CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of MTBE. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to MTBE, but may not be inclusive of the entire body of literature.

Summaries of the human observational studies are presented in Table 2-1. Animal inhalation studies are presented in Table 2-2 and Figure 2-2, animal oral studies are presented in Table 2-3 and Figure 2-3, and dermal data are presented in Table 2-4.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects (SLOAELs) are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR

acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of MTBE are indicated in Tables 2-2 and 2-3 and Figures 2-2 and 2-3.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The health effects of MTBE have been evaluated in 15 human studies and 84 animal studies. As illustrated in Figure 2-1, most of the health effects data come from inhalation exposure studies in animals. For the purposes of Figure 2-1, all human and animal inhalation studies were classified as such; however, it is acknowledged that dermal and ocular effects associated with inhalation studies are likely attributable to direct contact with MTBE vapors. Therefore, ocular and dermal effects from animal inhalation studies are counted as dermal exposure in Figure 2-1 and listed in the dermal LSE table. For animal data, inhalation and oral studies are available for all health effect and exposure duration categories. The dermal animal database is limited to five acute-duration studies and one intermediate-duration study, evaluating limited endpoints. The most examined endpoints were death, body weight, neurological, reproductive, and hepatic effects. The available human studies, most of which were in humans exposed to gasoline containing MTBE (not MTBE alone), were predominantly focused on evaluation of respiratory, neurological, and ocular effects.

The results of the animal studies, along with limited human data, suggest potential associations between MTBE exposure and the following health outcomes:

- **Respiratory effects:** Some occupational and population-based studies conducted in the early 1990s suggest an increase in respiratory symptoms with introduction of MTBE into fuel during the oxyfuel program. There is no evidence of respiratory symptoms in volunteers following acute-duration exposure to low levels of MTBE. In animal studies, evidence of respiratory

irritation and/or inflammation was observed at high inhalation and oral exposure levels. Hyperpnea, labored breathing, and respiratory failure were observed at lethal air concentrations. However, no evidence of lung damage was observed in animal studies.

- **Gastrointestinal effects:** Several epidemiology studies report nausea and/or vomiting with inhalation exposure to gasoline containing MTBE; however, these symptoms are likely related to neurological effects associated with MTBE exposure (see **Neurological effects** below). Other human studies are limited to patients receiving intracystic MTBE therapy for gallstone dissolution that report gastrointestinal side effects during and/or after treatment (e.g., vomiting, nausea, burning sensation, duodenal ulcer). In animals, the gastrointestinal tract only appears to be a target of toxicity following exposure to high gavage doses, including diarrhea and inflammation of the gastrointestinal tract. Observed effects in humans and animals are consistent with irritative effects on the gastrointestinal mucosa. Effects associated with intracystic MTBE therapy or bolus gavage exposure in animals may not be relevant endpoints for environmental exposures, in which oral exposure is expected to be predominantly via drinking water.

- **Hepatic effects:** Data from medical intervention studies report hepatic side effects in patients receiving intracystic infusions of MTBE for gallstone dissolution in cases of accidental overflow of MTBE or bile leakage during the procedure. Additional human data for this endpoint are limited to a single cross-sectional study reporting no association between non-alcoholic fatty liver disease (NAFLD) and low-level occupational MTBE exposure. In inhalation and oral animal studies, elevated liver weight, hepatocellular hypertrophy, and induction of hepatic enzymes was consistently observed at high exposure levels associated with overt clinical signs of toxicity (CNS depression). These effects may represent adaptive changes following MTBE exposure. Elevated serum cholesterol was also observed in some studies; however, the biological significance of this is unclear due to lack of associated hepatic lesions (e.g., fatty liver).

- **Renal effects:** Data from patients treated intracystically with MTBE for the dissolution of gallstones do not consistently report renal side effects. No additional human data are available. Renal toxicity has been consistently observed in male rats at inhalation and oral exposure levels at or below those associated with overt clinical signs (e.g., CNS depression). Renal toxicity has also been reported in female rats, but findings were less severe and at higher exposure levels compared to male rats. Findings in male rats are likely due, in part, to α2u-globulin accumulation, which is not relevant to human health.

- **Lymphoreticular effects:** No human data are available. Data from inhalation and oral studies in laboratory animals provide limited evidence of proliferation of lymphoreticular tissues in rats. These lesions may be preneoplastic in nature (see **Cancer effects** below).

- **Neurological effects:** Effects consistent with transient CNS depression have been reported in humans exposed to MTBE in fuel; however, should be interpreted with caution due to simultaneous exposure to other chemicals in gasoline. No changes in subjective symptoms or neurobehavioral function were observed in volunteers exposed to low air levels of pure MTBE. However, transient CNS depression has been reported following MTBE therapy for gallstone dissolution. In animals, the predominant and immediate effect of exposure to high levels of MTBE is CNS depression, including hypoactivity, ataxia, and anesthesia. Effects are transient, generally subsiding within hours of exposure and do not increase in severity with duration of study. There is no evidence of structural damage to the central or peripheral nervous systems in exposed animals.

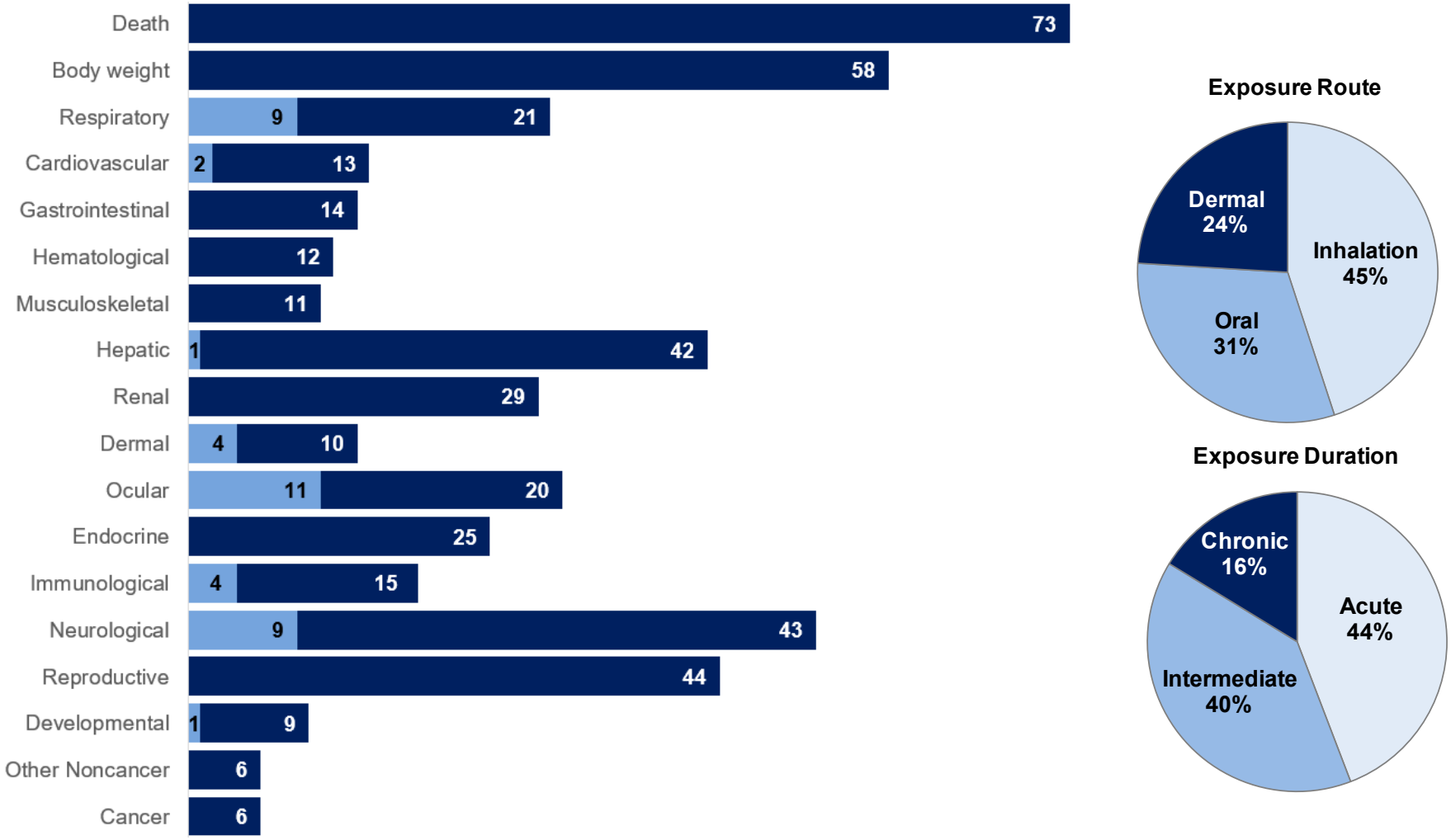
- **Reproductive system:** No human data are available. Based on animal oral studies, there is some evidence of male reproductive toxicity in rats (decreased fertility, decreased serum testosterone, abnormal sperm, decreased testicular weight, histopathological changes in the testes) at doses associated with overt clinical signs of toxicity (CNS depression); however, findings are inconsistent across studies and exposure durations. There is no evidence of impaired female fertility or damage to the female reproductive system following oral exposure. In animal inhalation studies, the male and female reproductive tract do not appear to be primary targets of MTBE toxicity, with alterations in female reproductive organs occurring in mice only at exposure levels associated with frank systemic toxicity.

