study, inhalation of MTBE for 1 h at 1.39 ppm led to peak blood concentrations of MTBE of 8.2 µg/L in one male and 14.1 µg/L in one female at the end of the exposure. No leveling off was evident during the 1 h exposure (Buckley *et al.* 1997; Prah *et al.* 1994). In two males and two females exposed by inhalation to MTBE for 1 h at 1.7 ppm, blood concentrations of MTBE peaked at the end of exposure at 17.1 ppb (average of individual peak blood concentrations of 14.85, 16.65, 17.35, and 19.70 ppb). Some leveling off of blood concentration was evident between 30 minutes and 60 minutes of exposure (Cain *et al.* 1996). Environmental studies have also indicated that exposure to MTBE results in increased blood levels (ATSDR 1996).

MTBE was also rapidly and extensively absorbed by rats in inhalation studies using both single and repeated exposure sessions. In rats exposed to 400 or 8000 ppm MTBE for 6 h, plasma concentrations of MTBE increased rapidly, then leveled off after about 2 h. Peak plasma concentrations of 14 and 493 mg/L were found for the low and high concentration. Little difference between males and females was found. At the end of repeated exposure for 6 h/d for 15 d at 400 ppm, a plasma concentration of 9 mg/L was

reported, suggestive of induction by MTBE of its own metabolism (Miller *et al.* 1997). Similar observations were made when rats were exposed to MTBE for 6 h/d, 5 d/week, for 2-15 weeks at 50-300 ppm. At the end of 2 weeks at 300 ppm, blood concentration was 67 nmol/g (5.9 mg/kg). Blood concentrations were approximately linearly related to MTBE concentrations in the air. MTBE blood concentrations varied somewhat after 6, 10, or 15 weeks exposure, but were roughly similar to those at 2 weeks (Savolainen *et al.* 1985).

No human data for oral or dermal absorption of MTBE were located. In rats exposed by gavage to 40 or 400 m/kg, absorption was rapid and complete. After a 40 mg/kg gavage, a peak plasma concentration of about 18 mg/L was achieved in about 15 minutes. In rats exposed dermally to 40 or 400 mg/kg for 6 h, absorption was 16 and 34%, respectively. The peak plasma concentration at 40 mg/kg after about 2 h was about 0.3 μg/L (Miller *et al.* 1997).

B.4.3 Distribution of MTBE

MTBE is a small organic molecule with high lipid solubility and some water solubility. As such, it is expected to easily cross biological membranes and be widely distributed (ATSDR 1996).

As discussed in the section above, MTBE is readily taken up and distributed via blood in humans exposed by inhalation (Buckley *et al.* 1997; Johanson *et al.* 1995; Nihlen *et al.* 1998; Prah *et al.* 1994). In humans treated with MTBE by intracystic infusion for dissolution of gallstones, MTBE was found in blood, fatty tissue, and breast milk. At treatment end, the concentration in breast milk was similar to that in blood; the concentration in fat was 3-4 times higher (Leuschner *et al.* 1991). No data on distribution of MTBE in humans following oral or dermal exposures were located.

Rats were exposed to MTBE at 50-300 ppm by inhalation for 6 h/d, 5 d/week for 2, 6, 10, or 15 weeks. MTBE was found in blood, brain, and perirenal fat at the end of exposure at all time points. Concentrations in brain were similar to those in blood. Concentrations in fat were on the order of 10-20 times those in blood (Savolainen *et al.* 1985).

PBPK models incorporate distribution of MTBE to numerous tissues (Borghoff *et al.* 1996; Rao and Ginsberg 1997). For rats, the experimentally measured tissue:air partition coefficient for blood was 11.5 (similar to saline). A similar blood:air partition coefficient (17.7) was found for humans (Johanson *et al.* 1995). Partition coefficients for rat liver, kidney and muscle ranged from 6.5 to 35.8, whereas that for fat was considerably higher at 115.6 (similar to oil) (Borghoff *et al.* 1996).

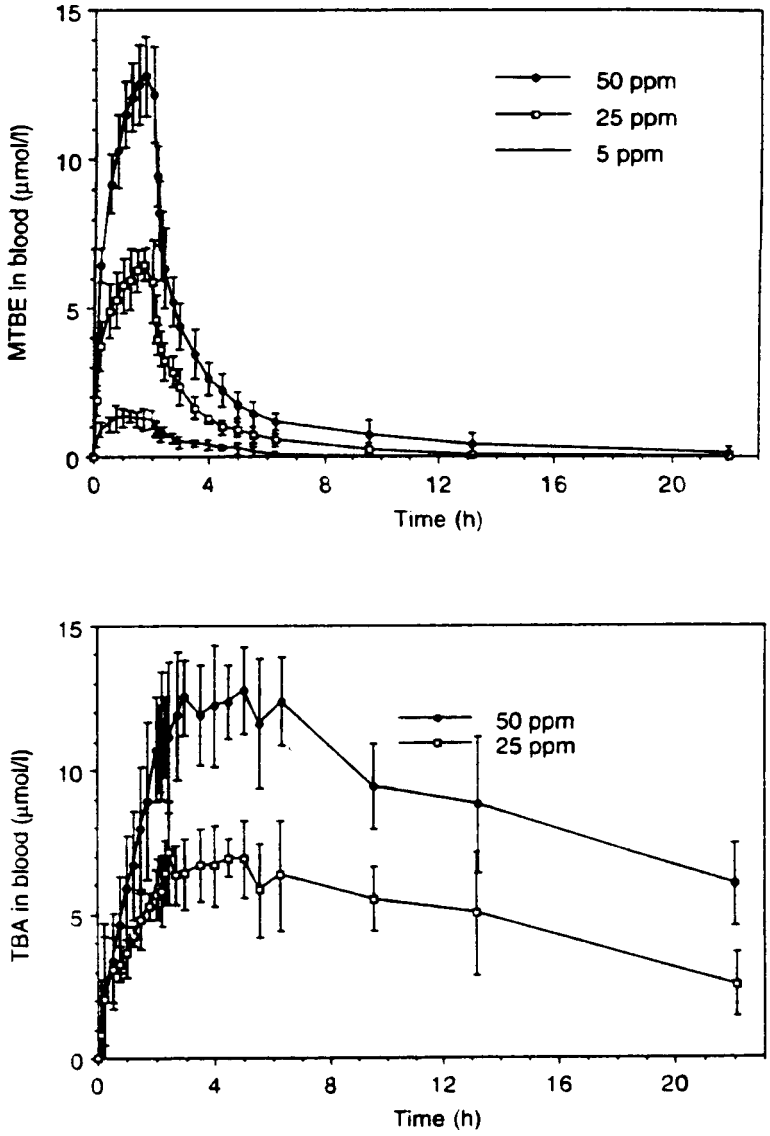


Figure 2. Concentrations of MTBE and TBA in blood samples from 10 male humans exposed to 5, 25, or 50 ppm MTBE for 2 hours.
Vertical lines indicate 1 standard deviation. From Johanson et al. 1995.

B.4.4 Distribution of TBA

Following human inhalation of MTBE, TBA has been found in the blood (Buckley *et al.* 1997; Johanson *et al.* 1995; Nihlen *et al.* 1998; Prah *et al.* 1994). Following intracystic infusion of MTBE for the dissolution of gallstones, TBA was found in the blood, and, in 1 subject, in breast milk, at a concentration slightly less than that in blood (Leuschner *et al.* 1991).

In rats exposed to MTBE by inhalation at 50-300 ppm for 6 h/d, 5 d/week for 2-15 weeks, TBA was found in blood, brain, and perirenal fat at all time points. Concentrations in brain were similar to those in blood, but concentrations in fat were considerably lower (Savolainen *et al.* 1985).

PBPK models for MTBE incorporate distribution of TBA to numerous tissues (Borghoff *et al.* 1996; Rao and Ginsberg 1997). For rats, the experimentally measured tissue:air partition coefficient for blood was 481 (similar to saline) and those for liver, kidney and muscle ranged from 400 to 543. The coefficient for fat was lower at 191 (similar to oil) (Borghoff *et al.* 1996). Thus, TBA has a relatively greater affinity for water compared to lipids than does MTBE.

B.4.5 Metabolism

A proposed metabolic scheme based upon the limited available evidence is shown in Figure 3. It should be noted that the evidence supporting this scheme is highly variable in quality.

The initial step in MTBE metabolism is the conversion of MTBE to TBA and formaldehyde. In humans, elevated TBA has been found in blood following MTBE exposure. The peak concentrations of TBA in blood were roughly comparable to those of MTBE (Buckley *et al.* 1997; Cain *et al.* 1996; Johanson *et al.* 1995; Leuschner *et al.* 1991; Nihlen *et al.* 1998; Prah *et al.* 1994). See Figure 2. Similarly elevated TBA levels have been found in rat blood and plasma following MTBE exposure (Savolainen *et al.* 1985; Miller *et al.* 1997).

Microsomes from human liver metabolized MTBE to TBA *in vitro* (Hong *et al*. 1997b). The specific activity varied about 2-fold for 8 liver samples obtained in the U.S. Of two samples from China, activity for one was at the high end of the U.S. range, and the other slightly exceeded the highest activity U.S. sample. The involvement of cytochromes P450 was indicated by microsomal location, NADPH dependence, and inhibition by carbon monoxide. Human CYP2A6 and 2E1, expressed in a baculovirus system, were able to metabolize MTBE, with 2A6 having about 9-fold higher activity per nmol protein than 2E1 (Hong *et al.* 1997b). Another study, reported as a meeting abstract (Poet and Borghoff 1998), also found that microsomes prepared from individual human livers metabolized MTBE *in vitro*. The kinetics of MTBE disappearance indicated a high affinity, low capacity pathway and a low affinity, high capacity pathway. The former correlated with activity markers for cytochrome P4502E1, and the latter correlated with

activity markers for 2A6. Considerable individual variability in activity was found. The V_{max} for the low capacity pathway varied by 3.7 fold, and for the high capacity pathway by 26 fold. Metabolic products were not reported (Poet and Borghoff 1998).

An *in vitro* study using rat liver microsomes incubated with 1 mM MTBE found equimolar production of TBA and formaldehyde (Brady *et al.* 1990). The involvement of cytochrome P4502E1 was indicated by an approximately 4-fold increase in metabolism following acetone pretreatment, and loss of 35% of activity following incubation with monoclonal antibodies for this protein. The involvement of P4502B1 was suggested by increased MTBE metabolizing activity following pretreatment with phenobarbital (an inducer of 2B1) and an increase in this protein (assessed by immunoblotting) following MTBE pretreatment (Brady *et al.* 1990). In studies of metabolic activity of purified rat P450s *in vitro*, 2B1 had a 16-fold higher demethylation activity per nmol protein than 2E1, which in turn had a 10-fold or greater demethylation activity than 2C11 and 1A1. A series of inhibition and induction studies also supported a substantial role for 2B1 and a lesser role for 2E1 (Turini *et al.* 1998). Another study, reported as a meeting abstract, also found production of formaldehyde from MTBE by rat liver microsomes, and induction with phenobarbital pretreatment (Poet and Borghoff 1997b). In a separate study, microsomes prepared from various rat tissues were tested for MTBE metabolizing activity. Olfactory mucosa were found to have the highest specific activity: 46-fold higher than liver. No activity was detected in rat lungs, kidneys, or olfactory bulb of the brain (Hong *et al.* 1997a).

A study of the apparent anti-estrogenic effects of MTBE in female mice found elevated liver P450 levels following MTBE exposure. Mice were treated with MTBE by inhalation at 8000 ppm for 6 h/d, 5 d/week, for up to 3 weeks. Total liver P450 protein increased by 40% after 3 d and 200% after 3 weeks. Induction of specific enzyme activities, including activity indicative of the P4502B family, was observed. Treatment by gavage at 1.8 g/kg/d for 3 d resulted in similar increases in P450 protein levels and enzyme activity. MTBE increased estrogen metabolism, but this study did not examine metabolism of MTBE *per se* (Moser *et al.* 1996).

A series of experiments in rats involving either inhalation (400 or 8000 ppm) or oral (gavage: 40 or 400 mg/kg) exposures to MTBE found a shift in elimination from urine to exhalation at the higher exposures (see below). This is suggestive of a saturation of some metabolic pathway (Miller *et al.* 1997; Bioresearch Laboratories 1990[b] as cited in ATSDR 1996). A PBPK model for rats found an improved fit to elimination data by using a 2-pathway model, with a high-affinity, low capacity component and a low affinity, high capacity component (Borghoff *et al.* 1996).

Further metabolism of TBA in rats and humans is indicated by the pattern of urinary metabolites. In rats exposed to ^{14}C-MTBE, approximately 70% of urinary radioactivity co-migrated with α-hydroxyisobutyric acid on a C-18 HPLC column. Another peak, co-migrating with 2-methyl-1,2-propanediol, accounted for 14% of radioactivity. Two other peaks, accounting for 10% and 5% of radioactivity were identified. No TBA was

identified in urine (Miller *et al.* 1997). In other studies, rats were exposed to [2-^{13}C]MTBE by inhalation, and urinary metabolites analyzed by nuclear magnetic resonance and gas chromatography/mass spectrometry. Qualitatively, the major urinary metabolites were α-hydroxyisobutyric acid, 2-methyl-1,2-propanediol, and (putative) TBA-sulfate. Quantitative analysis of MTBE metabolites was not performed. Minor metabolites were TBA, acetone and (putative) TBA-glucuronide. Similar urinary elimination products were found after administration of [2-^{13}C]TBA by gavage at 250 mg/kg. In a single male human volunteer administered [2-^{13}C]TBA as a single oral dose of 5 mg/kg, the major elimination products were α-hydroxyisobutyric acid and 2-methyl-1,2-propanediol. Minor products were TBA, (putative) TBA-glucuronide and (putative) TBA-sulfate (Bernauer *et al*. 1998). Urinary excretion of the MTBE metabolites found in the rat inhalation study above also occurs in humans after MTBE exposure by inhalation (Amberg *et al.* unpublished, cited in Bernauer *et al.* 1998; Dekant, personal communication).

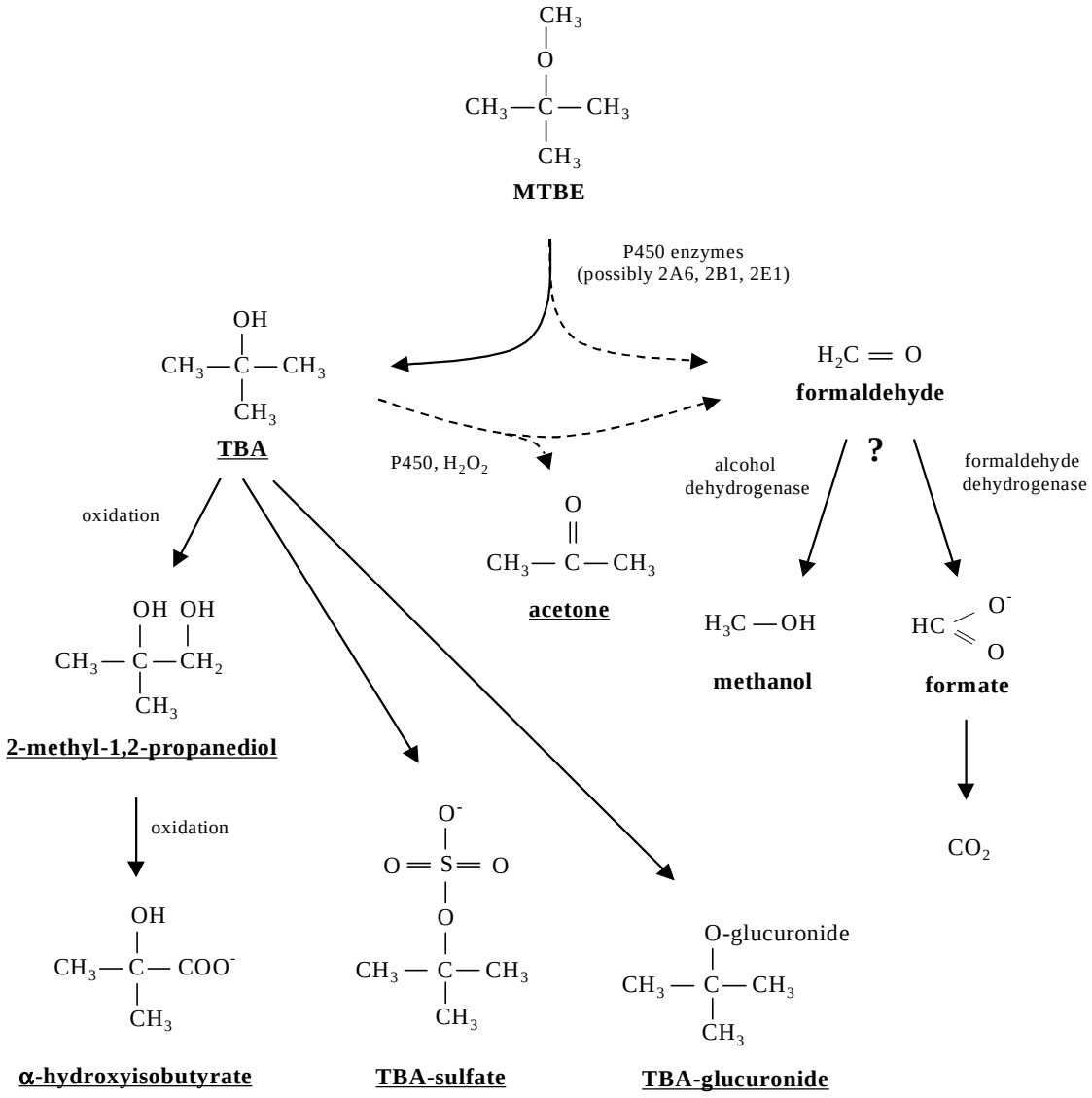
Metabolism of TBA has also been indicated by experiments where TBA was administered to rats by injection. Following injection of TBA at 1 g/kg, acetone was found in the blood. Similar results were obtained using [β-^{14}C]-TBA or [α-^{13}C]-TBA. Using [β-^{14}C]-TBA, ^{14}C-acetone and $^{14}CO_2$ were recovered as major products. However, the ^{14}C-acetone and $^{14}CO_2$ were not produced in equal amounts, and relative quantities varied greatly from experiment to experiment (Baker *et al.* 1982). Rat liver microsomes *in vitro* converted TBA to acetone and formaldehyde; the reaction was NADPH-dependent (indicating the involvement of cytochrome P450). The reaction was also inhibited by hydroxyl radical scavengers and catalase, indicating the involvement of H_2O_2 (Cederbaum and Cohen 1980; Cederbaum *et al.* 1983).