- **Developmental effects:** Human data are limited to a single cohort study reporting a potential association between MTBE exposure during birth year and diagnosis of autism spectrum disorder (ASD). In animals, developmental toxicity (litter resorption, post-implantation loss, reduced live fetuses, decreased offspring weight, delayed ossification, cleft palate) was only observed following inhalation exposure to high concentrations associated with overt parental toxicity (e.g., clinical signs of CNS depression). No adequate oral developmental toxicity studies in animals following gestational exposure were available. However, one study reported male reproductive effects (decreased serum testosterone, decreased number and size of Leydig cells) in rats following prepubertal exposure.

- **Cancer effects:** No human data are available. In animals, chronic-duration inhalation exposure was associated with increased renal tubular cell tumors in male rats and hepatocellular adenomas in female mice and chronic-duration gavage exposure was associated with increased testicular Leydig cell tumors in male rats and lymphomas and leukemia in female rats. No exposure-related tumors were observed following chronic-duration drinking water exposure in rats.

Figure 2-1. Overview of the Number of Studies Examining Methyl *tert*-Butyl Ether (MTBE) Health Effects*

Most studies examined the potential body weight, neurological, reproductive, and hepatic effects of MTBE
Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 99 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
Occupational studies		
Alaska DHSS 1992a Cross-sectional study United States (Fairbanks, Alaska) Exposure groups: taxi drivers (n=12) and health-care workers (n=90) who traveled routinely in motor vehicles Referent group: university students (n=101)	Subjects exposed to MTBE in oxyfuel gasoline during travel for an average of 69 hours/week (taxi drivers), 7.7 hours/week (health-care workers), or 0.8 hours/week (students). Exposure levels not reported; exposure expected to be primarily via inhalation, with potential for dermal exposure. Surveyed 1 or 2 months after oxyfuel program began.	Subjects meeting case definition (increase in headaches or an increase in two or more of the following: nausea or vomiting; burning sensation in the nose, mouth, or throat; cough; dizziness; spaciness or disorientation; eye irritation): Taxi drivers: 4/12 (33%) Symptoms reported during travel: 3/4 (75%) Symptoms reported during fueling: 1/4 (25%) Health-care workers: 26/90 (29%) Symptoms reported during travel: 11/26 (42%) Symptoms reported during fueling: 9/26 (35%) Students (referent): 15/101 (15%) Symptoms reported during travel: 3/15 (20%) Symptoms reported during fueling: 3/15 (20%) Specific complaints in subjects meeting case definition: Burning sensation in nose or throat: Taxi drivers: 0/4 (0%) Health-care workers: 2/26 (8%) Students: 3/15 (20%) Cough: Taxi drivers: 1/4 (25%) Health-care workers: 8/26 (31%) Students: 3/15 (20%) Eye irritation: Taxi drivers: 1/4 (25%) Health-care workers: 9/26 (35%) Students: 3/15 (20%) Headache: Taxi drivers: 3/4 (75%) Health-care workers: 21/26 (81%) Students: 10/15 (67%) Nausea or vomiting: Taxi drivers: 3/4 (75%) Health-care workers: 9/26 (35%) Students: 6/15 (40%)

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
		Spaciness: Taxi drivers: 1/4 (25%) Health-care workers: 1/26 (4%) Students: 2/15 (13%)
CDC 1993a Cross-sectional study United States (Albany, New York) Exposure groups: Group 1: 34 automobile repair shop workers and service station attendants exposed to gasoline fumes Group 2: 48 policemen, toll booth workers, and parking garage attendants exposed to automobile exhaust Referent group: Group 3: 182 office workers and college students who may have been exposed to minute amounts of automobile emissions, but who were not occupationally exposed to gasoline	Subjects exposed to MTBE in oxyfuel gasoline. Median (range) blood MTBE concentration (µg/L): Group 1: Nonsmokers (n=7): 0.38 (<LOD–0.58) Smokers (n=4): 0.46 (0.09–1.50) Group 2: Nonsmokers: (n=6): 0.05 (<LOD–0.15) Smokers (n=3): 0.08 (<LOD–0.11) Group 3: Nonsmokers (n=16): <LOD Smokers (n=4): <LOD Median (range) MTBE in ambient workplace air (µg/m^3): Group 1 (n=3): <LOD Group 2 (n=8): <LOD Median (range) MTBE in personal breathing zone workplace air (µg/m^3): Group 1 (n=13): <LOD (<LOD–505) Group 2 (n=11): <LOD	"Key" symptoms reported by subjects: Burning sensation in nose or throat: Group 1: 2/34 (6%) Group 2: 2/48 (4%) Group 3: 24/182 (13%) Cough: Group 1: 5/34 (15%) Group 2: 12/48 (25%) Group 3: 37/182 (20%) Nausea: Group 1: 2/34 (6%) Group 2: 3/48 (6%) Group 3: 14/182 (8%) Dizziness: Group 1: 3/34 (9%) Group 2: 6/48[a] (12%) Group 3: 5/182 (3%) Spaciness or disorientation: Group 1: 0/34 (0%) Group 2: 2/48 (4%) Group 3: 13/182 (7%) Headache: Group 1: 7/34 (21%) Group 2: 23/48[a] (47%) Group 3: 44/182 (24%) Any key symptom: Group 1: 14/34 (41%) Group 2: 28/48 (59%) Group 3: 95/182 (52%) Two or more key symptoms: Group 1: 3/34 (9%) Group 2: 13/48[a] (27%)