An early paper examined the conjugation of numerous alcohols in the rabbit. When TBA was administered by gavage at 350 mg/kg, additional glucuronide conjugated material was found in the urine. The additional conjugate amounted to 24.4% of the administered dose of TBA (Kamil *et al.* 1953).

There is less information concerning the fate of the methyl group of MTBE. As discussed above, the initial product *in vitro* is formaldehyde. However, this has not been clearly demonstrated *in vivo*. Formaldehyde is a normal metabolic intermediate in mammals. In the absence of exogenous exposure, formaldehyde has been found in blood from humans, monkeys, and rats at levels of 2-3 mg/L. It is oxidized to formic acid by two pathways. Formic acid, in turn, can be oxidized to CO_2, excreted in urine, or incorporated into various biological molecules. Formaldehyde can also react directly with biological macromolecules (e.g., DNA and proteins) (IARC 1995). There is very limited information concerning methanol as an MTBE metabolite; trace amounts of methanol were detected when blood and urine of gallstone patients treated with MTBE was analyzed (Leuschner *et al.* 1991).

Figure 3. Proposed metabolic pathways for MTBE.

Underlined compounds have been detected in urine after MTBE exposure (Bernauer *et al*., 1998); dashed pathways are based on *in vitro* evidence.



B.4.6 Elimination

Data are available on the elimination of MTBE and TBA in humans and rats, and also on elimination of TBA (following TBA exposure) in mice.

As described earlier, 10 male volunteers were exposed by inhalation to MTBE at 5, 25, or 50 ppm during 2 h of light exercise. Elimination in breath and urine was followed for 22 h. After the end of exposure, MTBE concentration in blood dropped rapidly. See Figure 2. Decay could be resolved into 4 phases with $t_{1/2}$ = 1 m, 10 m, 1.5 h, and 19 h. Respiratory excretion (including exposure and post-exposure) ranged from 32% to 47% of respiratory uptake. In contrast, blood TBA leveled off following the end of MTBE exposure, began to decline after about 6 h, and declined with a half-life of about 10 h. See Figure 2. Less than 0.1% of the inhaled MTBE was eliminated in urine as MTBE within 24 h. Less than 1% of the inhaled MTBE was excreted in the urine as TBA within 24 h, suggesting further metabolism of TBA (Johanson *et al.* 1995; Nihlen *et al.* 1998).

In a more limited study, 2 male and 2 female humans were exposed to MTBE by inhalation at 1.7 ppm for 1 h. The blood concentration of MTBE dropped rapidly after exposure, reaching one-half of the peak 40 minutes later. No reduction in blood TBA was evident 90 minutes after exposure (Cain *et al.* 1996).

In another small study including pharmacokinetic modeling, a man and a woman were exposed to MTBE by inhalation at 1.39 ppm for 1 h. The blood concentration of MTBE dropped rapidly after exposure. A one component model yielded clearances with $t_{1/2}$ = 36 min and 37 min, respectively. A three component model yielded residence times of 5 min, 60 min, and 32 h. In contrast, blood concentrations of TBA continued to increase after the end of MTBE exposure. In the male, TBA concentration in blood peaked about 1-2 h after the end of MTBE exposure, and dropped to about one-half 7 h after the end of exposure. In the female, TBA blood concentration was still increasing 7 h after the end of MTBE exposure. After exposure to MTBE, both MTBE and TBA were found in urine samples in concentrations similar to those in blood. An unexplained observation was that the highest concentrations of TBA in urine were found more than 1 h prior to MTBE exposure. It was thus not clear what the appropriate baseline for elimination of TBA to urine from MTBE exposure was. If it was assumed that all TBA eliminated to urine following MTBE exposure was due to that exposure, the total of MTBE and TBA eliminated to urine was less than 1% of the inhaled MTBE (Buckley *et al.* 1997; Prah *et al.* 1994).

Blood and urinary MTBE and TBA were followed in 20-27 humans treated with MTBE by intracystic infusion for gallstone dissolution. MTBE in blood dropped rapidly after treatment and was present in only trace amounts after 12-18 h. TBA was more persistent, and was present at slightly more than one-half the peak concentration 12-18 h after treatment. MTBE and TBA were found in the urine in concentrations similar to those in blood. In one patient, at the end of treatment, MTBE and TBA were found in breast milk at concentrations similar to those in blood (Leuschner *et al.* 1991).

Rats were exposed to MTBE by inhalation for 6 h at 400 or 8000 ppm (Miller *et al.* 1997). After exposure, plasma concentrations of MTBE dropped rapidly, with a $t_{1/2}$ of about 0.5 h. Plasma TBA began to drop shortly after the end of exposure to MTBE, with a $t_{1/2}$ of slightly over 3 h. Using ^{14}C-MTBE at 400 ppm, the majority of radioactivity was recovered in urine (64.7% urine, 21.2% expired air), whereas at 8000 ppm, the majority of radioactivity was recovered in expired air (41.6% urine, 53.6 % expired air). This suggests saturation of some metabolic pathway at the higher concentration. Negligible amounts of radioactivity were recovered in feces. When exposed repeatedly (15 d) at 400 ppm, elimination of MTBE was similar to single exposure ($t_{1/2}$ = 0.51 h), but elimination of TBA was somewhat faster ($t_{1/2}$ = 1.8 h). The radioactivity recovered in expired air consisted mainly of MTBE (66% to 79%), with the remainder being TBA. The urinary excretion was mainly (70%) a compound which co-migrated with α-hydroxyisobutyric acid on a C-18 HPLC column. A second peak (14%) co-migrated with 2-methyl-1,2-propanediol. Two other peaks (10% and 5%) were unidentified. No MTBE or TBA was detected in urine (Miller *et al.* 1997).

In a study where rats were exposed to MTBE by inhalation (described under metabolism, above), the major urinary excretion products identified were 2-methyl-1,2-propanediol, α-hydroxyisobutyric acid, and (putative) TBA-sulfate. The minor products identified were TBA, acetone, and (putative) TBA-glucuronide (Bernauer *et al*., 1998). Human exposures by inhalation have been reported to produce similar urinary products (Amberg *et al*. unpublished, cited in Bernauer *et al*. 1998; Dekant, personal communication).

Rats were treated with ^{14}C-MTBE by gavage at 40 or 400 mg/kg. Elimination of radioactivity by exhalation exceeded elimination in urine. Relative elimination by exhalation increased, and by urine decreased, at the higher dose. Elimination in feces was negligible. At the low dose, the $t_{1/2}$ for elimination of MTBE from plasma was 0.52 h and that for TBA was 0.95 h. At the high dose, the $t_{1/2}$ values were 0.79 h and 1.6 h, respectively (Miller *et al.* 1997; Bioresearch Laboratories 1990[b] as cited in ATSDR 1996).

Rats were treated dermally with MTBE at 40 or 400 mg/kg. The MTBE was injected into a metal chamber that covered an area of the rat's skin. Somewhat slower elimination of MTBE was found than by the inhalation or oral routes. The $t_{1/2}$ for MTBE was 2.3 and 1.8 h, and for TBA was 2.1 and 1.9 h for the low- and high-dose, respectively (Miller *et al*. 1997).

In addition to data on the elimination of TBA following MTBE exposure, data from rats, mice, and humans on the elimination of TBA following TBA exposure were located. In rats treated with TBA by single injection (1g/kg) or gavage (at a dose chosen to achieve similar blood levels), blood concentrations of TBA dropped to one-half of the peak after about 9-12 h, and to very low levels after about 24 h. In the gavage experiment, pretreatment with TBA appeared to speed elimination slightly (Baker *et al.* 1982; Thurman *et al.* 1980). These times for elimination are considerably longer than those reported above for TBA elimination following MTBE treatment. However, the peak

TBA blood levels in the TBA treatment experiments were much higher than in the MTBE treatment experiments.

In non-pregnant female mice receiving 780 mg TBA/kg by gavage, peak blood levels occurred about 1.5 h after administration. Blood levels dropped to one-half the peak after about 6 h and to very low levels after 12 h. Pretreatment with 5 additional doses of TBA did not significantly affect elimination (Faulkner *et al.* 1989). In mice treated by intraperitoneal injection with TBA at 370, 740, or 1480 mg/kg, peak blood concentrations occurred soon after injection. The time to drop to one-half of the peak increased with dose, from 3 - 4 h at 370 mg/kg to 9 - 10 h at 1480 mg/kg (Faulkner and Hussain 1989). In mice treated by single injection of TBA at 600 mg/kg, blood concentrations of TBA dropped to one-half of the peak value after about 4 - 5 h, and to very low levels after about 8 h. In contrast, after 3 d of TBA inhalation (concentration approximately 1200 ppm), TBA blood levels dropped to one-half of maximum after about 1 - 1.5 h, and to very low levels after 2.5 h. The blood levels for the injection and inhalation experiments were similar: whether the pronounced difference in rate of elimination was due to differing routes or duration of exposure was not clear (McComb and Goldstein 1979).

In rats given TBA by gavage (described under metabolism, above), the major urinary metabolites found were 2-methyl-1,2-propanediol, α-hydroxyisobutyric acid, and (putative) TBA-sulfate. Minor urinary metabolites were TBA, acetone, and (putative) TBA-glucuronide (Bernauer *et al*. 1998). In a single human given TBA orally, the major metabolites were 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid. Minor urinary metabolites were TBA, (putative) TBA-glucuronide, and (putative) TBA-sulfate (Bernauer *et al*., 1998).

B.4.7 PBPK models

A PBPK model has been developed for MTBE and TBA in male rats (Borghoff *et al*. 1996), and refined and extended to humans (Rao and Ginsberg 1997). These models used 7-8 compartments for MTBE and TBA. The best fit to the data for MTBE in rats was obtained by using two saturable metabolic pathways (a high-affinity, low capacity pathway and a low-affinity, high capacity pathway). In the initial model, a reasonably good fit to MTBE absorption and blood data for inhalation, oral (gavage) and injection (intravenous) administration was achieved. However, the fit for TBA in the initial model was poor. The fit of MTBE to humans was reasonable during exposure, but was low after exposure ceased. The authors concluded that additional data were needed on TBA distribution and elimination (Borghoff *et al.* 1996). The later model improved the fit by fitting MTBE metabolic parameters to both MTBE and TBA empirical data, and by fitting the slowly perfused compartment partition coefficient to the empirical data. Although an improved fit to TBA was obtained, there was still a considerable divergence from experimental values obtained for the rat. The model was extended to humans: the overall time dependence of MTBE and TBA blood concentrations was reproduced, although substantial divergences from experimental data were also found (Rao and Ginsberg 1997).

B.4.8 Comparison of doses from inhalation and oral routes

Toxicological studies with MTBE have most often used inhalation and oral (gavage) routes. It is therefore helpful in interpreting these studies to compare concentrations used in inhalation studies to doses used in oral studies. Two different approaches have been used to make this comparison. The first used the amount of MTBE inhaled, corrected by an assumed inhalation:oral absorption ratio of 0.5:1 (Dourson and Felter 1997). The second used a PBPK model to calculate equivalent oral doses based on area under the curve for blood concentrations (OEHHA 1998) (See Table 5). Neither measure reflects the time-dependence of blood concentration. For inhalation, over the 6 h exposure interval, the MTBE blood concentration rises to a plateau, followed by a rapid drop after the end of exposure. The gavage route produces a sharp peak about 15 minutes after administration, followed by a rapid drop (Miller *et al.* 1997).

Table 1. Estimated comparison of inhalation to oral (gavage) doses in rats.

MTBE inhalation (6 hr/d, 5 d/wk) (ppm)	Equivalent oral dose[1] (mg/kg/d)	Equivalent oral dose[2] (mg/kg/d)
0	0	0
400	130	82.9
3000	940	618.8
8000	2700	1848.3

[1] Assumes inhalation:oral absorption ratio 0.5:1 (Dourson and Felter 1997)
[2] Based upon area under the curve for blood MTBE using a PBPK model (OEHHA 1998)

B.5 NON-DART TOXICITIES

MTBE is not a potent toxicant in terms of acute lethality. MTBE has an unpleasant taste and smell at low air and water concentrations. It is an irritant at higher concentrations, and has anesthetic properties. With chronic administration, as determined in animal studies, MTBE produces nephrotoxicity and is carcinogenic. The majority of information on MTBE toxicity comes from laboratory animal studies, supplemented by 3 human experimental studies at low inhalation concentrations, and symptom reports of human populations exposed to MTBE or MTBE-containing gasoline. This summary covers primarily inhalation exposures as background to inhalation developmental toxicity and reproductive toxicity studies.

B.5.1 Acute toxicity[iii]

A 4-h inhalation LC50 in rats for MTBE has been calculated at 33,370 ppm (ATSDR 1996). A 10-min inhalation LC50 in mice has been calculated at 180,000 ppm (ATSDR 1996). There is no information concerning lethal doses for humans.

There are very limited data on the acute toxicity of MTBE in humans by any route of exposure. A recent literature review (Borak *et al.* 1998) summarized the exposure to MTBE and acute human health effects including 9 epidemiological studies, 10 industrial hygiene studies and 12 clinical studies. No studies were located regarding acute toxic effects of ingested or skin-contacted MTBE in humans. There are very limited data on the acute toxicity of MTBE in humans through inhalation exposures. Several studies undertaken over the past 4 to 5 years were unable to find any correlation between reported acute health effects and MTBE exposures experienced by the general public from MTBE's use in gasoline (ATSDR 1996, McCoy *et al.* 1995, NSTC 1996, 1997, U.S. EPA 1997a). Chronic effects have not been studied.

In 1993, the J. B. Pierce Laboratory of Yale University (Cain *et al.* 1996) and U.S. EPA (Prah *et al.* 1994), in 2 separate studies, exposed individuals to clean air and air mixed with MTBE. In cases where 37 or 43 human volunteers were exposed to low levels of MTBE in air (1.39 or 1.7 ppm) for 1-h, there was no significant increase in symptoms of eye, nasal, or pulmonary irritation when the results for periods of exposure to MTBE were compared to results from exposure to ambient air. There were also no significant effects on mood or in the results from several performance-based neurobehavioral tests. In both studies, the females ranked the general quality of the air containing MTBE lower than the control atmosphere. However, in the study by Cain *et al.* (1996), where the subjects were also exposed to an atmosphere containing a total of 7.1 ppm mixture of 17 volatile organic compounds (VOCs) that are frequent air contaminants in areas around gasoline stations, the air quality of the MTBE-containing atmosphere ranked higher than that with the VOC mixture. No increase in acute symptoms was observed in individuals exposed to MTBE at concentrations that would be encountered while refueling a car.

The National Institute for Working Life in Sweden (Nihlen *et al.* 1998a, 1998b) assessed acute effects up to the Swedish occupational exposure limit value both with objective measurements and questionnaires. The healthy male volunteers were exposed to MTBE vapor for 2 h at 5, 25, and 50 ppm during light physical work. In the questionnaire, only the ratings of solvent smell increased up to 50% of the scale as the volunteers entered the chamber and declined slowly with time. No ocular effects were observed. Nasal airway resistance blockage index increased but was not related to exposure levels. Decreased nasal volume was seen but with no dose-effect relationship. The authors concluded no or minimal acute effects of MTBE vapor upon short-term exposure at these relatively high levels.

[iii] This section is a summary of information from *Public Health Goal for Methyl Tertiary-Butyl Ether (MTBE) in Drinking Water* (OEHHA, 1998)

B.5.2 Irritant effects

Acute irritant effects of MTBE inhalation in humans were recently studied (Cain *et al.* 1996, Prah *et al.* 1994, Nihlen *et al.* 1997). There was no significant increase in symptoms of eye, nasal or pulmonary irritation or effects on mood or performance-based neurobehavioral tests with MTBE exposures of 1.39 and 1.7 ppm (Cain *et al.* 1996, Prah *et al.* 1994). Similarly no significant increase in symptoms of ocular or nasal irritation were observed by Nihlen *et al.* (1997) at 5, 25 and 50 ppm.