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
		Group 3: 36/182 (20%) "Other" symptoms reported by subjects: Difficulty breathing: Group 1: 0/34 (0%) Group 2: 7/48 (14%) Group 3: 25/182 (14%) Diarrhea: Group 1: 2/34 (6%) Group 2: 4/48 (8%) Group 3: 21/182 (12%) Skin irritation: Group 1: 3/34 (9%) Group 2: 5/48 (10%) Group 3: 17/182 (9%) Fatigue: Group 1: 6/34 (18%) Group 2: 8/48 (16%) Group 3: 44/182 (24%) Fainting: Group 1: 0/34 (0%) Group 2: 0/48 (0%) Group 3: 3/182 (2%)
Alaska DHSS 1992b Cross-sectional study United States (Anchorage, Alaska) Exposure group: taxi drivers (n=25) and health-care workers (n=137) who traveled routinely in motor vehicles Referent group: none	Subjects exposed to MTBE in oxyfuel gasoline during travel; average travel time per week not reported. Exposure levels not reported; exposure expected to be primarily via inhalation, with potential for dermal exposure. Surveyed 1 or 2 months after oxyfuel program began.	Subjects meeting case definition (increase in headaches or an increase in two or more of the following: nausea or vomiting; burning sensation in the nose, mouth, or throat; cough; dizziness; spaciness or disorientation; eye irritation): Taxi drivers: 12/25 (48%): Symptoms reported during travel: 12/12 (100%) Symptoms reported during fueling: 9/12 (75%) Health-care workers: 36/137 (26%): Symptoms reported during travel: 27/36 (75%) Symptoms reported during fueling: 19/36 (53%) Specific complaints in subjects meeting case definition: Burning sensation in nose or throat: Taxi drivers: 5/12 (42%) Health-care workers: 13/36 (36%)

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
		Cough: Taxi drivers: 4/12 (33%) Health-care workers: 13/36 (36%) Eye irritation: Taxi drivers: 8/12 (67%) Health-care workers: 13/36 (36%) Headache: Taxi drivers: 11/12 (92%) Health-care workers: 31/36 (86%) Nausea or vomiting: Taxi drivers: 5/12 (42%) Health-care workers: 9/36 (25%) Dizziness: Taxi drivers: 4/12 (33%) Health-care workers: 7/36 (19%) Spaciness: Taxi drivers: 4/12 (33%) Health-care workers: 3/36 (8%)
Duffy 1994 Cross-sectional study United States (Fairbanks, Alaska) Exposure group: 22 volunteers occupationally exposed to automobile emissions between late November and early December 1992 Referent group: none	Subjects exposed to gasoline containing MTBE during the oxyfuel program. Exposure levels not reported; exposure expected to be primarily via inhalation, with potential for dermal exposure.	Mean±SD interleukin-6 levels Pre-shift: 2.50±2.4 pg/mL Post-shift: 2.53±2.6 pg/mL Mean±SD interleukin-1 levels Pre-shift: <LOD Post-shift: <LOD

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
Mohr et al. 1994 Cross-sectional study United States (New Jersey) Exposure group: 115 automobile mechanics in northern New Jersey exposed to MTBE during wintertime oxyfuel program Referent group: 122 garage workers in southern New Jersey 10 weeks after the phase-out date for oxyfuel program	Exposure group was exposed to gasoline containing MTBE from November 15, 1992 to April 30, 1993. Survey data for this group were collected in April 1993. Referent group was exposed to gasoline containing MTBE only from November 15, 1992 to February 28, 1993. Survey data for this group were collected in May 1993. 8-Hour TWA MTBE in air (1-hour active sampling) Exposure: 1.66–6.1 ppm Referent: <0.28 (LOD)–0.83 ppm	Symptoms reported by subjects to have occurred at least once over the past 30 days: Cough: Exposed: 12% Referent: 17% p-value: 0.23 Eye irritation: Exposed: 22% Referent: 27% p-value: 0.39 Headache: Exposed: 36% Referent: 39% p-value: 0.82 Lightheadedness: Exposed: 10% Referent: 13% p-value: 0.41 Sleepiness while driving: Exposed: 20% Referent: 24% p-value: 0.37 Daytime sleepiness: Exposed: 13% Referent: 24% p-value: 0.001 Nausea: Exposed: 27% Referent: 35% p-value: 0.03 Mean summary score for all symptoms: Exposed: 4.3 Referent: 5.1 p-value: 0.09

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
		Mean postshift summary score for all symptoms in exposed subjects who pumped gas for >5 hours/day and matched referents: Exposed (n=11): 3.37 Control (n=11): 2.00 Time effect p-value 0.81 Group effect p-value: 0.66 Time x group effect p-value: 0.33
Moolenaar et al. 1994; CDC 1993c Cohort study United States (Fairbanks, Alaska) Exposure group (Phase I): 18 workers heavily exposed to gasoline fumes in December 1992, during oxyfuel program (10 mechanics/workers at service stations and automobile dealerships and 8 workers who spent most of their workdays in motor vehicles, including animal control officers, meter and telephone technicians, and a garbage collector) Referent group (Phase II): 28 workers heavily exposed to gasoline fumes in February 1993, after the oxyfuel program ended (12 subjects from Phase I plus16 additional workers from service stations and garages)	Subjects exposed to gasoline containing 15% MTBE during Phase I; gasoline did not contain MTBE during Phase II. Median 8-hour TWA (range) of MTBE in workplace air of service stations and dealership garages: Phase I: 0.2* (0.001–0.81) ppm Phase II: 0.04 (<LOD–0.14) ppm Median preshift concentrations (range) of MTBE in blood of exposed workers: Phase I: 1.15* (0.1–27.8) µg/L Phase II: 0.20 (0.05–4.35) µg/L Median postshift concentrations (range) of MTBE in blood of exposed workers: Phase I: 1.80* (0.2–37.0) µg/L Phase II: 0.24 (0.05–1.44) µg/L	Symptoms reported by subjects: Burning sensation in nose or throat: Phase I: 9/18[a] (50%) Phase II: 0/28 (0%) Cough Phase I: 5/18[a] (28%) Phase II: 0/28 (0%) Eye irritation: Phase I: 12/18[a] (67%) Phase II: 2/28 (7%) Nausea/vomiting: Phase I: 6/18[a] (33%) Phase II: 1/28 (4%) Dizziness: Phase I: 8/18[a] (44%) Phase II: 0/28 (0%) Spaciness or disorientation: Phase I: 6/18[a] (33%) Phase II: 0/28 (0%) Headache: Phase I: 13/18[a] (72%) Phase II: 1/28 (4%) Symptoms reported during Phase I by quartile (Q): Any symptom: Phase I Q4 (>9.6 µg/L postshift): 4/4 Phase I Q1-Q3: 9/14