At exposure concentrations used in animal toxicology studies (up to 8000 ppm), irritation has been noted as ocular and nasal discharge, and blepharospasm (eyeblinking) (see sections C.2, C.3 and D.2.1 below).

B.5.3 Central nervous system effects

MTBE belongs to the class of alkyl ethers, which includes volatile anesthetics such as diethyl ether. A full neurotoxicity evaluation of MTBE has been conducted in rats using U.S. EPA Neurotoxicity Testing Guidelines (Daughtrey *et al.* 1997). Testing methods included the Functional Observation Battery (FOB), automated spontaneous activity monitoring, and neuropathology. In keeping with an anesthetic profile, MTBE was found to produce ataxia, reduced auditory startle response, depressed pain reflex and a number of autonomic signs (piloerection, lacrimation) as well as labored breathing and reduced body temperature. These effects were seen 1 h after a single 6 h exposure; no effects were indicated 6 or 24 h after the exposure. Continuous monitoring of spontaneous motor activity after the single 6 h exposure demonstrated effects only within the first hour after exposure. When subchronic exposures (6 h/day, 5 days/week, 13 weeks) were given and FOB evaluation was delayed to 2 days after completion of the last exposure, a few effects were noted, but they were considered isolated and not of toxicological significance. The transient CNS depression was seen at exposure concentrations of 8000 ppm and 4000 ppm with a NOAEL at 800 ppm. The CNS depression caused by MTBE is consistent with the profile produced by the related agent diethyl ether (Glowa 1993).

B.5.4 Chronic toxicity[iv] (non-cancer)

In recent literature searches, no studies were located regarding death, cardiovascular effects, hematological effects, musculoskeletal effects, hepatic effects, renal effects, or body weight effects of MTBE in humans.

Animal studies conducted at inhalation exposures to MTBE greater than 1000 ppm reported increased liver, kidney, spleen, and adrenal weights, decreased brain weight, body weight, and body weight gain, swollen periocular tissue, and ataxia in rodents.

Kidney toxicity was also observed in both males and females in the 2-year inhalation study in F344 rats by Chun *et al.* (1992). U.S. EPA derived a RfC of 3 mg/m^3 based on

[iv] Text in this section is from *Public Health Goal for Methyl Tertiary-Butyl Ether (MTBE) in Drinking Water* (OEHHA, 1998)

the kidney and liver effects of MTBE (U.S. EPA 1993, 1997c). These data support the conclusion that, after MTBE exposure, kidney toxicity is of toxicological concern.

B.5.5 Carcinogenicity[v]

A full review of evidence on the carcinogenicity of MTBE is being developed by OEHHA for consideration of the Cancer Hazard Identification Committee of the Science Advisory Board. The American Conference of Governmental Industrial Hygienists (ACGIH) lists MTBE as an A3 Animal Carcinogen (ACGIH 1996). In 1998 the U.S. Environmental Protection Agency (US EPA) nominated MTBE for review by the U.S. National Toxicology Program (NTP) as a candidate to be considered for testing and listing in NTP's Ninth Edition Report on Carcinogens as "reasonably anticipated to be a human carcinogen based on positive carcinogenicity findings in laboratory animals studies" (NTP, 1998). U.S. EPA has not classified MTBE as to its carcinogenicity, however, in an earlier review of the data by the U.S. EPA, which did not include the more recent oral rodent cancer bioassays, the Agency proposed classifying MTBE as a group C carcinogen (U.S. EPA 1994a, 1994b). The International Agency for Research on Cancer (IARC) has neither evaluated nor classified MTBE as to its carcinogenicity (IARC 1995); however, this organization is planning to perform such an evaluation in the near future.

No data on long-term effects of human exposure to MTBE relevant to cancer risk were found in recent literature searches performed by OEHHA. The carcinogenic activity of MTBE has been investigated in male and female Sprague-Dawley rats administered MTBE by gavage (Belpoggi *et al.* 1995, 1997) and in male and female F344 rats (Chun *et al.* 1992, Bird *et al.* 1997) and $B6C3F_1$ mice (Burleigh-Flayer *et al.* 1992, Bird *et al.* 1997) exposed to MTBE by inhalation. In rats receiving MTBE by gavage for 24 months, statistically significant increases in Leydig interstitial cell tumors of the testes were observed in males, and statistically significant increases in lymphomas and leukemias (combined) were observed in females. An increase in the incidence of uterine sarcomas was also observed in MTBE-exposed female rats, but was not statistically significant at the $p<0.05$ level. In rats exposed to MTBE by inhalation for up to 24 months, statistically significant increases in the incidences of renal tubular tumors and Leydig interstitial cell tumors of the testes were observed in males. In mice exposed to MTBE by inhalation for up to 18 months, statistically significant increases in the incidences of liver tumors were observed in females (hepatocellular adenomas; hepatocellular adenomas and carcinomas combined) and males (hepatocellular carcinomas; hepatocellular adenomas and carcinomas combined). Although the published literature to date indicates that MTBE has little or no genotoxic activity (ATSDR 1996), one of MTBE's major metabolites, formaldehyde, is genotoxic.

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[v] Text in this section is adapted from *Public Health Goal for Methyl Tertiary-Butyl Ether (MTBE) in Drinking Water* (OEHHA, 1998)

B.6 ORGANOLEPTIC PROPERTIES[vi]

Taste and odor characteristics of MTBE have been studied in humans. A range of 5 to 53 ppb odor threshold in the air was reported in an American Petroleum Institute (API) document (API 1994). The same subjects described the taste of MTBE in water as "nasty", "bitter", "nauseating", and "similar to rubbing alcohol" (API 1994). The taste and odor responses reported in observed individuals for MTBE in water are in the 15 to 180 ppb range for odor and the 24 to 135 ppb range for taste (API 1994, Prah *et al.* 1994, Young *et al.* 1996, Dale *et al.* 1997, Shen *et al.* 1997, NSTC 1997). U.S. EPA (1997a) has analyzed these studies in detail in which the ranges are indicative of the variability in individual response, and recommended a range of 20 to 40 ppb as an approximate threshold for organoleptic responses.

C. DEVELOPMENTAL TOXICITY

C.1 OVERVIEW

No human studies relevant to MTBE developmental toxicity were located. There are 4 animal developmental toxicity studies, 2 studies in mice (Conaway *et al.* 1985, Bio/dynamics, Inc. 1984b) (Bevan *et al.* 1997b, Tyl and Neeper-Bradley 1989), 1 study in rats (Conaway *et al.* 1985, Bio/dynamics, Inc. 1984a), and 1 study in rabbits (Bevan *et al.* 1997b, Tyl 1989). All the developmental toxicity studies used the inhalation route of administration and limited exposures to the period of organogenesis. Developmental toxicity studies of the MTBE metabolite TBA have also been performed.

The developmental toxicity studies of MTBE were of good quality, generally conformed to U.S. EPA testing guidelines and were conducted in commercial laboratories under quality assurance protocols. These studies were available to OEHHA as the original reports of the contract laboratory performing the study (Tyl and Neeper-Bradley 1989, Tyl 1989, Biodynamics, 1984a, b) as well as peer-reviewed reports in the literature (Conaway *et al.* 1985, Bevan *et al.* 1997b). The group size was adequate in all cases. Due to safety considerations, inhalation concentrations used for these studies were limited to 8000 ppm (50% of the lower explosive limit).

Dose-dependent effects on fetal weight and fetal skeletal variations were reported in 1 of the 2 mouse studies (Tyl and Neeper-Bradley 1989, Bevan *et al.* 1997b); no fetal effects were reported in the rat and rabbit studies. Notably, for both the rat study and the mouse study that reported no effects (Conaway *et al.* 1985, Bio/dynamics, Inc. 1984b), the investigators used a concentration range lower than that used in the mouse study that reported developmental toxicity (Tyl and Neeper-Bradley, 1989). In this lower concentration range, there was no induction of minimal maternal toxicity, which is a departure from U.S. EPA testing guidelines.

[vi] Sentences in this section are from *Public Health Goal for Methyl Tertiary-Butyl Ether (MTBE) in Drinking Water* (OEHHA, 1998)

In addition to effects reported in fetuses at the end of gestation, effects of MTBE on postnatal viability and weight gain have been reported in multi-generation reproductive toxicity studies.

C.2 ANIMAL DEVELOPMENTAL TOXICITY STUDIES

The design and results of all studies discussed below are outlined in Appendix A at the end of the document.

C.2.1 Mice

In the first mouse developmental toxicity study, using the lower dose range (250, 1000 and 2500 ppm, gestation day 6-15, n=29-30 group) (Conaway *et al.* 1985, Bio/dynamics, Inc. 1984b), no statistically significant group differences were reported between controls and MTBE exposed groups for either maternal or fetal endpoints. Endpoints measured for maternal toxicity included mortality, food and water intake, body weight, body weight gain, liver weight, relative liver weight, and the results of gross post mortem examination. One dam in the 250 ppm group and 1 dam in the 2500 ppm group demonstrated total resorption of the litter. There were no statistically significant MTBE effects on implantation, early or late resorptions or live fetuses at term. No statistically significant MTBE effects were found for fetal toxicity endpoints including fetal weight and crown rump length, external abnormalities, soft tissue malformations (examined in 1/3 of fetuses), and skeletal malformations (examined in 2/3 of fetuses). However, in reviewing the skeletal malformation data, the U.S. EPA (1997a) pointed out an apparent concentration-related pattern of increased fetal skeletal malformations. These data are presented in Table 2.

The study report (Bio/dynamics, Inc. 1984b) also noted an apparent concentration related trend for fused sternebrae . The percents of fused sternebrae on a per fetus basis were 0, 0.6, 1.2 and 2.1% respectively in the control 250, 1000 and 2500 ppm groups (Bio/dynamics, Inc. 1984b). Historically, according to the authors of the study report, an incidence of fused sternebrae of 4/2449 fetuses or 0.16% was found at the Huntingdon Research Centre. The authors of the report did not consider the incidence of fused sternebrae to be treatment related because of the absence of “vertebral and/or rib defects occasionally associated with sternebral malformations”. Another skeletal endpoint, fetal ossification variations, was not evaluated statistically (Bio/dynamics, Inc. 1984b) but there were no apparent dose-related trends. Notably, ossification of the all sternebrae was complete in all the fetuses of the MTBE treated groups; in contrast, the incidence of fetuses with individual unossified sternebrae ranged from 1.6 % (sternebra 1) to 6.0 % (sternebra 6) in the control group.

Table 2. Effects of MTBE on fetal skeletal malformations in CD-1 mice.

Table from Conaway et al. 1985. No statistically significant group differences.

MTBE (ppm)	Fetal Malformation	Litters Affected	
		Number[1]	%
0	Cleft palate	1/27	3.7
	Fused ribs (unilateral) and misaligned thoracic centra	1/27	3.7
	Total[2]	2/27	7.4
250	Fused sternebrae	1/26	3.8
	Scrambled sternebrae	1/26	3.8
	Fused ribs (unilateral) and vertebral defects	1/26	3.8
	Total	3/26	11.5
1000	Fused sternebrae	2/25	8.0
	Fused ribs (unilateral)	1/25	4.0
	Angulated ribs (unilateral)	1/25	4.0
	Total	4/25	16.0
2500	Cleft palate	2/27	7.4
	Fused sternebrae	4/27	14.8
	Total	6/27	22.2

[1] (Number of litters containing fetuses with a malformation)/(number of litters evaluated).
[2] Each malformation occurred in a different litter, so that incidence can be summed across litters.

The second mouse developmental toxicity study (Bevan *et al.* 1997b, Tyl and Neeper-Bradley 1989), used higher MTBE concentrations (1000, 4000, 8000 ppm). At 8000 ppm, MTBE produced both maternal and fetal toxicity. Statistically significant lower pregnancy weight gain (approximately 30% lower) compared to controls was seen, as well as reduced corrected pregnancy weight gain (23%). Food consumption of dams was significantly lower during the exposure period. Clinical signs of toxicity, which were recorded daily for each mouse immediately after the exposure session (R. Tyl, personal communication), included a statistically greater incidence of lacrimation, periocular encrustation, hypoactivity, ataxia, prostration, and labored respiration. These clinical observations are consistent with the irritation and the transient CNS depression noted in MTBE neurotoxicology studies (Daughtrey *et al.* 1997) (see section B.5.5 above). In addition, 4 of 30 dams in the 8000 ppm group had total litter resorptions. Fetal toxicity at the 8000 ppm concentration included: increased postimplantation loss, decreased live fetuses/litter, increased percent of litters with external and visceral malformations, increased incidence of cleft palate, reduced fetal body weight (23%), and increased incidence of a number of skeletal variations reflecting delayed ossification. The authors attribute the increased incidence of cleft palate in the 8000 ppm group to the well-documented association between maternal stress, corticosteroid production and cleft palate induction in mice (Sullivan-Jones *et al.* 1992).

At the 4000 ppm MTBE exposure, 2 of the fetal effects observed at 8000 ppm, reduced fetal body weight and delayed ossification, were also found. The fetal body weight

effects and delayed ossification were generally concentration-related at 4000 and 8000 ppm, with no indication of treatment-related effects at 1000 ppm (see Table 3). No maternal toxicity in the form of body weights or clinical signs of toxicity occurred at 4000 ppm. Group observations of dams during exposure at the 4000 ppm concentrations included hypoactivity and ataxia. (For group observations, any occurrence of the toxic sign in any member of the group that could be observed through the window of the exposure chamber was noted.) At the 1000 ppm exposure, there was a statistically significant increased incidence of poorly ossified hindlimb interphalanges. The authors discounted this effect because it did not occur at the 4000 and 8000 ppm exposures.

Table 3. Fetal toxicity endpoints in mice exposed to MTBE on gestation day 6-15. (Bevan et al. 1997b, Tyl and Neeper-Bradley 1989)

	MTBE (ppm)			
	0	**1000**	**4000**	**8000**
Group size (litters)	27	29	26	26
Fetal Body Weight (g, mean±s.d.)				
males	1.441 ±.1094	1.400 ±.0995	1.340** ±.1053	1.108** ±.1149
females	1.391 ±0.1083	1.350 ±0.0980	1.288** ±0.0864	1.066** ±0.0843
Skeletal Variations[1]				
cervical centra #1, 2, 3 and/or 4 poorly ossified	2[2] (7.4)[3]	2 (6.9)	12** (46.2)	23** (88.5)
cervical centrum #5 poorly ossified	0 (0.0)	2 (6.9)	9** (34.6)	17** (65.4)
cervical centrum #6 unossified	1 (3.7)	1 (3.4)	9** (34.6)	14** (53.8)
some proximal phalanges (forelimb) unossified	0 (0.0)	1 (3.4)	6* (23.1)	9** (34.6)
some proximal phalanges (hindlimb) unossified	3 (11.1)	4 (13.8)	12* (46.2)	20** (76.9)
sternebra #3 poorly ossified	3 (11.1)	5 (17.2)	12* (46.2)	16** (61.5)
Any skeletal variation	27 (100)	29 (100)	26 (100)	26 (100)

[1] 6 categories of skeletal variations that demonstrated a statistically significant increase in incidence in both the 4000 and 8000 ppm MTBE groups (of 77 categories reported).
[2] Numbers of litters affected for this variation; approximately 50% of each litter were examined for skeletal defects.
[3] Percent of litters affected for this variation.
* Statistically different from control, p<.05.
** Statistically different from control, p<.01.

C.2.2 Rats

This study (Conaway *et al.* 1985, Bio/dynamics, Inc. 1984a) was conducted with MTBE inhalation concentrations of 0, 250, 1000, and 2500 ppm. A maternally toxic dose was not reached in the study, and no fetal toxicity was reported. The only maternal effect reported was a significantly lower mean food consumption from gestation day 9-12 (middle third of the exposure period) in all 3 MTBE groups relative to controls. No effects of MTBE on dams were reported from twice daily observation of gross signs of toxicologic or pharmacologic effects, from detailed physical exams on gestation day 0, 6, 9, 12, 15 and 20, or from gross post-mortem exams. Body weight, body weight gain, liver weight, relative liver weight and water consumption of dams were not found to be influenced by MTBE. The numbers of implantation sites, early and late resorptions, and live and dead fetuses were similarly unaffected. Measures of fetal toxicity including fetal weight and crown rump length, the frequencies of gross external malformations, soft tissue malformations (1/3 of fetuses examined) and skeletal malformations (2/3 of fetuses examined) demonstrated no statistically significant differences between controls and MTBE groups, and no noteworthy trends. Ossification variations were not evaluated statistically and did not appear to demonstrate concentration-related trends.

C.2.3 Rabbits

The rabbit developmental toxicity study (Bevan *et al.* 1997b, Tyl 1989), conducted at concentrations of 0, 1000, 4000 and 8000 ppm MTBE, reached a marginally maternally toxic dose. Body weight gain and food consumption were lower in the 8000 ppm group than in controls during the exposure period (gestation day 6-18) only. Also, maternal liver weight as a percent of body weight was significantly greater in the 8000 ppm group than in controls at term (3.1% vs. 2.7%). However, doe weight gain during gestation (gestation day 0-29), and food consumption after the exposure period (gestation day 18-29) were not influenced by MTBE. In addition, no signs of irritation or CNS depression were reported in any of the does at the time of daily observation for clinical signs. Hypoactivity and ataxia were seen in group observations on 6/13 exposure days in the 8000 ppm group only. Thus, rabbit dams appeared less sensitive than mouse dams to the 8000 ppm inhalation exposure.

No fetal toxicity was observed at any inhalation concentration. Fetal weights were similar in all groups. The frequencies of malformations and variations were not elevated relative to controls, and no trends toward elevated incidence of skeletal variations reflecting delayed ossification were noted across dose groups.

C.3 POSTNATAL ENDPOINTS FROM REPRODUCTIVE TOXICITY STUDIES

Viability and preweaning growth of offspring were developmental toxicity endpoints measured in both the one-generation (Biles *et al.* 1987, Bio/dynamics, Inc. 1984d) and the two-generation (Bevan *et al.* 1997a, Neeper-Bradley 1991) reproductive toxicity studies of MTBE. These studies are described in more detail in Section D.2. Both parents were exposed in these studies, and pups were exposed *in utero* and potentially during lactation. Thus identification of the exposure or exposures that produce postnatal effects cannot be

determined. Notably, in these chamber inhalation studies, exposure was discontinued shortly before parturition and resumed on postnatal day 5 for the dams. Thus, neither pups nor dams were exposed in the immediate perinatal period and pups were not exposed directly to MTBE vapor during the postnatal period, although they may have been exposed via their dam's milk.

There were findings concerning both postnatal viability and growth in both the one- and two-generation studies, but the information is difficult to integrate due to procedural differences between the studies. The dams received 1 more day of gestational exposure prior to parturition in the one-generation study than in the two-generation study. Also the indexes of postnatal viability and growth were different in the one- and two-generation studies.

C.3.1 Viability

In the two-generation study (Bevan *et al.* 1997a, Neeper-Bradley 1991), the number of dead (vs. surviving) pups from postnatal day 0-4 (excluding stillborns) was significantly greater in the 8000 ppm group than in the control group in both the F1 and F2 generations. In the 3000 ppm group, the number of dead pups from day 4 to weaning at day 28 was greater than in the control group in the F1 generation. Individual litters with high mortality rates on a single day had a major influence on the outcome of these group-based statistics. Survival indices calculated on a per litter basis (% of litter surviving) did not show statistically significant differences between control and MTBE exposed groups in either generation. However, average litter size was smaller in the 8000 ppm group than in controls at weaning of the F2 generation, reflecting greater pup loss on a litter basis during lactation.

Postnatal viability effects of MTBE were more prominent in the one-generation study (Biles *et al.* 1987, Bio/dynamics, Inc. 1984d) even though exposure concentrations (250, 1000, 2500) were lower than in the two-generation study (400, 3000, 8000 ppm). This may be related to the continuation of gestational exposure to day 20 in the one-generation study rather than day 19 in the two-generation study. The viability effect was seen as a greater incidence of early pup death. The number of dead vs. live pups at birth was significantly greater in the 1000 and 2500 ppm groups than in controls in the F1b litters, while the number of dead vs. surviving pups from postnatal day 0-4 was greater in the 250 and 1000 groups than in controls in the F1a litters (Biles *et al.* 1987). The difference in timing of the early postnatal deaths in the F1a and F1b litters may be due to the arbitrary distinction between pups "born dead" and those dying on postnatal day 0. Viability was expressed in the report on the basis of pups pooled across litters; statistical comparison of survival on a litter basis (as % of litter surviving or in terms of postnatal litter size) was not conducted.

C.3.2 Growth

Postnatal growth effects in the two-generation study (Bevan *et al.* 1997a, Neeper-Bradley 1991) were reported both as pup weight and pup weight gain (see Figure 4). For the F1 litters, average litter weights were significantly lower in the 8000 ppm group than in

controls on postnatal day 14, 21 and 28. The average litter weights for males and females considered separately were also affected. Litter weights were also lower in the 8000 ppm group than controls on postnatal day 1, 4 and 7, but these differences were not statistically significant. Birth (postnatal day 0) weights were not provided. Average litter weight gain was less in the 8000 ppm group than controls for the intervals from postnatal day 7-14 and 14-21. Weight gains were also lower from postnatal day 1-4 and 4-7 but these differences were not statistically significant.

In the 3000 ppm group, average litter weights were significantly lower than controls only on postnatal day 4 and 14. Weights were lower on postnatal day 7, 21 and 28, and intermediate between control and 8000 ppm groups, but not statistically significant. Weight gains were significantly lower for 3000 ppm litters than for controls from postnatal day 1-4 and 7-14. Weight gains at other intervals were intermediate between those of the control and the 8000 ppm group, but differences were not statistically significant. Notably maternal gestational and lactational weight gain were not statistically lower than controls at any timepoint.

A similar pattern of postnatal growth retardation was seen in the F2 generation in the 3000 and 8000 ppm groups. In this generation, however, the body weight effects in the 3000 ppm group were statistically significant beginning at postnatal day 14 and continuing to weaning. As was the case for the F1 generation, maternal gestational and lactational weight gains in the MTBE exposed group were not statistically lower than controls at any timepoint.

Postnatal growth was evaluated in the one-generation study (Biles *et al.* 1987, Bio/dynamics, Inc. 1984d) as average male and female weights per litter. The ANOVA for male weights was significant on day 21, but none of the individual groups differed from controls. No other significant effects of MTBE on postnatal body weight were found. Neither average combined-sex litter weights nor weight gains were reported and analyzed.

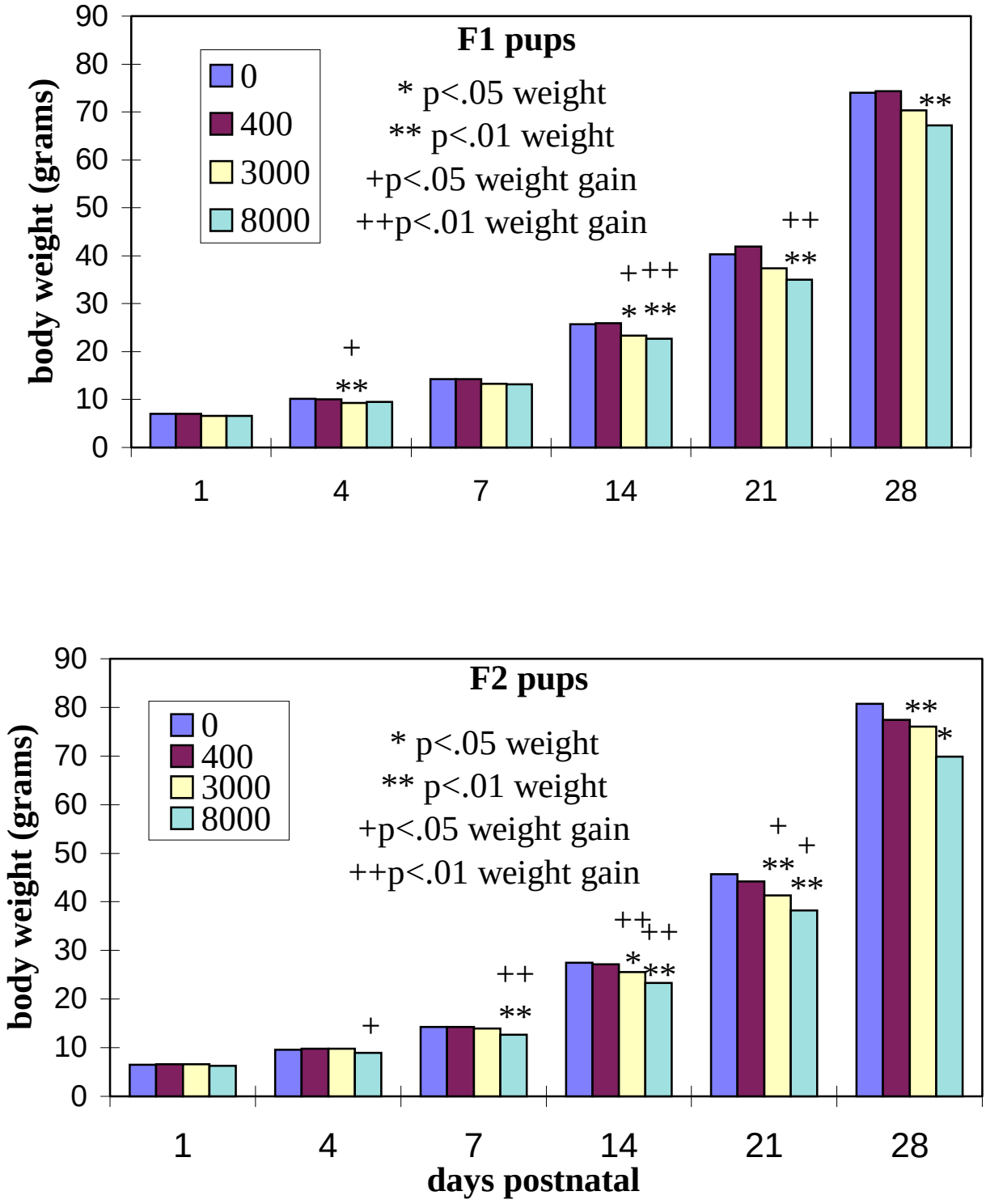

Figure 4. Postnatal growth in the rat two-generation MTBE inhalation study.

C.4 OTHER RELEVANT DATA

No studies examining the distribution, metabolism, etc. of MTBE or TBA in the placenta or fetus were located. As discussed in section B.4, MTBE and TBA are expected to be widely distributed in the body. It is therefore plausible that they reach the placenta and fetus. Numerous xenobiotics have been found to be metabolized by embryonic tissues (Juchau *et al.* 1992). It is possible, then, that embryonic and/or fetal metabolism of MTBE and/or its metabolites occurs.

This section will briefly review the studies of the developmental effects of TBA. As discussed in section B.4, there is ample evidence that TBA is produced from MTBE and is widely distributed. In contrast, there has been no direct examination of the fate of formaldehyde, the putative other first metabolite of MTBE. It is not clear whether formaldehyde appears in circulation following MTBE exposure, or is metabolized to other compounds, or what the fate of those compounds might be. Given this uncertainty, a review of developmental effects of formaldehyde is not included in this document.

Several studies have examined the developmental toxicity of TBA in mice (oral) and rats (oral and inhalation). TBA has been studied in part because of interest in the chemical as a solvent and chemical intermediate, and in part because it is an analogue of ethanol, but with differing metabolism. Most of the studies of TBA have deficiencies in terms of reporting and/or analysis of results. In general, however, developmental toxicity, sometimes accompanied by maternal toxicity of varying degrees, has been observed following TBA exposure in both mice and rats.

Two strains of mice (CBA/J and C57BL/J) were treated with TBA by gavage at 0 or 780 mg/kg, twice daily, on gestation days 6-18. Mice were sacrificed on gestation day 18. Significantly increased resorptions and reduced litter size were found. No significant differences between strains were found. No significant effect on malformations, minor variations, or fetal weight was found. No data on maternal effects was presented (Faulkner *et al.* 1989).

Swiss mice were treated with TBA in food (liquid diet) at 0, 0.5%, 0.75%, or 1.0% w/v from gestation days 6 to 20. Mice in the 0, 0.5%, and 0.75% groups were pair-fed to the 1.0% group, which led to a 20-25% reduction in intake compared to *ad libitum* feeding. Mice were allowed to give birth, and half of the litters in the TBA treated groups were fostered to unexposed dams. Pups were evaluated for neurobehavioral endpoints. In the 0.75% and 1.0% groups, reduced maternal weight gain, reduced number of litters, reduced live litter size, increased stillborn pups, and reduced pup weight on postnatal day 2 were observed. These effects were dose-related, as illustrated in Table 4, but the authors did not test for statistical significance. Reduced postnatal weight gain was observed in pups from the 0.75% and 1.0% TBA groups (the statistical significance is unclear in the text). Testing on various postnatal days between 4 and 20 found significant effects in the 0.75% and 1.0% groups on cliff avoidance, righting reflex, and open field

activity. Rotorod performance was significantly affected at 1.0%. Fostering to non-TBA-treated dams significantly improved pup postnatal weight gain and performance on the righting reflex, open field, and rotorod tests. The authors interpreted the neurobehavioral effects as developmental delay, after which all groups acquired the same level of performance (Daniel and Evans 1982).

Table 4. Maternal and parturition data from Daniel and Evans 1982.

	Control	0.5% TBA	0.75% TBA	1.0% TBA
Number of maternal animals	15	15	15	15
Maternal food consumption (% of control)	100%[1]	102%[1]	97%[1]	96%
Maternal weight gain (g) (%)	16.9 (64%)	17.0 (62%)	14.0 (52%)	13.2 (51%)
Number of litters (% of total)	11 (77%)	12 (80%)	8 (53%)	7 (47%)
Neonates/litter (Mean ± S.D.)	10.4 ± 4.0	10.3 ± 4.4	7.4 ± 2.3	5.3 ± 2.8
Total stillborn	3	6	14	20
Neonatal weight at day 2 (g) (mean ± S.D.)	1.78 ± 0.21	1.66 ± 0.24	1.45 ± 0.14	1.10 ± 0.10

[1]These groups were pair fed to the 1.0% TBA group resulting in food intake 20-25% below that of *ad libitum* feeding

In a study reported only in abstract, rats were treated with TBA in liquid diets at 0, 0.65%, 1.3%, or 10.9% v/v (equivalent to 0, 0.5%, 1.0% or 8.5% w/v, based on density of 0.78 g/ml: Merck 1989) from gestation day 8 until parturition. TBA "reduced maternal weight gain, litter sizes (from 11 to 3 pups per litter), birth weights, and weights at weaning and increased perinatal mortality (from 2% to 14%) and postnatal mortality (from 6% to 100%)." No other data was presented (Abel and Belitzke 1992).

In a well-reported study, rats were treated with TBA by inhalation at 0, 2000, 3500, or 5000 ppm for 7 h/day from gestation days 1-19. Rats were sacrificed on gestation day 20. Dose-related reductions in maternal weight gain were observed at all concentrations of TBA, but were statistically significant only at 5000 ppm. At 5000 ppm, narcosis was observed, and unsteady gait was observed at all concentrations of TBA. No effects on resorptions, live litter size, or malformations were found. Dose-related reductions in fetal

weight were observed at all concentrations: all were statistically significant. A dose-related increase in the number of skeletal variations (mainly reduced ossification) was found; these were statistically significant at 3500 and 5000 ppm (Nelson *et al.* 1989a).

In another study by the same group, reported only in abstract, rats were treated with TBA by inhalation at 0, 1660, or 3320 ppm (0, 6000, or 12000 mg/m^3) for 7 h/day from gestation days 1-19. Litters were culled to 8 pups at birth, and pups cross-fostered to untreated control dams. Reduced maternal food intake and weight gain was observed at 3330 ppm. In pups, few differences from controls were observed for behavioral measures (coordination, activity or learning). However, elevations in some brain neurotransmitters were observed. No other data were presented (Nelson *et al.* 1989b).

Neonatal rats were treated with TBA in milk formula by gastric cannula at 0 or 0.6-2.7 g/kg/d on postnatal days 4 to 7. No effect on pup weight gain was observed. Visible intoxication and reduced brain weight were observed. Interpretation of this study is complicated by the stress of the procedure: out of 48 pups, 15 died within 48 h due to the cannulation surgery, and 7 more died of gastric bloating (Grant and Samson 1982).

C.5 INTEGRATIVE EVALUATION

The literature on MTBE developmental toxicity includes 2 reports of developmental retardation in good quality animal studies that contain no inconsistencies or complications of interpretation due to maternal toxicity. The literature does not contain any studies of humans by any route of administration, any studies of animals using the oral route of administration, any studies of postnatal functional endpoints or any investigation of mechanism of toxicity.

In mice, inhalation of MTBE during organogenesis led to developmental delay as reflected in lower fetal weights at term, and in increased incidence of reduced skeletal ossification. These effects were dose dependent and represent adverse developmental effects. Two other developmental toxicity studies conducted with lower inhalation concentrations, 1 in rats and 1 in mice, did not identify fetal effects. An additional study in rabbits used the higher concentration range, but did not identify fetal effects. Thus, when dose and species are taken into account, there was no inconsistency between the studies.

In a two-generation study conducted in rats by the inhalation route, dose dependent postnatal growth retardation was observed. Notably, maternal weights during gestation and lactation were not influenced by MTBE exposure. Offspring were not directly exposed to MTBE vapor after birth in this study, but their postnatal development could have been indirectly influenced by maternal gestational and lactational MTBE exposure in addition to their *in utero* exposure and lactational exposure via milk. The existence and relative contribution of these influences cannot be determined. Together, the mouse developmental toxicity study and the rat two-generation study indicate that MTBE has the potential to produce developmental retardation.