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
CDC 1993b; White et al. 1995 Cross-sectional study United States (Stamford, Connecticut) Exposure groups: Group 1 (mechanics): 48 male mechanics or gasoline station attendants Group 2 (professional drivers): 57 male taxi or limousine drivers Group 3 (other): 12 male workers who spent most of their time around traffic or motor vehicles (e.g., meter readers) Referent group: Group 4 (commuters): 59 men not occupationally exposed, but commuted to work in their cars	Subjects exposed to gasoline containing 15% MTBE during work and/or travel from April 5 to 16, 1993 (~5 months after oxyfuel program began). MTBE levels in personal breathing zones (range) Mechanics: <0.03 to 12.04 ppm Drivers: NR Other: <0.03 (LOD) Commuters: NR Median post-shift concentration (range) of MTBE in blood Mechanics (n=21): 1.73 (0.17–36.7) µg/L Gas station (n=3): 15 (7.6–28.9) µg/L Drivers: NR Other (n=6): 0.1 µg/L (range not reported) Commuters: (n=14): 0.11 (<0.05–2.60) µg/L	Symptoms reported by subjects: Burning sensation in nose or throat: Group 1: 7/48 (15%) Group 2: 0/57 (0%) Group 3: 4/12[a] (33%) Group 4: 4/59 (7%) Cough: Group 1: 7/48 (15%) Group 2: 3/57 (5%) Group 3: 5/12 (42%) Group 4: 9/59 (15%) Eye irritation: Group 1: 10/48 (21%) Group 2: 4/57 (7%) Group 3: 2/12[a] (17%) Group 4: 11/59 (19%) Nausea: Group 1: 1/48 (2%) Group 2: 0/57 (0%) Group 3: 1/12 (8%) Group 4: 0/59 (0%) Dizziness: Group 1: 3/48 (6%) Group 2: 3/57 (5%) Group 3: 2/12 (17%) Group 4: 1/59 (2%) Spaciness or disorientation: Group 1: 5/48 (10%) Group 2: 1/57 (2%) Group 3: 1/12 (8%) Group 4: 2/59 (3%) Headache (1 or more times): Group 1: 13/48 (27%) Group 2: 15/57 (26%) Group 3: 5/12 (42%) Group 4: 15/59 (25%) Headache (2 or more times): Group 1: 12/48 (25%) Group 2: 12/57 (21%)

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
		Group 3: 5/12 (42%) Group 4: 14/59 (24%) Headache (5 or more times): Group 1: 4/48 (8%) Group 2: 5/57 (9%) Group 3: 1/12 (8%) Group 4: 3/59 (5%) Odds ratio (CI) to report of one or more key symptoms (listed above): Median blood level ≥2.4 µg/L (n=11) = 8.9 (1.2–75.6)* Median blood level >3.8 µg/L (n=8) = 21.0 (1.8–539)*
Yang et al. 2016 Cross-sectional study China Exposure group: 71 gas station attendants (41 males, 30 females) employed in Southern China from April to September 2014 for >3 years; any workers with alcohol intake >20 g/day, hepatitis B, hepatitis C, autoimmune hepatitis, primary biliary cirrhosis, or other chronic liver disease with a clear cause were excluded	Mean (SD) personal MTBE air exposure concentrations (3 consecutive 8-hour workdays) NAFLD group (n=11): 292.98±154.90 µg/m^3 (0.081 ppm) Non-NAFLD group (n=60): 286.64±122.28 µg/m^3 (0.079 ppm)	Adjusted[c] odds ratio (CI) for diagnosis of NAFLD: Males+females (11 NAFLD, 60 non-NAFLD): MTBE ≤100 µg/m^3 (n=11): 1.00 (referent) MTBE 100–200 µg/m^3 (n=12): 1.31 (0.85–1.54) MTBE 200–300 µg/m^3 (n=34): 1.14 (0.81–1.32) MTBE ≥300 µg/m^3 (n=14): 1.52 (0.93–1.61) Males (10 NAFLD, 31 non-NAFLD): MTBE ≤100 µg/m^3 (n=4): 1.00 (referent) MTBE 100–200 µg/m^3 (n=4): 1.64 (0.84–1.83) MTBE 200–300 µg/m^3 (n=25): 1.32 (0.80–1.63) MTBE ≥300 µg/m^3 (n=7): 1.21 (0.77–1.73) Females (1 NAFLD, 29 non-NAFLD) MTBE ≤100 µg/m^3 (n=7): 1.00 (referent) MTBE 100–200 µg/m^3 (n=8): 1.17 (0.79–1.32) MTBE 200–300 µg/m^3 (n=9): 1.02 (0.79–1.26) MTBE ≥300 µg/m^3 (n=7): 1.11 (0.75–1.41)

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
General population studies		
Wisconsin DHSS 1995 Cross-sectional study United States (Milwaukee, Wisconsin; Chicago, Illinois) Exposure groups: residents of metropolitan Milwaukee (n=527; 43% male) and metropolitan Chicago (n=485; 45% male); interviewed between February 24, 1995 and March 19, 1995 Referent group: residents of Wisconsin, excluding metropolitan areas participating in oxyfuel program (n=501; 45% male); interviewed between February 24, 1995 and March 19, 1995	Subjects living in metropolitan areas were exposed to gasoline containing MTBE during the oxyfuel program; gasoline in non-metropolitan areas did not contain MTBE. Mean (range) of ambient MTBE exposure at University of Wisconsin, North Campus (metropolitan Milwaukee): 0.20 (<0.025 ppb (LOD)–0.85) ppb Range of ambient MTBE exposure at gas stations: Milwaukee: 0.25–4.58 ppb Chicago: NR Referent: <0.025 ppb (LOD) Range of personal breathing zone MTBE exposure at gas stations while fueling: Milwaukee: 70–5,930 ppb Chicago: NR	Adjusted[d] risk ratios for symptoms in individuals who reported buying oxyfuel since induction of program on November 1, 1994, compared to referent group: Throat irritation: Milwaukee: 3.8* Chicago: not calculated (incidence too small) Difficulty breathing: Milwaukee: 1.8 Chicago: not calculated (incidence too small) Sinus congestion: Milwaukee: 2.6* Chicago: 0.8 Rashes: Milwaukee: 2.2 Chicago: not calculated (incidence too small) Eye irritation: Milwaukee: 3.8* Chicago: not calculated (incidence too small) Headache: Milwaukee: 4.2* Chicago: 1.8 Nausea: Milwaukee: 2.9 Chicago: 3.0 Dizziness: Milwaukee: 1.8 Chicago: 1.2 Spaciness: Milwaukee: 2.1 Chicago: 1.5 Any unusual symptom: Milwaukee: 2.8* Chicago: 2.3 Incidence of any “unusual” symptoms: Milwaukie: 23% Chicago: 6% Referent: 6%

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
Gordian et al. 1995 Ecological study United States (Fairbanks and Anchorage, Alaska) Study population: Alaska state employees, retirees, and dependents in Anchorage (n=~15,000) and Fairbanks (n=~4,900) Exposure period: November 1992–February 1993 (Anchorage); November 1992–December 1992 (Fairbanks) Referent period: November 1990–February 1991 and November–February 1992 (both cities)	Subjects were exposed to gasoline containing 16% MTBE during winter of 1992–1993; gasoline in prior years did not contain MTBE. Exposure levels were not reported; exposure expected to be primarily via inhalation.	Odds ratios (95% CI) for winter outpatient visits during the oxyfuel program (1992–1993) versus prior to the oxyfuel program: Upper respiratory illness: Anchorage: 1992–1993 versus 1990–1991: 0.94 (0.84–1.05) 1992–1993 versus 1991–1992: 0.94 (0.84–1.05) Fairbanks: 1992 versus 1990–1991: 0.95 (0.81–1.13) 1992 versus 1991–1992: 1.07 (0.90–1.27) Bronchitis: Anchorage: 1992–1993 versus. 1990–1991: 0.90 (0.73–1.11) 1992–1993 versus 1991–1992: 0.85 (0.069–1.05) Fairbanks: 1992 versus 1990–1991: 1.33 (0.90–1.99) 1992 versus 1991–1992: 0.96 (0.67–1.38) Asthma: Anchorage: 1992–1993 versus 1990–1991: 1.05 (0.76–1.47) 1992–1993 versus 1991–1992: 0.99 (0.72–1.37) Fairbanks: 1992 versus 1990–1991: 1.16 (0.75–1.81) 1992 versus 1991–1992: 1.00 (0.66–1.52) Headaches: Anchorage: 1992–1993 versus 1990–1991: 1.54 (0.94–2.52) 1992–1993 versus 1991–1992: 0.52 (0.35–0.75) Fairbanks: 1992 versus 1990–1991: 1.43 (0.71–2.94) 1992 versus 1991–1992: 1.30 (0.67–2.58)