Another potential endpoint of MTBE developmental toxicity is early postnatal pup death. Decreased viability prior to postnatal day 4 was reported in MTBE exposed groups in the one- and two-generation rat studies. This effect is attributable to *in utero* MTBE exposure, since the inhalation exposures were discontinued prior to parturition and reinstituted for the dams on postnatal day 5. The appearance of this effect in all the dose groups of the one-generation study (250, 1000, 2500 ppm), but in only the highest dose group (8000 ppm) of the two-generation study, may have been due to the continuation of exposure to within 1 day of parturition in the former study, as compared to 2 days prior to parturition in the latter. However, an entirely consistent interpretation of early postnatal death across experiments and dose groups is difficult to provide.

No studies have been conducted of potential mechanisms of action of MTBE in producing developmental toxicity. In rats and humans, MTBE is metabolized to tertiary butyl alcohol (TBA). There are no data on MTBE or TBA distribution to the fetus. Hence it is reasonable to assume that both compounds could potentially reach the fetus. TBA has not been mentioned in the literature as an active metabolite in connection with MTBE toxicity; however, TBA has been tested for developmental toxicity along with ethyl alcohol and other alcohols, and has been found to produce developmental delay when administered by inhalation to rats throughout gestation.

D. FEMALE REPRODUCTIVE TOXICITY

D.1 OVERVIEW

No human studies relevant to MTBE female reproductive toxicity were located. In experimental animals there are 2 inhalation reproductive toxicity studies, a one-generation study (Biles *et al.* 1987, Bio/dynamics, Inc. 1984d), and a two-generation study (Bevan *et al.* 1997a, Neeper-Bradley 1991). Both these studies were conducted in rats, and no reproductive toxicity studies in other species were identified. As with the developmental toxicity studies, both reproductive toxicity studies were conducted in commercial laboratories under quality control protocols, and the original study reports as well as reports from the peer-reviewed literature were available to OEHHA. Neither study reported any indication of effects on female fertility indices. The two-generation study reported postnatal effects of MTBE on offspring, which may have resulted from prenatal, postnatal or combined prenatal and postnatal exposure of dam and/or offspring. The study designs and results are outlined in Appendix 2 at the end of this document. Also included are chronic and subchronic toxicity studies with information on reproductive organ weight and/or histopathology.

Recently, possible endocrine-related effects of MTBE have been investigated in female mice (Moser *et al.* 1996, 1998). These studies are consistent with reduced estrogen action in association with MTBE exposure as reflected in reproductive organ weights and estrous cycle data. Mechanism studies have not been identified an MTBE biological action responsible for this effect. Also, studies of potential endocrine disruption by MTBE are currently being conducted in the laboratories of Drs. Ann de Peyster (Lee *et al.* 1995, Okahara *et al.* 1998) and Barry Wilson (Wilson *et al.* 1998).

D.2 ANIMAL FEMALE REPRODUCTIVE TOXICOLOGY STUDIES

D.2.1 Fertility, general toxicity and reproductive organ weights

No effects on female fertility indexes were reported in either the one-generation study rat inhalation study (250-2500 ppm MTBE) or the two-generation rat inhalation study (400-8000 ppm MTBE) (Table 5). The mating protocol in the one-generation study began with a 5-day period during which 1 male was placed with 2 females. Any females not mated at the end of the first 5-day period were placed with a different male for a second or third 5-day period. In the two-generation study, 1 male was placed with 1 female of the same treatment group for 7 days; partners in unmated pairs were switched for the remainder of the 3-week mating period.

An MTBE concentration toxic to female breeders was not reached in the one-generation study (Biles *et al.* 1987), but was included in the two-generation study (Bevan *et al.* 1997a, Neeper-Bradley 1991). The effects at the minimally toxic dose (8000 ppm) in the two-generation study, included reduced body weight, food intake, and body weight gain, and increased incidence of clinical observations including perioral salivation and encrustation. Also CNS depression was observed during exposure sessions in the group as a whole. Increased absolute and relative liver weights were reported in the 8000 ppm females of the F1 generation. The occurrence of these effects in female breeders of the two-generation study is detailed in Appendix 2 at the end of the document. The 3000 ppm concentration produced little male or female adult general toxicity. Body weights (but not body weight gain or food intake) of the 3000 ppm group were lower than controls during the first 4 weeks of the prebreeding period in the F_1 parental generation (F0 offspring) only. Notably, the F1 parental generation was growth retarded at weaning, 2-3 weeks prior to the beginning of the prebreeding period.

The two-generation study found greater lactational body weight gain in the 3000 ppm dams (F1) and 8000 ppm dams (F0 and F1) relative to controls. This was due to *less maternal weight loss* at the end of the lactation period, postnatal days 14 to 28. It is possible that this weight difference was due to an endocrine or other reproductive toxicity effect of MTBE. However, pups in the 3000 and 8000 ppm groups were smaller than controls during lactation and this may have resulted in lower energy requirements for lactation and reduced body weight loss in dams in late lactation.

Table 5. Fertility measures from rat MTBE reproductive toxicity studies.

A. One-Generation Study of Inhaled MTBE in CD Sprague-Dawley Rats

	MTBE (ppm)			
	0 (control)	**250**	**1000**	**2500**
Reproductive Parameters for F1a litter				
No. males cohoused	15	15	15	15
No. males mating	12	14	14	15
No. males fertile	11	14	13	14
No. females cohoused	30	30	30	30
Days to mating (females)(mean)	5.5	3.0	3.0	2.0
No. females mated	27	30	27	29
No. females pregnant	25	28	20	24
Gestation length in days (mean)	22.3	22.2	22.2	22.3
Reproductive Parameters for F2a litter				
No. males cohoused	15	15	15	15
No. males mating	12	14	14	12
No. males fertile	11	13	13	11
No. females cohoused	30	30	30	30
Days to mating (females)(mean)	3.0	3.0	2.5	6.0
No. females mated	25	29	29	25
No. females pregnant	22	23	23	19
Gestation length in days (mean)	22.1	22.1	22.2	22.2

B. Two-Generation Study of Inhaled MTBE In CD Sprague-Dawley Rats

	MTBE (ppm)			
	0 (control)	**400**	**3000**	**8000**
Reproductive Parameters for F0 Parents				
No. F0 pairs at start of F1 breed	25	25	25	25
No. plug/sperm-positive females	24	25	25	25
No. pregnant	23	22	25	22
No. live litters on postnatal day 0	23	22	25	21
Gestation length in days (mean)	22.1	22.0	22.0	21.9
Reproductive parameters for F1 Parents				
No. F0 pairs at start of F2 breed	25	25	25	25
No. plug/sperm-positive females	24	23	24	21
No. pregnant	22	23	22	21
No. live litters on postnatal day 0	22	23	22	21
Gestation length in days (mean)	22.0	22.0	22.0	21.9

Information on reproductive organs of rats from one- and two-generation studies was varied and incomplete, but there were no indications of MTBE effects. No effects on weight or histopathology of the ovaries were reported in the rat one-generation study (Biles *et al.* 1987). Female reproductive organs other than the ovaries were not examined. Reproductive organ weights were not obtained in the rat two-generation study; no exposure-related histopathology of female reproductive organs (vagina, uterus, ovaries) was reported when 25 females/generation in the control and 8000 ppm group were examined (Bevan *et al.* 1997a, Neeper-Bradley 1991). Information on organ weights from other studies is reviewed in Section D.2.3.1 below.

D.2.2 Postnatal effects in offspring

Postnatal offspring toxicity in the one- and two-generation rat studies included lower pup viability and body weights in MTBE groups compared to controls (Bevan *et al.* 1997a, Neeper-Bradley 1991). These results are outlined in Appendix 2 and described in detail in Section C.3. Because dams in these studies were exposed to MTBE during gestation and lactation, it is not possible to rule out a role for female reproductive toxicity in producing these effects.

D.2.3 Endocrine effects

Moser *et al.* (1996, 1998) have conducted studies in mice of potential antiestrogenic effects of MTBE. Endocrine modulating effects of MTBE were suggested by the tumor profile of endocrine sensitive organs in rodent oncogenicity studies (Moser *et al.* 1996). An additional suggestive finding was reduced incidence of uterine endometrial hyperplasia in the mouse inhalation cancer bioassays (Burleigh-Flayer *et al.* 1991), which suggested reduced estrogen action on the endometrium throughout the lifetime (MacGregor *et al.* 1993).

To follow up on the suggestion of reduced estrogen action from cancer bioassays, Moser *et al.* (1996, 1998) conducted studies in B6C3F1 mice aimed at both confirming this finding and elucidating its mechanism. These studies typically used the maximum inhalation exposure target concentration of 8000 ppm, as did the two-generation reproductive toxicity study reviewed above. However, the endocrine studies used only mice, while the reproductive toxicity studies used only rats.

The endocrine studies demonstrated a number of adverse effects of MTBE on the reproductive system of mice after 3 or 21 days, or 4 or 8 months exposure (6 h/day, 5 days/week) to 8000 ppm MTBE beginning at 8 weeks of age.

- lower relative uterine and ovarian weights compared to controls.
- lower rate of cell proliferation in the uterine, cervical and vaginal epithelium.
- changes in histology of the uterus, cervix and vagina indicative of decreased estrogen action
- increase in estrous cycle length.

The exposure concentrations, exposure periods and endpoints are outlined in Appendix 2 at the end of this document. In addition to negative controls (air or corn oil), ovariectomized mice were included as positive controls for hypoestrogenicity in these studies.

D.2.3.1 Uterine and ovarian weights

Moser *et al.* (1996, 1998) reported lower relative uterine and ovarian weights in the absence of body weight changes in mice exposed to MTBE at 8000 ppm for 21 days. Only relative uterine weights were affected after a 3-day exposure to 8000 ppm. After 4 and 8 month exposures, body weights, uterine and ovarian weights, and relative uterine and ovarian weights were significantly lower than in controls. These data are shown in Table 6.

Table 6. MTBE effects on female reproductive organ weights. (from Moser et al. 1996, 1998). Relative organ weight refers to organ weight/ body weight (g/g)

duration of exposure	Control	MTBE 8000 ppm
3 Days[1] (n = 5, 6)		
body weight (g)	20.5 ± 0.5[2]	19.7 ± 1.0
relative uterus weight	0.27 ±. 0.12	0.14 ± .02*
relative ovary weight	0.028 ± .006	0.028 ± .004
21 Days[1] (n = 5, 6)		
body weight (g)	22.3 ± 2.6	23.7 ± 2.8
relative uterus weight	0.31 ± 0.14	0.11 ± 0.04*
relative ovary weight	0.032 ± .007	0.020 ± 0.004*
4 Months[3] (n = 10 - 12)		
body weight (g)	36.8 ± 4.3	30.5 ± 1.5*
relative uterus weight	0.524 ± 0.210	0.109 ± 0.077*
relative ovary weight	0.030 ± 0.004	0.019 ± 0.004*
8 Months[3] (n = 10 - 12)		
body weight (g)	39.9 ± 7.0	32.4 ± 2.3*
relative uterus weight	0.738 ± 0.325	0.169 ± 0.050*
relative ovary weight	0.023 ± 0.006	0.012 ± 0.003*

[1] Absolute uterus and ovary weight not provided.
[2] Mean ± SD.
[3] MTBE effect also significant for absolute uterus and ovary weights.
*difference from controls is statistically significant, p<.05.

Data on uterine and ovarian weight from subchronic, chronic, or reproductive toxicity studies of MTBE have not reported similar effects. Notably, none of the available subchronic, chronic and reproductive toxicity studies used mice; all were conducted in

rats. (The inhalation oncogenicity study in mice conducted by Burleigh-Flayer *et al.* [1992] did not report data on ovarian or uterine weight.) Also, none of these studies used the same doses and durations of exposure as Moser *et al.* (1996, 1998). As noted above (Section D.2.1) no effects on ovarian weight were found in rat one- and two-generation reproductive toxicity studies conducted by inhalation. In a 13-week inhalation toxicology study in rats using exposure concentrations of 0, 250, 500 and 1000 ppm, no MTBE effects on ovarian or uterine absolute or relative weights were reported (Greenough *et al.* 1980). No effects on ovary weights were reported in 3 subchronic rat oral gavage studies; a 14-day study using 0, 357, 714, 1071, and 1428 mg MTBE/kg/day (Robinson *et al.* 1990), a 28-day study using 90, 440 and 1750 mg MTBE/kg/day (IIT, 1992); or a 90-day study using 0, 100, 300, 900, and 1200 mg MTBE/kg/day (Robinson *et al.* 1990). Uterine weights were not obtained in these studies.

D.2.3.2 Uterine and ovarian histology and cell proliferation

Uterine glands of MTBE exposed mice were described as less tortuous than those of untreated controls, and similar to those of ovariectomized controls (Moser *et al.* 1998). Cell proliferation studies identified decreased DNA synthesis in luminal and glandular epithelial cells, but not in stromal cells, of the uterus in MTBE treated mice. As described by the authors, epithelial cell proliferation is responsive to estrogen while stromal cells are more responsive to progesterone. Thus, the authors concluded that the effect was cell specific and indicative of reduced estrogen action. Ovarian histology did not differ from controls in terms of follicular development and BrdU labeling (cell proliferation) between MTBE-treated and control mice.

D.2.3.3 Pituitary and adrenal

Weights, histopathology and cell proliferation were measured in the pituitary and the adrenal gland because of their involvement in regulation of steroid metabolism. Absolute and relative pituitary weights were lower in mice exposed to MTBE for 4 or 8 months (Moser *et al.* 1998). No interpretation was provided by the authors; however, decreased pituitary weights can result from decreased estrogen stimulation (U.S. EPA, 1996). Adrenal weights were not affected, but histopathological examination revealed loss of the zona reticularis. The zona reticularis of the adrenal cortex is involved in the synthesis of adrenal androgens as well as glucocorticoids (corticosterone and cortisol) and is sensitive to gonadal hormone regulation. However, loss of the zona reticularis was not seen in ovariectomized mice. No MTBE influences on BrdU labeling in pituitary or adrenal were noted.

D.2.3.4 Estrous cycle length

Moser *et al.* (1998) collected vaginal lavages on successive days (at least 10) from mice exposed for 8 months to 8000 ppm MTBE and determined the length of the estrous cycle as the number of days between slides indicating estrus (solely cornified epithelial cells). A significantly longer estrous cycle was reported in the MTBE-exposed mice (average 6.4 days) relative to controls (average 4.2 days). In discussing these results, the authors state that lengthened estrous cycles are compatible with an antiestrogenic effect of MTBE, but also with MTBE-induced decreased body weight gain, anesthesia-like effects, or stress.

D.2.3.5 Mechanism of action

Moser *et al.* (1996) found that estrogen metabolism was increased twofold in hepatocytes isolated from mice exposed to 1800 mg MTBE /kg/day by gavage for 3 days. This change was associated with greater liver weight and hepatic cytochrome P450 content. This series of experiments suggested that MTBE might lower circulating estrogen concentrations by enhancing estrogen metabolism. Later studies failed to find changes in serum estrogen concentrations when virgin female mice were exposed by inhalation to 8000 ppm MTBE for 4 or 8 months (Moser *et al.* 1998). However, because of ovarian cyclicity, group differences in circulating estrogen are difficult to identify. An independent study (Lee *et al.* 1995) failed to find changes in serum estrogen concentration in CD-1 mice treated by gavage with 200 or 600 mg MTBE/kg every other day for 28 days.

A further series of *in vitro* studies (Moser *et al.* 1998) failed to find evidence that MTBE endocrine effects were mediated by the estrogen receptor. The binding of MTBE, TBA and formaldehyde to the estrogen receptor, and MTBE effects on estrogen receptor activation and translocation in a transfection assay were evaluated. Also, no changes in expression of estrogen receptor were found in the uterus, cervix or vagina of MTBE exposed mice. The authors suggested that MTBE may exert an antiestrogenic action by a mechanism which does not involve a change in circulating estrogen or in estrogen receptor binding.

Moser *et al.* (1998) also considered whether MTBE disrupted the pituitary-ovarian axis. They reported that absolute and relative pituitary weights were decreased after 4 and 8 months exposure to 8000 ppm MTBE. However, the presence of estrous cycles and normal circulating estrogen concentrations suggested to the authors that MTBE was not directly toxic to the ovary or pituitary, but rather acted on estrogen target tissues to produce antiestrogenic effects.

The authors further considered whether reduced food intake (caloric restriction) was an indirect mechanism of MTBE effects on reproductive organs. This was not considered an adequate explanation because the 3- and 21-day exposures used by Moser *et al.* (1996) influenced uterine and ovarian weights in the absence of effects on body weight.

Nonspecific adrenal activation has also received attention as a possible mediating mechanism. Glucocorticoids inhibit luteinizing hormone (LH), estrogen and progesterone production and also the responsiveness of target tissues to estrogen (Magiakou *et al.* 1997). Moser *et al.* (1998) reported an increased number of hyaline droplets in the pituitary that were immunoreactive for adrenocorticotropic hormone (ACTH). They also reported absence of the zona reticularis of the adrenal in MTBE exposed mice; however, not enough information was provided concerning effects on the zona reticularis to determine whether this effect might be related to glucocorticoid production.