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
Joseph and Weiner 2002 Ecological study United States (Philadelphia, Pennsylvania) Study population: Patients visiting the General Medicine Division of the Clinical Practices at the University of Pennsylvania in 1992 (n=14,900) and 1997 (n=26,644) Number and fraction of visits in 1997 (6th year of oxyfuel program) compared to number and fraction of visits in 1992 (1st year of oxyfuel program).	Subjects were exposed to gasoline containing 11–15% MTBE from 1992 to 1997 (4-month winter period of November–February only for 1992, 1993, and 1994; year-round 1995–1997). Exposure levels were not reported; exposure expected to be primarily via inhalation.	Number (fraction) of diagnostic codes pertaining to MTBE-related symptoms[b]: Burning throat: 1992: 198 (0.013) 1997: 1,104 (0.041)* Burning nose: 1992: 179 (0.012) 1997: 774 (0.029)* Cough: 1992: 59 (0.0039) 1997: 333 (0.0125)* Eye irritation: 1992: 20 (0.0013) 1997: 62 (0.0023)* Headache: 1992: 139 (0.0093) 1997: 659 (0.024)* Nausea: 1992: 14 (0.00094) 1997: 107 (0.0040)* Dizziness: 1992: 143 (0.0096) 1997: 430 (0.0161)* Spaciness 1992: 0 (0.0) 1997: 30 (0.0006) Number (fraction) of diagnostic codes for symptoms related to respiration (not directly linked to MTBE): Wheezing: 1992: 45 (0.003) 1997: 484 (0.018)* Upper Respiratory Infection: 1992: 397 (0.026) 1997: 1,531 (0.057)* Asthma: 1992: 393 (0.026) 1997: 737 (0.028)

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
		Otitis media: 1992: 34 (0.0023) 1997: 116 (0.0043)* Allergic rhinitis: 1992: 179 (0.012) 1997: 774 (0.029)* Number (fraction) of diagnostic codes for symptoms attributed anecdotally to MTBE in gasoline: Skin rash: 1992: 74 (0.005) 1997: 1,062 (0.040)* General allergy: 1992: 13 (0.0009) 1997: 115 (0.0043)* Anxiety: 1992: 27 (0.0018) 1997: 390 (0.0146) Insomnia: 1992: 61 (0.0041) 1997: 259 (0.0097)* Cardiac (tachycardia, palpitations, murmurs): 1992: 84 (0.0056) 1997: 239 (0.0089)* Malaise and fatigue: 1992: 162 (0.011) 1997: 871 (0.032)* Number (fraction) of diagnostic codes for symptoms unrelated to air pollution (considered “referent” conditions”): Diabetes: 1992: 1,100 (0.0738) 1997: 1,853 (0.0695) Hypertension (summed across years): 1992–1993: 9,554 (0.314) 1996–1997: 16,862 (0.327)*

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
		Liver disease: 1992: 146 (0.0097) 1997: 296 (0.0111) Back pain: 1992: 225 (0.0151) 1997: 338 (0.0127) Abdominal pain: 1992: 492 (0.0330) 1997: 973 (0.0365) Diverticulosis: 1992: 65 (0.0043) 1997: 129 (0.0048)
Nobles et al. 2019a, 2019b Retrospective cohort study United States Subjects: 49,607 women with 110,985 singleton pregnancies between 2002 and 2010, including 1,987 women with gestational hypertension and 1,712 women with pre-eclampsia	Exposure estimated before and during pregnancy using a modified Community Multiscale Air Quality Model and the following inputs: meteorological data from the Weather Research and Forecasting model, emission data from the EPA National Emissions Inventory, and photochemical properties of pollutants. Median (25^{th}–75^{th} percentile): 0.0011 (0.00074–0.0030) ppb	Adjusted[e] relative risk (CI) for diagnosis of gestational hypertension per interquartile increase in MTBE concentration: 3 months preconception: 0.97 (0.89–1.05) Whole pregnancy: 0.97 (0.90–1.05) 1^{st} trimester: 0.98 (0.91–1.06) 3^{rd} trimester: 0.99 (0.91–1.07) Adjusted[e] relative risk (CI) for diagnosis of pre-eclampsia per interquartile increase in MTBE concentration: 3 months preconception: 1.23 (1.12–1.35)‡ Whole pregnancy: 1.24 (1.14–1.36)‡ 1^{st} trimester: 1.30 (1.19–1.42)‡ 3^{rd} trimester: 1.27 (1.16–1.39)‡

Table 2-1. Health Effects in Humans Exposed to Methyl *tert*-Butyl Ether (MTBE)—Epidemiological Studies

Reference and study population	Exposure	Outcomes
Kalkbrenner et al. 2018 Case-control study United States Subjects: 1,540 cases of ASD and 477 controls from the AGRE family-based study cohort 1,272 cases and controls (combined) were evaluated for autism severity using the CSS and 1,380 cases and controls (combined) were evaluated for autism-related traits using the SRS	Exposure estimated based on birth year from average annual concentration reported in the EPA emissions-based National-scale Air Toxics Assessment.	Adjusted[f] odds ratio (CI) between measure of ASD and log-transformed MTBE concentration: ASD diagnosis: 2.33 (1.31–4.15)† Change in CSS: 0.07 (-0.54–0.68) Change in SRS: 5.88 (-0.30–12.36) Adjusted[f] odds ratio (CI) between ASD diagnosis and log-transformed MTBE concentration, adjusted for additional air toxics: Adjusted for log-transformed diesel particular matter: 2.03 (1.02–4.05)† Adjusted for log-transformed xylene: 2.10 (1.03–4.27)†

[a]Statistically significant ($p<0.05$) compared to the reference group, as calculated for this review (2-tailed Fischer's Exact Probability Test).
[b]Symptoms linked to MTBE (headache, eye irritation, burning nose or throat, cough, nausea, dizziness, spaciness), as determined by the CDC.
[c]Adjusted for age, physical exercise, body mass index, systolic and diastolic blood pressure, ALT, white blood cells, total cholesterol, triglycerides, LDL, and HDL.
[d]Adjusted for age, race, sex, smoking status, asthma diagnosis, having a cold since 11/1/94, perception of living in a reformulated gas area, and heard of MTBE.
[e]Adjusted for maternal age, race/ethnicity, prepregnancy body mass index, smoking, alcohol use, parity, insurance type, marital status, history of asthma, and temperature.
[f]Adjusted for participant's birth year, median mean exposure level in the family, and census block group variables (population density, education, median rent).