Systemic concentrations of adrenocortical hormones were not measured in the Moser *et al.* studies. However, some information is available from other studies The only available information on mice comes from sampling conducted after 80 weeks of an inhalation oncogenicity study using 0, 400, 3000 and 8000 ppm MTBE (Burleigh-Flayer *et al.* 1992). Significantly elevated corticosterone concentrations were found in males (n=10/group) exposed to 8000 ppm MTBE. Corticosterone concentrations were also elevated in the females, but control values were higher and more variable than in males, and group differences were not significant. Relative adrenal weights of the 8000 ppm group males, but not females, were greater than controls at the end of the study. Corticosterone measures were also included in several studies of rats. At the conclusion of a 13-week exposure study using inhalation concentrations of 800, 4000 and 8000 ppm MTBE, elevated corticosterone was found in both male and female rats (Dodd and Kintigh 1989). In the MTBE rat inhalation oncogenicity study (Chun *et al.*, 1992), serum corticosterone concentrations were elevated in males, but not females in the 3000 ppm exposure group; however serum corticosterone was significantly lower than controls in the males of the 8000 ppm exposure group. Interpretation of group comparisons for corticosterone is complicated in this study because sampling was conducted after different durations of exposure in the various groups. Finally, in a recent study reported as an abstract, MTBE gavage (40, 400, 800 mg/kg, 28 days) of male rats lead to greater plasma corticosterone concentrations relative to controls (Day *et al.* 1998). Adrenal activation by MTBE would be consistent with a similar well known effect of diethyl ether, another alkyl ether (Glowa 1993).

The consequences of the implied reduction in estrogen action associated with MTBE in mice are not known; unfortunately, there are no reproductive toxicity studies in mice. Some general information is available concerning the relationship between fertility and changes in ovarian weight and estrous cycle length of mice. In summarizing 72 reproductive toxicity studies in CD-1 mice using the RACB (Reproductive Assessment by Continuous Breeding) protocol, Chapin *et al.* (1997) found no relationship between ovarian weight and fertility parameters, but stated that “if estrous cycle length was increased, it was likely that female reproduction would be adversely affected”.

It is also not clear whether similar effects occur in other species, at other doses, or with longer exposures, since parallel studies have not been done. One of the effects demonstrated by Moser *et al.* (1996, 1998) was on uterine and ovarian weights. Although weighing of reproductive organs is frequently included in subchronic and chronic toxicity studies, the studies available for MTBE do not include these data at doses comparable to those used by Moser *et al.* (see Appendix 2). The specificity of the effect also needs to be determined: unleaded gasoline has been found to have some antiestrogenic effects similar to MTBE (MacGregor *et al.* 1993; Moser *et al.* 1996; Standeven *et al.* 1994).

Endocrine disrupting effects of MTBE have recently been investigated in mice in the laboratory of Dr. Ann de Peyster at San Diego State University. The hypothesis was that MTBE could function as an antiestrogen by inducing liver cytochrome P450, thus increasing estradiol metabolic breakdown. A 1995 abstract (Lee *et al.* 1995) reported

increased hepatic P450 content in adult female CD-1 mice treated by gavage with 600 mg MTBE/kg 3 times/week for 1 month. A lower dose (200 mg/kg) led to a similar mean P450 content, but the difference was not significant at this dose. CYP3A2 was also measured but interpretation of MTBE effects was complicated by differences between untreated and corn oil (vehicle) gavage controls, as well as variability attributed to stage of estrus cycle. Serum estradiol concentrations, uterine, ovarian, liver or kidney weights, the number of corpora lutea and vaginal cytology were not influenced by MTBE treatment. Body weights of the MTBE groups were not lower than those of corn oil gavage controls, but were lower than those of untreated controls.

A more recent study reported as an abstract (Okahara *et al.* 1998) used the immature mouse uterotrophic assay to evaluate endocrine disrupting effects of MTBE. CD-1 mice were given 600 or 1500 mg MTBE/kg by gavage. There were no effects on uterine or ovarian weights or uterine peroxidase activity of MTBE alone, and there was no enhancement or antagonism of the effect of estradiol on these endpoints. Similarly there was no consistent, statistically significant effect on time of vaginal opening or on vaginal cytology. Liver and kidney weights were also comparable to controls. The authors noted that, grossly, the uteri of both MTBE and estradiol treated mice had a translucent surface and that follow-up histological studies were being done. The authors pointed out that the immature mouse assay might not be sensitive to antiestrogenic agents which act indirectly via altered hepatic metabolism, rather than directly on estrogen target tissues, due to the immaturity of hepatic metabolism.

D.3 OTHER RELEVANT DATA

Studies examining the distribution of MTBE or TBA to female reproductive organs are not available. As discussed in section B.4, MTBE and TBA are expected to be widely distributed in the body. It is therefore likely that they reach the female reproductive organs.

This section will briefly review the studies of the female reproductive effects of TBA. As discussed in section B.4, there is ample evidence that TBA is produced from MTBE and is widely distributed. In contrast, there has been no direct examination of the fate of formaldehyde, the putative other first metabolite of MTBE. It is not clear whether formaldehyde appears in circulation following MTBE exposure, or is metabolized to other compounds, or what the fate of those compounds might be. Given this uncertainty, a review of female reproductive effects of formaldehyde is not included in this document.

No studies examining female reproduction with TBA were located. The National Toxicology Program (NTP) has performed 13-week and 2-year studies in rats and mice, which examined some female reproductive endpoints. These studies were performed because of interest in the TBA as a solvent and chemical intermediate. In the 13-week studies, alterations in estrous cycle length in rats and mice were observed at the high exposure concentration. Systemic toxicity, including increased death and reduced body

weight, was also observed at this exposure concentration. These studies did not report data on ovarian weight.

Female rats were treated for 13 weeks with TBA in drinking water at 0, 2.5, 5, 10, 20, or 40 mg/mL. At 40 mg/mL, 6/10 rats died. Other toxic effects, including reduced body weight, increased kidney and liver weight, hypoactivity and ataxia were observed at various concentrations. No effects on estrous cycle length were found up to 20 mg/mL (range 4.9 to 5.5 days). At 40 mg/mL, of the 4 surviving rats, 3 had a cycle length that was longer than 7 days or was unclear, and 1 had no cycle (NTP 1995).

In another study, female rats were treated for 2 years with TBA in drinking water at 0, 2.5, 5, or 10 mg/mL. Reduced survival was observed at 10 mg/mL. Other toxic effects, including reduced body weight, increased kidney weight, increased severity of nephropathy, and hyperactivity were observed at various concentrations. No increase in ovarian histopathological effects was found (NTP 1995).

Female mice were treated for 13 weeks with TBA in drinking water at 0, 2.5, 5, 10, 20, or 40 mg/mL. Increased death (1/10) was attributed to TBA exposure at 40 mg/mL; 3 other deaths in this group were attributed to other causes. Other toxic effects, including reduced body weight and increased kidney weight were observed at various concentrations. Estrous cycle length was significantly increased at 40 mg/mL (control 3.90 days, 40 mg/ml 5.00 days) (NTP 1995).

In another study, female mice were treated for 2 years with TBA in drinking water at 0, 5, 10, or 20 mg/mL. No reduced survival was observed. Reduced body weight was observed at 10 and 20 mg/mL. No increase in ovarian histopathological effects was found (NTP 1995).

D.4 INTEGRATIVE EVALUATION

No human studies were located in the area of female reproductive toxicity. The animal literature contains 2 major types of data, studies using standard one-and two-generation reproductive toxicity designs, and more recent studies of endocrine disrupting effects. These are difficult to integrate because the reproductive toxicity studies were conducted in rats and the endocrine studies in mice.

No effects on female fertility indexes were found in rat inhalation one- and two-generation studies. Reduced postnatal survival and postnatal offspring growth retardation were reported in the one- and two-generation studies. These postnatal effects could potentially be due to reproductive toxicity in the dams (altered lactational performance).

A series of studies of potential antiestrogenic effects of MTBE in mice reported effects on ovarian and uterine weights, uterine histology and estrous cycles. A very striking 50% reduction in ovarian weight was accompanied by a lengthening of the estrous cycle, but unfortunately, nothing is known about the fertility of these mice. Only 1 MTBE inhalation dose was used in these studies, so that dose-dependence cannot be evaluated.

Other ongoing studies using gavage exposure in mice did not identify antiestrogenic effects, but administered doses were lower than for the inhalation studies.

Potential mechanisms of antiestrogenic effects were also studied. MTBE exposure induces hepatic P450 and has been shown to enhance estradiol metabolism *in vitro*. MTBE was not found to influence estrogen receptor binding, activity or expression. Endocrine suppression secondary to adrenal activation has been proposed as a possible mechanism, but has not been studied. There is no empirical information concerning distribution of MTBE and TBA to reproductive organs, but physical properties suggest wide distribution to soft tissues.

E. MALE REPRODUCTIVE TOXICITY

E.1 OVERVIEW

No human studies relevant to male reproductive toxicity were identified. A rat one-generation reproductive toxicity study and a rat two-generation reproductive toxicity study (see section D above) are the only relevant animal studies, and they did not report effects on male fertility indices. Very limited data are available on male endocrine function. Reproductive organ weights and histopathology are available from some chronic and subchronic studies of MTBE and TBA. The studies are outlined in Appendix 3.

E.2 ANIMAL MALE REPRODUCTIVE TOXICITY STUDIES

E.2.1 Fertility and general toxicity

There were no effects on male fertility indexes in 2 available rat reproductive toxicity studies (see Appendix 3). Sperm parameters were not evaluated in these studies. There were no effects on testes weights in the male breeders in the one-generation study; testes weights were not reported in the two-generation study. No statistically significant effects on male reproductive organ histopathology (testes, epididymides, seminal vesicles, prostate) were reported in either study. No reproductive organ weight or histopathology effects were reported in MTBE subchronic or chronic studies, as detailed in Appendix 3. Increased atrophy of the seminiferous tubules was found in the rat inhalation oncogenicity study (Chun *et al.* 1992) but this study involved extensive mortality in males prior to completion of the study. This effect was not seen in the mouse inhalation toxicity study (Burleigh-Flayer *et al.* 1992), which did not involve extensive mortality.

An MTBE concentration toxic to male breeders was not reached in the one-generation study (Biles *et al.* 1987), but was included in the two-generation study (Bevan *et al.* 1997a, Neeper-Bradley 1991). The occurrence of general toxicity at the minimally toxic dose (8000 ppm) in the male breeders is detailed in Appendix 3. The 3000 ppm concentration did not produce general toxicity in the male breeders. Increased absolute liver weights (8000 ppm) and increased relative liver weights (3000 and 8000 ppm) were reported in the F1 generation.

E.2.2 Endocrine effects

Male endocrine effects have not been addressed in detail. The increased incidence of interstitial cell hyperplasia and Leydig cell tumors in oncogenicity studies (Chun *et al.* 1992, Belpoggi *et al.* 1995) has led to investigation of potential hormonal disrupting effect of MTBE in males. Because MTBE is not genotoxic, other mechanisms of carcinogenesis require investigation. A recent abstract from the laboratory of Dr. Ann de Peyster (Day *et al.* 1998) reported a study in which male rats were gavaged daily for 28 days with 40, 400 or 800 mg MTBE/kg. After 28 days plasma testosterone was significantly lower in the 800 mg/kg group (about 50% of control values). The authors characterize testosterone as "slightly reduced". There were no group differences in plasma LH or prolactin. No changes were reported in "androgen dependent organ weights" or in testicular mitochondrial or microsomal P450 or 17-α hydroxyprogesterone content as a consequence of MTBE exposure. The abstract also reported an increase in plasma corticosterone in all 3 MTBE exposed groups relative to controls after 14 days exposure, and in the 800 mg/kg group only after 28 days exposure. In *in vitro* studies, there was no increase in hCG stimulated testosterone production when decapsulated testes were incubated in MTBE or TBA. Liver enzyme induction and corticosteroid suppression of endocrine function were mentioned as potential mechanisms.

Ongoing studies in mice at the University of California Davis are evaluating testosterone production in male mice treated by gavage with MTBE (Wilson *et al.* 1998).

E.3 OTHER RELEVANT DATA

Studies examining the distribution of MTBE or TBA to male reproductive organs are not available. As discussed in section B.4, MTBE and TBA are expected to be widely distributed in the body, and it is therefore likely that they reach the male reproductive organs.

This section will briefly review the studies of the male reproductive effects of TBA. As discussed in section B.4, there is ample evidence that TBA is produced from MTBE and is widely distributed. In contrast, there has been no direct examination of the fate of formaldehyde, the putative other first metabolite of MTBE. It is not clear whether formaldehyde appears in circulation following MTBE exposure, or is metabolized to other compounds, or what the fate of those compounds might be. Given this uncertainty, a review of male reproductive effects of formaldehyde is not included in this document.

No studies examining male reproduction with TBA were located. NTP has performed 13-week and 2-year studies in rats and mice, which examined some male reproductive endpoints. There is also 1 *in vitro* study of mouse sperm fertilization capacity. These studies were performed because of interest in the TBA as a solvent and chemical intermediate, and for comparison to ethanol. In the 2-year mouse study, an increase in the incidence of degeneration of the germinal epithelium of testes was observed at the high exposure concentration. Systemic toxicity, including reduced survival and transiently reduced body weight, was also observed at this exposure concentration. Effects on

testicular histopathology were not found in the 2-year rat study. No adverse effects on testis weight, sperm concentration, motility, or abnormalities were found in the 13-week studies. In the 13-week mouse study, absolute testes weights were reduced at the high concentration, but relative testes weights were increased, due to reduced body weight. Increased death and other toxic effects were also observed at this concentration. Adverse effects on testes weights were not found in the 13-week rat study. No effect of TBA on *in vitro* mouse sperm fertilization capacity was found.

Male rats were treated for 13 weeks with TBA in drinking water at 0, 2.5, 5, 10, 20, or 40 mg/mL. At 40 mg/mL, 10/10 rats died. Other toxic effects, including reduced body weight, increased kidney and liver weight, hypoactivity and ataxia were observed at various concentrations. Absolute testis weight was not altered, but relative testis weight was significantly increased due to reduced body weight at 10 and 20 mg/mL. No effects on sperm concentration, motility, or abnormalities were found (NTP 1995).

In another study, male rats were treated for 2 years with TBA in drinking water at 0, 1.25, 2.5, or 5 mg/mL. Reduced survival was observed at 5 mg/mL. Other toxic effects, including reduced body weight, increased kidney weight, and increased kidney mineralization were observed at various concentrations. No testicular histopathological effects were found (NTP 1995).

Male mice were treated for 13 weeks with TBA in drinking water at 0, 2.5, 5, 10, 20, or 40 mg/mL. Increased death (2/10) was attributed to TBA exposure at 40 mg/mL; 4 other deaths in this group were attributed to other causes. Other toxic effects, including reduced body weight, ataxia and hypoactivity were observed at various concentrations. Significantly reduced absolute testes weight was found at 40 mg/mL. However, relative testes weight was significantly increased at 20 and 40 mg/mL due to reduced body weight. No effects on sperm concentration, motility, or abnormalities were found (NTP 1995).

In another study, male mice were treated for 2 years with TBA in drinking water at 0, 5, 10, or 20 mg/mL. Reduced survival was observed at 20 mg/mL. A transient reduction in average body weight was also found at 20 mg/mL. An increase in the incidence of degeneration of germinal epithelium of testes was also observed (10% at 20 mg/mL compared to 3% in controls) (NTP 1995).

An *in vitro* study examined the ability of mouse sperm to fertilize oocytes after treatment of sperm with TBA or ethanol. At 87 mM TBA, no effect was found. In contrast, ethanol at the same concentration significantly reduced fertilization (Anderson *et al.* 1982).

E.4 INTEGRATIVE EVALUATION

No relevant human studies were located. Very restricted information is available from animal studies. In rat inhalation one- and two-generation studies, no effects on male fertility indexes were found. No effects on testis weight or histopathology were reported

in subchronic and chronic toxicity studies. MTBE led to lower plasma testosterone after a 28-day gavage treatment in rats, as reported in a recent abstract. Liver enzyme induction and adrenal corticosteroid response were mentioned as possible mechanisms. No studies of MTBE effects on sperm parameters were available. Studies of TBA effects on sperm parameters did not find effects.

F. SUMMARY

F.1 DEVELOPMENTAL TOXICITY

A developmental toxicity study conducted by inhalation in mice reported a dose-dependent effect of MTBE on fetal weight and skeletal ossification. A two-generation study in rats reported effects of MTBE on early postnatal death and postnatal growth. Although the literature is limited, there are no contradictory findings. These data can be taken to indicate the potential of MTBE for producing developmental toxicity.

F.2 FEMALE REPRODUCTIVE TOXICITY

No effects of MTBE on female fertility were reported in 2 available reproductive toxicity studies in rats. Recent studies of potential endocrine effects reported lower relative uterine and ovarian weights and lengthened estrous cycles in female mice treated with 8000 ppm MTBE by inhalation. Lower doses by the oral route did not influence uterine weight in mice. Mechanisms of action have been studied but not identified.

F.3 MALE REPRODUCTIVE TOXICITY

Relevant studies are limited in variety, but a rat two-generation study using a minimally toxic inhalation dose failed to identify effects of MTBE on male fertility indexes. Chronic and subchronic studies did not identify effects on reproductive organs. Potential MTBE effects on testosterone are currently being investigated.