* = statistically significant ($p<0.05$), as reported by the study authors; † = statistically significant from the null after correcting for multiple comparisons using the false discovery rate (set at 0.1), as reported by the study authors; ‡ = statistically significant from the null after correcting for multiple comparisons using the false discovery rate (set at 0.05), as reported by the study authors; AGRE = Autism Genetic Resource Exchange; ALT = alanine aminotransferase; ASD = autism spectrum disorder; CDC = Centers for Disease Control and Prevention; CI = confidence interval; CSS = calibrated severity score; EPA = U.S. Environmental Protection Agency; HDL = high-density lipoprotein; LDL = low-density lipoprotein; LOD = level of detection; NAFLD = non-alcoholic fatty liver disease; NR = not reported; SD = standard deviation; SRS = Social Responsiveness Score; TWA = time-weighted average

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Cain et al. 1996									
1	Human 22 M, 21 F	1 hour	0, 1.7	CS, NX, OP	Resp	1.7			
					Neuro	1.7			
(data also available in unpublished report by Cain et al. 1994)									
Johanson et al. 1995; Nihlén et al. 1998a									
2	Human 10 M	2 hours	5, 25, 50	CS, NX	Resp	50			
					Neuro	50			
Prah et al. 1994									
3	Human 19 M, 18 F	1 hour	0, 1.39	CS, NX, BI	Resp	1.39			
					Neuro	1.39			
ARCO 1980									
4	Rat (NS) NS	4 hours (WB)	18,892, 34,127, 38,657, 41,860, 63,953	LE, CS	Death			33,370	
					Resp			18,892	Nasal discharge, tachypnea, respiration slowing until death
					Neuro			18,892	Incoordination, loss of righting reflex
(commercial MTBE [99.1% purity])									
ARCO 1980									
5	Rat (NS) (NS)	4 hours (WB)	19,621, 29,681, 39,406, 55,784	LE, CS	Death			39,395	4-hour LC_{50}
					Resp			19,621	Irregular respiration, hyperpnea
					Neuro			19,621	Ataxia, loss of righting reflex, incoordination, prostration
(ARCO MTBE [96.2% purity])									

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bird et al. 1997									
6	Rat (Fischer-344) 5 M, 5 F	5 days 6 hours/day (WB)	0, 400, 3,000, 8,000	BI	Renal	8,000 F 400 M	3,000 M		Increased proliferation of epithelial cells in the proximal convoluted tubules
(data also available in unpublished report by Chun and Kintigh 1993)									
Conaway et al. 1985									
7	Rat (Sprague-Dawley) 25 F	10 days GDs 6–15 6 hours/day (WB)	0, 250, 1,000, 2,500	LE, CS, BW, FI, WI, GN, OW, DX	Bd wt Hepatic Develop	2,500 2,500 2,500			
Daughtrey et al. 1997									
8	Rat (Fischer-344) 22 M, 22 F	6 hours (WB)	0, 800, 4,000, 8,000	LE, CS, BW, NX	Bd wt Neuro	8,000 800[b] F 4,000 M	4,000 F 8,000 M	8,000 F	Females: Altered gait and decreased hind-leg grip strength at ≥4,000 ppm; ataxia, incoordination, and altered motor activity at 8,000 ppm Males: Ataxia, altered gait, hindleg splay, decreased muscle tone, incoordination, and altered motor activity ($BMCL_{10}$ for altered gait in female rats = 454 ppm)
(data also available in unpublished report by Gill 1989)									
Dodd and Kintigh 1989									
9	Rat (Fischer 344) 5 M, 5 F	13 days 6 hours/day (WB)	0, 2,000, 4,000, 8,000	LE, CS, BW, OW, GN, NX	Bd wt	4,000 F 2,000 M		8,000 F 4,000 M	36% decrease in body weight gain on days 1–7 in females; 65% decrease in body weight gain on days 1–3 in males
					Resp	8,000			
					Hepatic	2,000	4,000		10–13% increase in relative liver weight

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal	8,000			
					Endocr	4,000	8,000		Increased relative adrenal weight in males (39%) and females (13%)
					Immuno	8,000			
					Neuro		2,000	4,000	Hypoactivity at ≥2,000 ppm; ataxia at ≥4,000 ppm; decreased reflexes, decreased muscle tone at 8,000 ppm
MTBE Committee 1990a									
10	Rat (Fischer-344) 1–6 M, 1–6 F	6 hours (N)	0, 400, 8,000	LE, CS	Neuro	400		8,000	Ataxia, drowsiness
Prescott-Mathews et al. 1997									
11	Rat (Fischer-344) 5 M, 5 F	10 days 6 hours/day (WB)	0, 400, 1,500, 3,000	OW, HP, BI	Bd wt	3,000			
					Renal	3,000 F			
						400 M	1,500 M		Proximal tubule necrosis, α2u-globulin droplet accumulation, and cell proliferation in males; epithelial cell exfoliation in the tubule lumen at 3,000 ppm in males
Savolainen et al. 1985									
12	Rat (Wistar) 5 M	2 weeks 5 days/week 6 hours/day (WB)	0, 50, 100, 300	BW	Bd wt	300			

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Texaco Inc. 1981									
13	Rat (Sprague-Dawley) 20 M, 20 F	9 days 5 days/week 6 hours/day (WB)	0, 100, 300, 1,000, 3,000	LE, CS, BW, BC, HE, UR, OW, GN, HP	Bd wt	3,000			
					Resp		1,000		Inflammation of nasal mucosa and trachea
					Cardio	3,000			
					Gastro	3,000			
					Hemato	3,000			
					Musc/skel	3,000			
					Hepatic	3,000			
					Renal	3,000			
					Endocr	3,000			
					Immuno	3,000			
					Neuro	3,000			
					Repro	3,000			
Vergnes and Morabit 1989									
14	Rat (Fischer-344) 5 M, 5 F	5 days 6 hours/day (WB)	0, 800, 4,000, 8,000	CS, BW	Bd wt	4,000 F		8,000	90% decrease in body weight gain in females, body weight loss in males
					Neuro	4,000		8,000	Ataxia
Bevan et al. 1997a									
15	Mouse (CD-1) 30 F	10 days (GDs 6–15) 6 hours/day (WB)	0, 1,000, 4,000, 8,000	CS, BW, FI, GN, OW, DX	Bd wt	4,000	8,000		>10% reduction in maternal body weight and >25% reduction in body weight gain during and post-exposure; reduced food consumption ~30% during exposure only
					Resp	4,000		8,000	Labored breathing
					Hepatic	8,000			
					Neuro	1,000		4,000	Hypoactivity, ataxia

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Develop	1,000	4,000	8,000	7% decrease in fetal weights and decreased skeletal ossification at 4,000 ppm; increased litter resorption and post-implantation loss, 29% reduction in live fetuses, 21% decrease in fetal weights, and cleft palate at 8,000 ppm
(data also available in unpublished report by Tyl and Neeper-Bradley 1989)									
Bird et al. 1997									
16	Mouse (CD-1) 5 M, 5 F	5 days 6 hours/day (WB)	0, 400, 3,000, 8,000	BI	Hepatic	3,000 F 8,000 M	8,000 F		Increased hepatic cell proliferation
(data also available in unpublished report by Chun and Kintigh 1993)									
Conaway et al. 1985									
17	Mouse (CD-1) 30 F	10 days GDs 6–15 6 hours/day (WB)	0, 250, 1,000, 2,500	LE, CS, BW, FI, WI, OW, GN, DX	Bd wt	2,500			
					Hepatic	2,500			
					Develop	2,500			
Dodd and Kintigh 1989									
18	Mouse (CD-1) 5 M, 5 F	13 days 6 hours/day (WB)	0, 2,000, 4,000, 8,000	LE, CS, BW, OW, GN	Bd wt	8,000			
					Hepatic	4,000 M	2,000 F 8,000 M		13% increased relative liver weight
					Endocr	8,000			
					Neuro		2,000	4,000	Hypoactivity at ≥2,000 ppm; ataxia at ≥4,000 ppm
					Repro	8,000 M			