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APPENDIX 1. Study summary tables: developmental effects of MTBE**.**

APPENDIX

Animal studies of the developmental effects of MTBE.

(studies in alphabetical order by author)

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Bevan *et al.* 1997b, Tyl and Neeper-Bradley 1989	Mouse (CD-1) Inhalation Gd 6-15, 6 h/day Target concentrations: 0, 1000, 4000, 8000 ppm Analytical concentrations: 0, 1035, 4076, 8153 ppm n=30/group	No increased pre-implant loss, early resorptions, or skeletal malformations. 8000 ppm: Increased post-implant loss (late resorptions and dead fetuses) (SS), reduced live litter size (SS), altered sex ratio (less males) (SS), increased cleft palate (SS) (resulting in increased pooled external malformations, soft tissue malformations, and total malformations (SS)), reduced fetal weight (SS), increased incidence of some skeletal variations (mainly reduced ossification) (SS). 4000 ppm: Reduced fetal weight (SS), increased incidence of some skeletal variations (mainly reduced ossification) (SS).	No maternal death, or altered liver weight. 8000 ppm: Reduced maternal body weight (SS), reduced body weight gain (SS), reduced food consumption during treatment period (SS). Clinical signs (individual observations): maternal hypoactivity (SS), ataxia (SS), prostration (SS), labored respiration (SS), lacrimation (SS), periocular encrustation (SS). Clinical signs (group observations during daily exposure periods): maternal hypoactivity, ataxia, labored breathing. 4000 ppm: Clinical signs (group observations during daily exposure periods): maternal hypoactivity, ataxia.
Bevan *et al.* 1997b, Tyl 1989	Rabbit (New Zealand White) Inhalation Gd 6-18, 6 hours/day Target concentrations: 0, 1000, 4000, 8000 ppm Analytical concentrations: 0, 1021, 4058, 8021 ppm n=15/group	No increased pre- or post-implant loss, reduced litter size, altered sex ratio, reduced fetal weight, increased malformations, or increased skeletal variations.	No maternal death, reduced body weight, or clinical signs of toxicity before or after daily exposure periods. 8000 ppm: Reduced maternal body weight gain (gd 6-12) (SS) (resulting in reduced body weight gain gd 6-18 (SS)), reduced food consumption (gd 6-11, 13-14) (SS) (resulting in reduced food consumption gd 6-18 (SS)), increased relative liver weight (SS). Clinical signs (group observations during daily exposure periods): hypoactivity, ataxia. 4000 ppm: Reduced maternal body weight gain (gd 6-9) (SS), reduced food consumption (gd 6-8, 9-10) (SS).

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Bevan *et al.* 1997a, Neeper-Bradley 1991	Rat (Sprague-Dawley) Inhalation 2 generation reproductive Target concentrations: 0, 400, 3000, 8000 ppm Analytical concentrations: 0, 402, 3019, 8007 ppm Male: 6 hours/day, 10 weeks (5 days/week) + mating + gestation Female: 6 hours/day, 10 weeks (5 days/week) + mating + gestation (gd 1-19) + lactation (pnd 5-28) Exposures for F_0 starting at pnd 42, and F_1 starting on pnd 29-31. Pups not placed in inhalation chambers during lactation. n=25 males/group, 25 females/group	No ovarian, uterine, or vaginal histopathological effects, testicular or other male reproductive organ histopathological effects, reduced mating (F_0, F_1), reduced fertility (F_0, F_1), reduced live litter size (F_1, F_2), reduced postnatal survival after pnd 4 (F_2), reduced live birth, 4-day survival, or lactation indices (F_1, F_2), reduced litter size at end of lactation (F_1), or reduced lactation day one weight (F_1, F_2). 8000 ppm: Increased dead pups pnd 4 (F_1, F_2) (SS), reduced litter size at end of lactation (F_2) (SS), reduced postnatal weight (F_1: pnd 14-28, F_2: pnd 7-28) (SS), reduced postnatal weight gain (F_1: pnd 7-21, F_2: pnd 1-21) (SS). 3000 ppm: Increased dead pups pnd 4-28 (F_1) (SS) (NOT at 8000 ppm), reduced postnatal weight (F_1: pnd 4, 14, F_2: pnd 14-28) (SS), reduced postnatal weight gain (F_1: pnd 1-4, 7-14, F_2: pnd 7-21) (SS).	No adult male or female deaths (F_0 or F_1), reduced adult female body weight (F_0), reduced adult female body weight gain (F_1), or reduced adult female food consumption (F_0). 8000 ppm: Reduced adult male body weight (F_0, F_1) (SS), reduced adult male body weight gain (F_0: weeks 0-3, 5-7; F_1: weeks 0-2, 5-6), reduced adult female body weight (F_1: weeks 0-8, not gestation or lactation) (SS), reduced adult female body weight gain (F_0: weeks 0-1, 5-6, not gestation or lactation) (SS), increased female body weight gain during lactation (F_0, F_1) (SS), increased adult male and female absolute and relative liver weights (F_1) (SS), reduced adult female food consumption (F_1: lactation days 7-14, not pre-breed or gestation) (SS). Clinical signs (individual observations): adult male, perioral wetness (F_0, F_1), perioral encrustation and salivation (F_1); adult female, perioral wetness (F_0, F_1), perioral encrustation, salivation and urine stains (F_1). Clinical signs (group observations during daily exposure periods): adult male and female, ataxia (F_0, F_1), hypoactivity (F_0, F_1), blepharospasm, lack of startle reflex (F_0, F_1). 3000 ppm: Reduced adult male body weight (F_1: weeks 0-3) (SS), reduced adult male body weight gain (F_0: weeks 1-2) (SS), reduced adult female body weight (F_1: weeks 0-4) (SS), increased adult female body weight gain (F_1: lactation) (SS), increased adult male relative liver weights (F_1) (SS). Clinical signs (group observations during daily exposure periods): adult male and female, hypoactivity (F_0, F_1), blepharospasm, lack of startle reflex (F_0, F_1).

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Biles *et al*. 1987 Bio/ dynamics 1984d	Rat (Sprague-Dawley) Inhalation Reproductive: 1 generation, 2 litter Male: 6 hours/day; 12 weeks (5 days/week), + first mating (2 weeks, daily), + 8 weeks (5 days/week), + second mating (2 weeks, daily). Female: 6 hours/day; 3 weeks (5 days/week), + first mating (daily) + first gestation (gd 0-20) + first lactation (pnd 5-21) + 2 weeks (5 days/week) + second mating (daily) + second gestation (gd 0-20) + second lactation (pnd 5-21) Target concentrations: 0, 250, 1000, 2500 ppm. Nominal concentrations[3] (M/F): 0/0, 290/300, 1300/1300, 3400/3400 ppm. Analytical concentrations[3] (M/F): 0/0, 290/300, 1190/1240, 2860/2980 ppm. n=15 males/group, 15 females/group	No altered testes or ovary weight (F_0), adverse histopathological effects on ovaries or testes (F_0), reduced mating, reduced male fertility, reduced female fertility (pregnancy rate), reduced litter size (live or total) (F_{1a}, F_{1b}), altered sex ratio (F_{1a}, F_{1b}), reduced pup viability at birth (live/total) (F_{1a}), reduced birth weight (F_{1a}, F_{1b}). reduced pup survival on pnd 4 (F_{1b}), or reduced pup survival on pnd 21 (F_{1a}, F_{1b}). 2500 ppm: Reduced pup viability at birth (live/total) (F_{1b}) (SS). (Note high in controls: control 99%, 1000 and 2500 ppm 95.5%. Authors discount biological significance), reduced postnatal weight on pnd 14, 21 (F_{1a}, F_{1b}) (NOT SS). 1000 ppm: Reduced pup viability at birth (live/total) (F_{1b}) (SS). (Note high in controls: control 99%, 1000 and 2500 ppm 95.5%. Authors discount biological significance), reduced pup survival from pnd 0-4 (F_{1a}) (NOT 2500 ppm), reduced postnatal weight on pnd 14, 21 (F_{1a}, F_{1b}) (NOT SS) 250 ppm: Reduced pup survival from pnd 0-4 (F_{1a}) (NOT 2500 ppm) (SS).	No adult male or female death, or reduced adult male or female body weight. 2500, 250 ppm: Increased incidence dilated renal pelves in females (NOT 1000 ppm).

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Conaway *et al.* 1985, Bio/dynamics 1984a	Rat (Sprague-Dawley) Inhalation Gd 6-15, 6 hours/day Target concentrations: 0, 250, 1000, 2500 ppm Analytical concentrations[3]: 0, 250, 1000, 2430 ppm. Nominal concentrations[3]: 0, 260, 1100, 3300 ppm. n=25/group	No increased pre- or post-implant loss, reduced live litter size, reduced fetal weight, reduced crown-rump distance, altered sex ratio, increased malformations, or increased ossification variations.	No maternal death, reduced maternal weight, altered water consumption, or altered liver weight. 2500, 1000, 250 ppm: Reduced maternal food consumption on gd 9-12 (SS).
Conaway *et al.* 1985, Bio/dynamics 1984b	Mouse (CD-1) Inhalation Gd 6-15, 6 hours/day Target concentrations: 0, 250, 1000, 2500 ppm Analytical concentrations[3]: 0, 280, 1110, 2710 ppm. Nominal concentrations[3]: 0, 280, 1200, 3500 ppm. n=30/group	No increased pre- or post-implant losses, reduced live litter size, reduced fetal weight, reduced crown-rump distance, altered sex ratio, or increased malformations. [Fetuses with skeletal malformations: control, 1.6%; 250 ppm, 1.7%; 1000 ppm, 2.4%; 2500 ppm, 3.1% (NOT SS). Litters with skeletal malformations: control, 7.4%, 250 ppm, 11.5%, 1000 ppm 16.0%, 2500 ppm, 22.2% (NOT SS).]	No maternal death, reduced maternal weight, altered food or water consumption, or altered liver weight.

[1] Abbreviations: gd = gestation day, pnd = postnatal day.
[2] Effects reported by authors to be statistically significant (SS) or discussed by authors in text.
[3] Analytical concentrations measured every 30 minutes by calibrated Miran infrared analyzer. Nominal concentrations calculated from daily weight of MTBE used divided by daily air volume. Authors comment that a manifold leak and aerosol particles may have caused an underestimation in the analytical concentrations (Biles *et al.* 1987, Bio/dynamics 1984a, 1984b, 1984d, Conaway *et al.* 1985).

APPENDIX

APPENDIX 2. Study summary tables: female reproductive effects of MTBE.

APPENDIX

Animal studies of the female reproductive effects of MTBE

(studies in alphabetical order by author)

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Belpoggi *et al.* 1995	Rat (Sprague-Dawley) Oral (gavage) Female 104 weeks, 4 days/week 0, 250, 1000 mg/kg/day n=60/group	No ovarian histopathological effects.	Female: No reduced weight gain, or reduced food consumption. 250, 1000 mg/kg/day: Increased death (dose-related, SS not addressed).

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Bevan *et al.* 1997a, Neeper-Bradley 1991	Rat (Sprague-Dawley) Inhalation 2 generation reproductive Target concentrations: 0, 400, 3000, 8000 ppm Analytical concentrations: 0, 402, 3019, 8007 ppm Male: 6 hours/day, 10 weeks (5 days/week) + mating + gestation Female: 6 hours/day, 10 weeks (5 days/week) + mating + gestation (gd 1-19) + lactation (pnd 5-28) Exposures for F_0 starting at pnd 42, and F_1 starting on pnd 29-31. Pups not placed in inhalation chambers during lactation. n=25 males/group, 25 females/group	No ovarian, uterine, or vaginal histopathological effects, testicular or other male reproductive organ histopathological effects, reduced mating (F_0, F_1), reduced fertility (F_0, F_1), reduced live litter size (F_1, F_2), reduced postnatal survival after pnd 4 (F_2), reduced live birth, 4-day survival, or lactation indices (F_1, F_2), reduced litter size at end of lactation (F_1), or reduced lactation day one weight (F_1, F_2). 8000 ppm: Increased dead pups pnd 4 (F_1, F_2) (SS), reduced litter size at end of lactation (F_2) (SS), reduced postnatal weight (F_1: pnd 14-28, F_2: pnd 7-28) (SS), reduced postnatal weight gain (F_1: pnd 7-21, F_2: pnd 1-21) (SS). 3000 ppm: Increased dead pups pnd 4-28 (F_1) (SS) (NOT at 8000 ppm), reduced postnatal weight (F_1: pnd 4, 14, F_2: pnd 14-28) (SS), reduced postnatal weight gain (F_1: pnd 1-4, 7-14, F_2: pnd 7-21) (SS).	No adult male or female deaths (F_0 or F_1), reduced adult female body weight (F_0), reduced adult female body weight gain (F_1), or reduced adult female food consumption (F_0). 8000 ppm: Reduced adult male body weight (F_0, F_1) (SS), reduced adult male body weight gain (F_0: weeks 0-3, 5-7; F_1: weeks 0-2, 5-6), reduced adult female body weight (F_1: weeks 0-8, not gestation or lactation) (SS), reduced adult female body weight gain (F_0: weeks 0-1, 5-6, not gestation or lactation) (SS), increased female body weight gain during lactation (F_0, F_1) (SS), increased adult male and female absolute and relative liver weights (F_1) (SS), reduced adult female food consumption (F_1: lactation days 7-14, not pre-breed or gestation) (SS). Clinical signs (individual observations): adult male, perioral wetness (F_0, F_1), perioral encrustation and salivation (F_1); adult female, perioral wetness (F_0, F_1), perioral encrustation, salivation and urine stains (F_1). Clinical signs (group observations during daily exposure periods): adult male and female, ataxia (F_0, F_1), hypoactivity (F_0, F_1), blepharospasm, lack of startle reflex (F_0, F_1). 3000 ppm: Reduced adult male body weight (F_1: weeks 0-3) (SS), reduced adult male body weight gain (F_0: weeks 1-2) (SS), reduced adult female body weight (F_1: weeks 0-4) (SS), increased adult female body weight gain (F_1: lactation) (SS), increased adult male relative liver weights (F_1) (SS). Clinical signs (group observations during daily exposure periods): adult male and female, hypoactivity (F_0, F_1), blepharospasm, lack of startle reflex (F_0, F_1).

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Biles *et al*. 1987 Bio/ dynamics 1984d	Rat (Sprague-Dawley) Inhalation Reproductive: 1 generation, 2 litter Male: 6 hours/day; 12 weeks (5 days/week), + first mating (2 weeks, daily), + 8 weeks (5 days/week), + second mating (2 weeks, daily). Female: 6 hours/day; 3 weeks (5 days/week), + first mating (daily) + first gestation (gd 0-20) + first lactation (pnd 5-21) + 2 weeks (5 days/week) + second mating (daily) + second gestation (gd 0-20) + second lactation (pnd 5-21) Target concentrations: 0, 250, 1000, 2500 ppm. Nominal concentrations[3] (M/F): 0/0, 290/300, 1300/1300, 3400/3400 ppm. Analytical concentrations[3] (M/F): 0/0, 290/300, 1190/1240, 2860/2980 ppm. n=15 males/group, 30 females/group	No altered testes or ovary weight (F_0), adverse histopathological effects on ovaries or testes (F_0), reduced mating, reduced male fertility, reduced female fertility (pregnancy rate), reduced litter size (live or total) (F_{1a}, F_{1b}), altered sex ratio (F_{1a}, F_{1b}), reduced pup viability at birth (live/total) (F_{1a}), reduced birth weight (F_{1a}, F_{1b}). reduced pup survival on pnd 4 (F_{1b}), or reduced pup survival on pnd 21 (F_{1a}, F_{1b}). 2500 ppm: Reduced pup viability at birth (live/total) (F_{1b}) (SS). (Note high in controls: control 99%, 1000 and 2500 ppm 95.5%. Authors discount biological significance), reduced postnatal weight on pnd 14, 21 (F_{1a}, F_{1b}) (NOT SS). 1000 ppm: Reduced pup viability at birth (live/total) (F_{1b}) (SS). (Note high in controls: control 99%, 1000 and 2500 ppm 95.5%. Authors discount biological significance), reduced pup survival from pnd 0-4 (F_{1a}) (NOT 2500 ppm), reduced postnatal weight on pnd 14, 21 (F_{1a}, F_{1b}) (NOT SS) 250 ppm: Reduced pup survival from pnd 0-4 (F_{1a}) (NOT 2500 ppm) (SS).	No adult male or female death, or reduced adult male or female body weight. 2500, 250 ppm: Increased incidence dilated renal pelves in females (NOT 1000 ppm).