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Moser et al. 1996									
19	Mouse (B6C3F1) 6 F	3 days 6 hours/day (WB)	0, 8,000	CS, BW, BC, BI, OW, HP	Bd wt	8,000			
					Hepatic		8,000		20% increase in relative liver weight, slight centrilobular hypertrophy, increased hepatic DNA synthesis, liver enzyme induction
					Neuro		8,000		Transient abnormal gait, hypoactivity, and decreased muscle tone
Moser et al. 1996									
20	Mouse (CD-1) 6 F	3 days 6 hours/day (WB)	0, 8,000	CS, BW, BC, OW, HP	Bd wt	8,000			
					Hepatic		8,000		19% increase in relative liver weight, slight centrilobular hypertrophy, increased hepatic DNA synthesis, liver enzyme induction
					Neuro		8,000		Transient abnormal gait, hypoactivity, and decreased muscle tone
Snamprogetti 1980									
21	Mouse (Swiss albino) 20 M	10 minutes (WB)	82,700, 122,000, 167,100, 200,500, 219,100	LE	Death			180,000	10-minute LC_{50}
Snamprogetti 1980									
22	Mouse (Swiss albino) 40 M	3–12 minutes (WB)	209,300	LE	Death			209,300	LT_{50} = 5.6 minutes

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Tepper et al. 1994									
23	Mouse (Swiss-Webster) 4 M	1 hour (WB)	83, 277, 832, 2,774, 8,321	CS, BI, OF	Resp		4,604		RD_{50} (indicative of sensory irritation)
Vergnes and Chun 1994									
24	Mouse (CD-1) 10 M, 10 F	2 days 6 hours/day (WB)	0, 400, 3,000, 8,000	CS	Neuro	400	3,000	8,000	Hypoactivity, lack of startle response at ≥3,000 ppm; ataxia, prostration at 8,000 ppm
Vergnes and Kintigh 1993									
25	Mouse (CD-1) 5–10 M, 5–10 F	1–2 days 6 hours/day (WB)	0, 400, 3,000, 8,000	CS, BW	Bd wt	8,000			
					Neuro	8,000			
Bevan et al. 1997a									
26	Rabbit (New Zealand) 15 F	13 days (GDs 6–18) 6 hours/day (WB)	0, 1,000, 4,000, 8,000	CS, BW, FI, GN, OW, HP, DX	Bd wt	8,000			
					Hepatic	4,000	8,000		15% increase in maternal relative liver weight
					Neuro	4,000		8,000	Hypoactivity and ataxia
					Develop	8,000			
(data also available in unpublished report by Tyl 1989)									
INTERMEDIATE EXPOSURE									
Bevan et al. 1997b									
27	Rat (Sprague-Dawley) 25 M, 25 F	2 generations ~14–19 weeks per generation 5 days/week 6 hours/day (WB)	0, 400, 3,000, 8,000	CS, BW, FI, DX, GN, OW, HP, NX, RX	Bd wt	8,000 F 3,000 M	8,000 M		11–12% decrease in F0 and F1 adult male body weight
					Resp	8,000			
					Gastro	8,000			
					Hepatic	3,000 F 400 M	8,000 F 3,000 M		>10% increase in relative liver weight in F1 adult animals
					Endocr	8,000			

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno	8,000			
					Neuro	400[c]	3,000	8,000	Hypoactivity, blepharospasms, lack of startle response in F0 and F1 adults at ≥3,000 ppm; ataxia at 8,000 ppm
					Repro	8,000			
					Develop	400	3,000		~10% decrease in body weight during lactation in F1 females and F2 males and females
(data also available in unpublished report by Neeper-Bradley 1991)									
Biles et al. 1987									
28	Rat (Sprague-Dawley) 15 M, 30 F	16–28 weeks 5–7 day/week 6 hours/day (WB)	0, 250, 1,000, 2,500	LE, CS, BW, OW, GN, HP, DX	Bd wt	2,500			
					Resp	2,500			
					Repro	2,500			
					Develop	2,500			
Bird et al. 1997									
29	Rat (Fischer-344) 10–15 M, 10–15 F	28 days 5 days/week 6 hours/day (WB)	0, 400, 3,000, 8,000	BW, OW, GN, HP, BC, CS, BI, UR, LE	Bd wt	8,000 F 3,000 M		8,000 M	24–35% decreased body weight gain
					Hepatic	400[c]	3,000		8–13% increase in relative liver weight
					Renal	8,000 F 400 M	3,000 M		Increased protein accumulation and proliferation of epithelial cells in proximal convoluted tubules
					Endocr	400 F 3,000 M	3,000 F 8,000 M		8–23% increase in relative adrenal weight in females at ≥3,000 ppm; 53% increase in relative adrenal weight in males at 8,000 ppm

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	400[c]		3,000	Ataxia, hypoactivity, lack of startle response, blepharospasm
(data also available in unpublished report by Chun and Kintigh 1993)									
Daughtrey et al. 1997									
30	Rat (Fischer-344) 15 M, 15 F	13 weeks 5 days/week 6 hours/day (WB)	0, 800, 4,000, 8,000	CS, BW, NX, OW, HP	Bd wt	8,000			
					Neuro	4,000		8,000	Ataxia (weeks 1–4 only)
(data also available in unpublished report by Dodd and Kintigh 1989)									
Greenough et al. 1980									
31	Rat Sprague-Dawley 10 M, 10 F	13 weeks 5 days/week 6 hours/day (WB)	0, 250, 500, 1,000	LE, CS, BW, FI, WI, BC, UR, HE, OP, GN, HP	Bd wt	1,000			
					Resp	1,000			
					Cardio	1,000			
					Gastro	1,000			
					Hemato	1,000			
					Musc/skel	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Endocr	1,000			
					Immuno	1,000			
					Repro	1,000			
					Other noncancer	1,000			
Lington et al. 1997									
32	Rat (Fischer-344) 25 M, 25 F	13 weeks 5 days/week 6 hours/day (WB)	0, 800, 4,000, 8,000	CS, BW, HE, BC, GN, HP, OW	Bd wt	8,000			
					Resp	8,000			
					Cardio	8,000			
					Gastro	8,000			
					Hemato	8,000			

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Musc/skel	8,000			
					Hepatic	800 F	4,000 F 800 M		>8–39% increase in relative liver weights in males at ≥800 ppm; 13–15% increase in relative liver weights in females at ≥4,000 ppm
					Renal	8,000 F	800 M		>5–19% increase in relative kidney weight at ≥800 ppm; increased size of hyaline droplets at ≥4,000 ppm
					Endocr	800	4,000		13–55% increase in relative weights of adrenal glands at ≥4,000 ppm; 3-fold increase in corticosterone levels at 8,000 ppm
					Immuno	8,000 F	8,000 M		Increased incidence of lymphoid hyperplasia (not examined at 800 or 4,000 ppm).
					Neuro	800	4,000	8,000	Transient hypoactivity at ≥4,000 ppm; ataxia at 8,000 ppm
					Repro	8,000			
					Other noncancer	8,000			
(data also available in unpublished report by Dodd and Kintigh 1989)									
Savolainen et al. 1985									
33	Rat (Wistar) 15 M	6–15 weeks 5 days/week 6 hours/day (WB)	0, 50, 100, 300	BW, BI	Bd wt	300			

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bird et al. 1997									
34	Mouse (CD-1) 10–15 M, 10–15 F	28 days 5 days/week 6 hours/day (WB)	0, 400, 3,000, 8,000	LE, CS, BW, BC, BI, UR OW, GN, HP,	Bd wt	8,000			
					Hepatic	400 F 3,000 M	8,000 M		9–13% increase in relative liver weight in females at ≥3,000 ppm; 12% increase in relative liver weight in males at 8,000 ppm
					Renal	8,000			
					Endocr	8,000			
					Neuro	400		3,000	Ataxia, hypoactivity, lack of startle response
(data also available in unpublished report by Chun and Kintigh 1993)									
Moser et al. 1996									
35	Mouse (B6C3F1) 6 F	3 weeks 5 days/week 6 hours/day (WB)	0, 8,000	CS, BW, BC, BI, OW, HP	Bd wt	8,000			
					Hepatic		8,000		26% increase in relative liver weight, increased enzyme activity, decreased hepatic DNA synthesis
					Neuro		8,000		Transient abnormal gait, hypoactivity, and decreased muscle tone
Moser et al. 1996									
36	Mouse (CD-1) 6 F	3 weeks 5 days/week 6 hours/day (WB)	0, 8,000	CS, BW, BC, BI, OW, HP	Bd wt	8,000			
					Hepatic		8,000		13% increase in relative liver weight, increased enzyme activity, increased hepatic DNA synthesis
					Neuro		8,000		Transient abnormal gait, hypoactivity, and decreased muscle tone