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Burleigh-Flayer *et al.* 1992	Mouse (CD-1) Inhalation Female 18 months 5 days/week 6 hours/day 0, 400, 3000, 8000 ppm n=50/group	No ovarian (or other reproductive organ) histopathological effects.	Female: No increased death. 8000 ppm: Reduced body weight (SS), increased liver weight (SS), blepharospasm, hypoactivity, ataxia, lack of startle reflex, prostration. 3000 ppm: Increased liver weight (SS), blepharospasm, hypoactivity, ataxia, lack of startle reflex, stereotypy.
Chun *et al.* 1992	Rat (Fischer 344) Inhalation Female 104 weeks, 5 days/week 6 hours/day 0, 400, 3000, 8000 ppm n=50/group	No ovarian (or other reproductive organ) histopathological effects.	Female: No increased death. 8000 ppm: Reduced body weight (SS), increased liver weight (SS), ataxia, hypoactivity, blepharospasm, lack of startle reflex, nephropathy. 3000 ppm: Increased liver weight (SS), ataxia, hypoactivity, blepharospasm, lack of startle reflex, nephropathy.
Greenough *et al.* 1980	Rat (Sprague-Dawley) Inhalation Female 13 weeks, 5 days/week, 6 hours/day 0, 250, 500, 1000 ppm n=10/group	No alterations in ovarian or uterine weight, or ovarian or uterine histopathological effects.	Female: No increased death, reduced body weight or body weight gain, or reduced food consumption. Concentrations unspecified: "...an increase in the depth of anaesthesia with increasing chamber concentrations of MTB[E]."

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
IIT Research Institute 1992	Rat (Sprague-Dawley) Oral (gavage) 4 weeks, 5 days/week 0, 90, 440, 1750 mg/kg/d n=10/group	No altered ovary weight, or ovarian or uterine histopathological effects.	Female: No deaths (attributed to MTBE exposure), or reduced body weight or body weight gain. 1.75 g/kg/d: Increased relative liver and kidney weight (SS), hypoactivity, ataxia, salivation. 0.44 g/kg/d Salivation. 0.09 g/kg/d Increased relative kidney weight (SS), salivation.
Moser *et al.* 1996	Mouse (B6C3F1) Inhalation Female 3 days, 6 hours/day 21 days, 5 days/week, 6 hours/day Target concentrations: 0, 8000 ppm Analytical concentrations: 0, 7814 ppm n=5-6/group	No ovarian or uterine histopathological effects (but see Moser *et al.* 1998 for cervix and vagina). 8000 ppm: Reduced relative ovary weight (at 21 days SS, but not 3 days), reduced relative uterine weight (3 and 21 days SS)	Female: No reduced body weight. 8000 ppm: Increased relative liver weight (at 3 days SS, but not 21 days), altered gait, hypoactivity, reduced muscle tone, increased lacrimation.

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Moser *et al.* 1998	Mouse (B6C3F1) Inhalation Female 4 or 8 months, 5 days/week, 6 hour/day 0, 8000 ppm n=12/group	No ovarian histopathological effects, or altered serum estrogen level. 8000 ppm: Reduced ovary weight (absolute and relative, 4 and 8 months, SS), reduced uterine weight (absolute and relative, 4 and 8 months, SS), lengthened estrus cycle (8 months, SS), altered estrous cycle distribution (4 and 8 months, SS), altered histopathology of uterus (4 and 8 months), cervix and vagina (4 and 8 months). Also reported: altered histopathology of cervix and vagina (3 and 21 days: data collected as part of Moser *et al.* 1996)	Female: 8000 ppm: Reduced adult body weight (4 and 8 months, SS), body weight gain (4 and 8 months, SS).
Robinson *et al.* 1990	Rat (Sprague-Dawley) Oral (gavage) Female 14 days 0, 357, 714, 1071, 1428 mg/kg/day n=10/group	No altered ovary weight, or ovarian histopathological effects.	Female: No increased death. 1,428 mg/kg/day: Reduced body weight gain (SS), anesthesia, loose stools. 1,071 mg/kg/day: Reduced body weight gain (SS), loose stools. 714, 357 mg/kg/day: Loose stools.

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Robinson *et al.* 1990	Rat (Sprague-Dawley) Oral (gavage) Female 90 days 0, 100, 300, 900, 1200 mg/kg/day n=10/group	No altered ovary weight, or ovarian histopathological effects.	Female: No increased death. 1,200 mg/kg/day: Reduced body weight (SS), anesthesia, reduced body weight (SS), loose stools. 900, 300 mg/kg/day: Reduced body weight (NOT SS), loose stools. 100 mg/kg/day: loose stools.

[1] Abbreviations: gd = gestation day, pnd = postnatal day.
[2] Effects reported by authors to be statistically significant (SS) or discussed by authors in text.
[3] Analytical concentrations measured every 30 minutes by calibrated Miran infrared analyzer. Nominal concentrations calculated from daily weight of MTBE used divided by daily air volume. Authors comment that a manifold leak and aerosol particles may have caused an underestimation in the analytical concentrations (Biles *et al.* 1987, Bio/dynamics 1984a, 1984b, 1984d, Conaway *et al.* 1985).

APPENDIX

APPENDIX 3. Study summary tables: male reproductive effects of MTBE.

APPENDIX

Animal studies of the male reproductive effects of MTBE

(studies in alphabetical order by author)

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Belpoggi *et al.* 1995	Rat (Sprague-Dawley) Oral (gavage) Male 104 weeks, 4 days/week 0, 250, 1000 mg/kg/day n=60/group	No testicular histopathological effects.	Male: No increased death, reduced body weight gain, or reduced food consumption.

APPENDIX

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Bevan *et al.* 1997a, Neeper-Bradley 1991	Rat (Sprague-Dawley) Inhalation 2 generation reproductive Target concentrations: 0, 400, 3000, 8000 ppm Analytical concentrations: 0, 402, 3019, 8007 ppm Male: 6 hours/day, 10 weeks (5 days/week) + mating + gestation Female: 6 hours/day, 10 weeks (5 days/week) + mating + gestation (gd 1-19) + lactation (pnd 5-28) Exposures for F_0 starting at pnd 42, and F_1 starting on pnd 29-31. Pups not placed in inhalation chambers during lactation. n=25 males/group, 25 females/group	No ovarian, uterine, or vaginal histopathological effects, testicular or other male reproductive organ histopathological effects, reduced mating (F_0, F_1), reduced fertility (F_0, F_1), reduced live litter size (F_1, F_2), reduced postnatal survival after pnd 4 (F_2), reduced live birth, 4-day survival, or lactation indices (F_1, F_2), reduced litter size at end of lactation (F_1), or reduced lactation day one weight (F_1, F_2). 8000 ppm: Increased dead pups pnd 4 (F_1, F_2) (SS), reduced litter size at end of lactation (F_2) (SS), reduced postnatal weight (F_1: pnd 14-28, F_2: pnd 7-28) (SS), reduced postnatal weight gain (F_1: pnd 7-21, F_2: pnd 1-21) (SS). 3000 ppm: Increased dead pups pnd 4-28 (F_1) (SS) (NOT at 8000 ppm), reduced postnatal weight (F_1: pnd 4, 14, F_2: pnd 14-28) (SS), reduced postnatal weight gain (F_1: pnd 1-4, 7-14, F_2: pnd 7-21) (SS).	No adult male or female deaths (F_0 or F_1), reduced adult female body weight (F_0), reduced adult female body weight gain (F_1), or reduced adult female food consumption (F_0). 8000 ppm: Reduced adult male body weight (F_0, F_1) (SS), reduced adult male body weight gain (F_0: weeks 0-3, 5-7; F_1: weeks 0-2, 5-6), reduced adult female body weight (F_1: weeks 0-8, not gestation or lactation) (SS), reduced adult female body weight gain (F_0: weeks 0-1, 5-6, not gestation or lactation) (SS), increased female body weight gain during lactation (F_0, F_1) (SS), increased adult male and female absolute and relative liver weights (F_1) (SS), reduced adult female food consumption (F_1: lactation days 7-14, not pre-breed or gestation) (SS). Clinical signs (individual observations): adult male, perioral wetness (F_0, F_1), perioral encrustation and salivation (F_1); adult female, perioral wetness (F_0, F_1), perioral encrustation, salivation and urine stains (F_1). Clinical signs (group observations during daily exposure periods): adult male and female, ataxia (F_0, F_1), hypoactivity (F_0, F_1), blepharospasm, lack of startle reflex (F_0, F_1). 3000 ppm: Reduced adult male body weight (F_1: weeks 0-3) (SS), reduced adult male body weight gain (F_0: weeks 1-2) (SS), reduced adult female body weight (F_1: weeks 0-4) (SS), increased adult female body weight gain (F_1: lactation) (SS), increased adult male relative liver weights (F_1) (SS). Clinical signs (group observations during daily exposure periods): adult male and female, hypoactivity (F_0, F_1), blepharospasm, lack of startle reflex (F_0, F_1).

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Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Biles *et al*. 1987 Bio/ dynamics 1984d	Rat (Sprague-Dawley) Inhalation Reproductive: 1 generation, 2 litter Male: 6 hours/day; 12 weeks (5 days/week), + first mating (2 weeks, daily), + 8 weeks (5 days/week), + second mating (2 weeks, daily). Female: 6 hours/day; 3 weeks (5 days/week), + first mating (daily) + first gestation (gd 0-20) + first lactation (pnd 5-21) + 2 weeks (5 days/week) + second mating (daily) + second gestation (gd 0-20) + second lactation (pnd 5-21) Target concentrations: 0, 250, 1000, 2500 ppm. Nominal concentrations[3] (M/F): 0/0, 290/300, 1300/1300, 3400/3400 ppm. Analytical concentrations[3] (M/F): 0/0, 290/300, 1190/1240, 2860/2980 ppm. n=15 males/group, 30 females/group	No altered testes or ovary weight (F_0), adverse histopathological effects on ovaries or testes (F_0), reduced mating, reduced male fertility, reduced female fertility (pregnancy rate), reduced litter size (live or total) (F_{1a}, F_{1b}), altered sex ratio (F_{1a}, F_{1b}), reduced pup viability at birth (live/total) (F_{1a}), reduced birth weight (F_{1a}, F_{1b}). reduced pup survival on pnd 4 (F_{1b}), or reduced pup survival on pnd 21 (F_{1a}, F_{1b}). 2500 ppm: Reduced pup viability at birth (live/total) (F_{1b}) (SS). (Note high in controls: control 99%, 1000 and 2500 ppm 95.5%. Authors discount biological significance), reduced postnatal weight on pnd 14, 21 (F_{1a}, F_{1b}) (NOT SS). 1000 ppm: Reduced pup viability at birth (live/total) (F_{1b}) (SS). (Note high in controls: control 99%, 1000 and 2500 ppm 95.5%. Authors discount biological significance), reduced pup survival from pnd 0-4 (F_{1a}) (NOT 2500 ppm), reduced postnatal weight on pnd 14, 21 (F_{1a}, F_{1b}) (NOT SS) 250 ppm: Reduced pup survival from pnd 0-4 (F_{1a}) (NOT 2500 ppm) (SS).	No adult male or female death, or reduced adult male or female body weight. 2500, 250 ppm: Increased incidence dilated renal pelves in females (NOT 1000 ppm).

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Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Burleigh-Flayer *et al.* 1992	Mouse (CD-1) Inhalation Male, 18 months, 6 hours/day, 5 days/week, 0, 400, 3000, 8000 ppm n=50/group	No altered testes weight, or testicular (or other reproductive organ) histopathological effects.	Male: 8000 ppm: Increased death (SS), reduced body weight (SS), increased liver weight (SS), blepharospasm, hypoactivity, ataxia, lack of startle reflex, prostration. 3000 ppm: Increased liver weight (SS), blepharospasm, hypoactivity, ataxia, lack of startle reflex, stereotypy. 400 ppm: Increased liver weight (SS).
Chun *et al.* 1992	Rat (Fischer 344) Inhalation Male 6 hours/day, 5 days/week 0, 400 ppm; 104 weeks 3000 ppm; 97 weeks 8000 ppm; 82 weeks n=50/group	No altered testes weight to 400 ppm (see note). 8000, 3000, 400 ppm: Increased testicular mineralization (see note).	Male: No altered liver weight to 400 ppm (see note). 8000 ppm: Increased death (SS), reduced body weight (SS), (increased) nephropathy, ataxia, hypoactivity, blepharospasm, lack of startle reflex,. 3000 ppm: Increased death (SS), nephropathy, ataxia, hypoactivity, blepharospasm, lack of startle reflex. 400 ppm: Increased death (SS), nephropathy. Note: Remaining males in 8000 and 3000 ppm groups were sacrificed early due to high group mortality. Authors attribute mortality and mineralization of "numerous tissues" to nephropathy. No statistical evaluation of testes or other organ weight, or, apparently, histopathological changes, was performed by the authors for the 8000 or 3000 ppm groups.

Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Dodd and Kintigh 1989	Rat (Fischer 344) Inhalation 13 weeks, 5 days/week 6 hours/day: 0, 800, 4000, 8000 ppm n=25/group	No altered testes weight.	Male: No increased death. 8000 ppm: Reduced body weight (SS), reduced body weight gain (SS), increased liver, kidney, and adrenal weight (relative and absolute) (SS), ataxia. 4000: Reduced body weight (SS), reduced body weight gain (SS), increased liver, kidney, and adrenal weight (relative and absolute) (SS). 800: Increased liver, kidney, and adrenal weight (relative and absolute) (some SS, some NOT SS).
Dodd and Kintigh 1989	Rat (Fischer 344) Inhalation (Range finding) 13 days, 6 hours/day: 0, 2000, 4000, 8000 ppm n=5/group	No altered testes weight.	Male: No increased death. 8000 ppm: Reduced body weight (NOT SS), reduced body weight gain (SS), increased liver, kidney and adrenal weight (relative and absolute) (some SS, some NOT SS). Clinical observations during exposure: hypoactivity, ataxia. Clinical and behavioral observations after exposure: ataxia, reduced startle response, pain reflex, muscle tone. 4000 ppm: Reduced weight (NOT SS), reduced weight gain (SS), increased liver, kidney and adrenal weight (relative and absolute) (some SS, others NOT SS) Clinical observations during exposure: hypoactivity, ataxia. 2000 ppm: Clinical observations during exposure: hypoactivity (2 exposures only).

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Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Dodd and Kintigh 1989	Mouse (CD-1) Inhalation (Range finding) 13 days, 6 hours/day: 0, 2000, 4000, 8000 ppm n=5/group	No altered testes weight.	Male: No increased death, or reduced body weight or body weight gain. 8000 ppm: Increased liver weight (absolute NOT SS, relative SS). Clinical observations during exposure: hypoactivity, ataxia. Clinical observations after exposure: ataxia. 4000 ppm: Clinical observations during exposure: hypoactivity, ataxia. 2000 ppm: Clinical observations during exposure: hypoactivity (2 exposures only).
Greenough *et al.* 1980	Rat (Sprague-Dawley) Inhalation Male 13 weeks, 5 days/week, 6 hour/day 0, 250, 500, 1000 ppm n=10/group	No alterations in testes or prostate weight, or testicular or prostate histopathological effects.	Male: No increased death, reduced body weight or body weight gain, or reduced food consumption. Concentrations unspecified: "...an increase in the depth of anaesthesia with increasing chamber concentrations of MTB[E]."

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Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
IIT Research Institute 1992	Rat (Sprague-Dawley) Oral (gavage) 4 weeks, 5 days/week 0, 90, 440, 1750 mg/kg/day n=10/group	No altered testes weight.	Male: No increased death, or reduced body weight or body weight gain. 1,750 mg/kg/day: Increased liver, kidney, adrenal weight (absolute NOT SS, relative SS). Clinical observations after dosing: hypoactivity, ataxia, salivation. 440 mg/kg/day: Increased kidney weight (absolute NOT SS, relative SS). Clinical observations after dosing: hypoactivity, ataxia, salivation. 90 mg/kg/day Clinical observations after dosing: salivation.
Robinson *et al.* 1990	Rat (Sprague-Dawley) Oral (gavage) Male 14 days 0, 357, 714, 1071, 1,428 mg/kg/day n=10/group	No altered absolute testes weight, or testicular histopathological effects. 1,071, 714 mg/kg/day: Increased relative testes weight (NOT at 1,428 mg/kg/d) (SS).	Male: No increased death. 1,428 mg/kg/day: Reduced body weight gain (SS), increased relative kidney weight, anesthesia, loose stools 1,071, 714 mg/kg/day: Reduced body weight gain (SS), loose stools 357 mg/kg/day: loose stools

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Reference	Study design[1]	Reported effects: DART[2]	Reported effects: maternal and systemic[2]
Robinson *et al.* 1990	Rat (Sprague-Dawley) Oral (gavage) Male 90 days 0, 100, 300, 900, 1200 mg/kg/day n=10/group	No altered testes weight, or testicular histopathological effects.	Male: No increased death. 1,200 mg/kg/day: Reduced body weight (NOT SS), increased liver weight (relative, SS), increased kidney weight (absolute and relative, SS), anesthesia, diarrhea 900 mg/kg/day: Increased liver weight (relative, SS), kidney weight (absolute and relative, SS), diarrhea. 300, 100 mg/kg/day: diarrhea

[1] Abbreviations: gd = gestation day, pnd = postnatal day.
[2] Effects reported by authors to be statistically significant (SS) or discussed by authors in text.
[3] Analytical concentrations measured every 30 minutes by calibrated Miran infrared analyzer. Nominal concentrations calculated from daily weight of MTBE used divided by daily air volume. Authors comment that a manifold leak and aerosol particles may have caused an underestimation in the analytical concentrations (Biles *et al.* 1987, Bio/dynamics 1984a, 1984b, 1984d, Conaway *et al.* 1985).