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Moser et al. 1996									
37	Mouse (B6C3F1) 12 F	32 weeks 5 days/week 6 hours/day (WB)	0, 8,000	CS, BW, BC, GN, OW, HP	Bd wt		8,000		19–24% decrease in body weight
					Hepatic		8,000		50–90% increase in relative liver weight, mild hepatic hypertrophy
					Neuro		8,000		Transient abnormal gait, hypoactivity, and decreased muscle tone
(initiation-promotion study: half of control and exposed animals were initiated with DEN ~6 weeks prior to MTBE exposure)									
Moser et al. 1996									
38	Mouse (B6C3F1) 12 F	16 weeks 5 days/week 6 hours/day (WB)	0, 8,000	CS, BW, BC, BI, GN, OW, HP	Bd wt		8,000		17% decrease in body weight
					Hepatic		8,000		34–41% increase in relative liver weight, mild hepatic hypertrophy, elevated liver enzymes
					Neuro		8,000		Transient abnormal gait, hypoactivity, and decreased muscle tone
(initiation-promotion study: half of control and exposed animals were initiated with DEN ~6 weeks prior to MTBE exposure)									
Moser et al. 1998									
39	Mouse (B6C3F1) 12 F	4 months 5 days/week 6 hours/day (WB)	0, 8,000	BW, BC, OW, HP	Bd wt		8,000		17% decrease in body weight
					Endocr		8,000		32% decrease in relative pituitary weight; histopathological changes in adrenal and pituitary glands
					Repro		8,000		79% decrease in relative uterus weight; 48% decrease in relative ovary weight; histopathological changes in uterus, cervix, and vagina; decreased cell proliferation in uterus

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Moser et al. 1998									
40	Mouse (B6C3F1) 12 F	8 months 5 days/week 6 hours/day (WB)	0, 8,000	BW, OW, HP	Bd wt		8,000		19% decrease in body weight
					Endocr		8,000		20% decrease in relative pituitary weight; histopathological changes in adrenal and pituitary glands
					Repro		8,000		77% decrease in relative uterus weight; 46% decrease in relative ovary weight; histopathological changes in uterus, cervix, and vagina; decreased cell proliferation in uterus
Snamprogetti 1980									
41	Mouse (Swiss albino) 30 M	30 days 5 day/week 5–10 minutes/ day (WB)	0, 50,000, 80,000	LE, CS, NX	Neuro	80,000			
CHRONIC EXPOSURE									
Bird et al. 1997									
42	Rat (Fischer-344) 50 M, 50 F	24 months 5 days/week 6 hours/day (WB)	0, 400, 3,000, 8,000	LE, CS, BW, BC, HE, UR, OW, GN, HP	Death			3,000 M	Decreased survival due to chronic progressive nephropathy
					Bd wt	3,000		8,000	13–19% decrease in terminal body weight; 22–29% decrease in body weight gain
					Resp	8,000			
					Cardio	8,000			
					Gastro	8,000			

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hemato	8,000			
					Musc/skel	8,000 F	400 M		Osteodystrophy (secondary to nephropathy)
					Hepatic	400 F 8,000 M	3,000 F		24% increase in relative liver weight
					Renal	400[d] F	3,000 F 400 M		22% increase in relative kidney weight in females; increased incidence and severity of chronic progressive nephropathy
					Endocr	8,000 F	400 M		Hyperplasia of parathyroid (secondary to nephropathy); and altered corticosterone levels at ≥3,000 ppm
					Immuno	8,000			
					Neuro	3,000		8,000	Ataxia in both sexes, salivation in males
					Repro	8,000			
					Cancer			3,000 M	CEL: Renal tubular adenomas and carcinomas in males; no exposure-related tumors in females

(data also available in unpublished report by Chun et al. 1992)

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bird et al. 1997									
43	Mouse (CD-1) 50 M, 50 F	18 months 5 days/week 6 hours/day (WB)	0, 400, 3,000, 8,000	LE, CS, BW, WI, BI, HE, UR, OW, GN, HP	Death			8,000 M	Decreased survival due to obstructive uropathy
					Bd wt	3,000			
							8,000 M	8,000 F	24% decrease in body weight gain in females; 16% decrease in body weight gain in males
					Resp	8,000			
					Cardio	8,000			
					Gastro	8,000			
					Hemato	8,000			
					Musc/skel	8,000			
					Hepatic	3,000	8,000		39% increase in relative liver weight in females; hepatocellular hypertrophy in males
					Renal	3,000	8,000 F	8,000 M	13% increase in relative kidney weight in females at 8,000 ppm; obstructive uropathy and decreased urinary pH and increased urinary gamma globulin fraction in males at 8,000 ppm
					Endocr	8,000 F 3,000 M	8,000 M		3-fold increase in corticosterone levels; 60% increase in relative adrenal weight
					Immuno	8,000			
					Neuro	3,000		8,000	Ataxia
					Repro	8,000			

Table 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			8,000 F	CEL: Increased hepatocellular adenomas (10/50) compared to controls (2/50); no exposure-related tumors in males

(data also available in unpublished report by Burleigh-Flayer et al. 1992)

Shaded rows indicate the MRL principal studies.

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration inhalation minimal risk level (MRL) of 2 ppm for MTBE; based on a rat $BMCL_{10}$ of 454 ppm, adjusted to continuous exposure and converted to a human equivalent concentration ($BMCL_{HEC}$) of 70.1 ppm, and divided by an uncertainty factor of 30 (3 for animal to human with dosimetric adjustments and 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[c]Used to derive an intermediate-duration inhalation MRL of 1 ppm for MTBE. The NOAEL of 400 ppm was adjusted to continuous exposure and converted into a $NOAEL_{HEC}$ of 43.9 ppm and divided by and uncertainty factor of 30 (3 for animal to human with dosimetric adjustments and 10 for human variability); see Appendix A for more detailed information regarding the MRL.
[d]Used to derive a chronic-duration inhalation MRL of 1 ppm for MTBE. The NOAEL of 400 ppm was adjusted to continuous exposure and converted into a $NOAEL_{HEC}$ of 43.9 ppm and divided by and uncertainty factor of 30 (3 for animal to human with dosimetric adjustments and 10 for human variability; see Appendix A for more detailed information regarding the MRL.

BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; BMC = benchmark concentration; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; DEN = *N*-nitrosodiethylamine; Develop = developmental; DNA = deoxyribonucleic acid; DX = developmental effects; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathology; Immuno = immunological; LC_{50} = median lethal concentration; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LT_{50} = exposure time producing 50% death; M = male(s); Musc/skel = muscular/skeletal; (N) = nose-only; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OP = ophthalmology; OW = organ weight; RD_{50} = concentration that results in 50% decrease in respiratory rate; Repro = reproductive; Resp = respiratory; RX = reproductive function; UR = urinalysis; (WB) = whole body; WI = water intake

Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation
Acute (≤14 days)

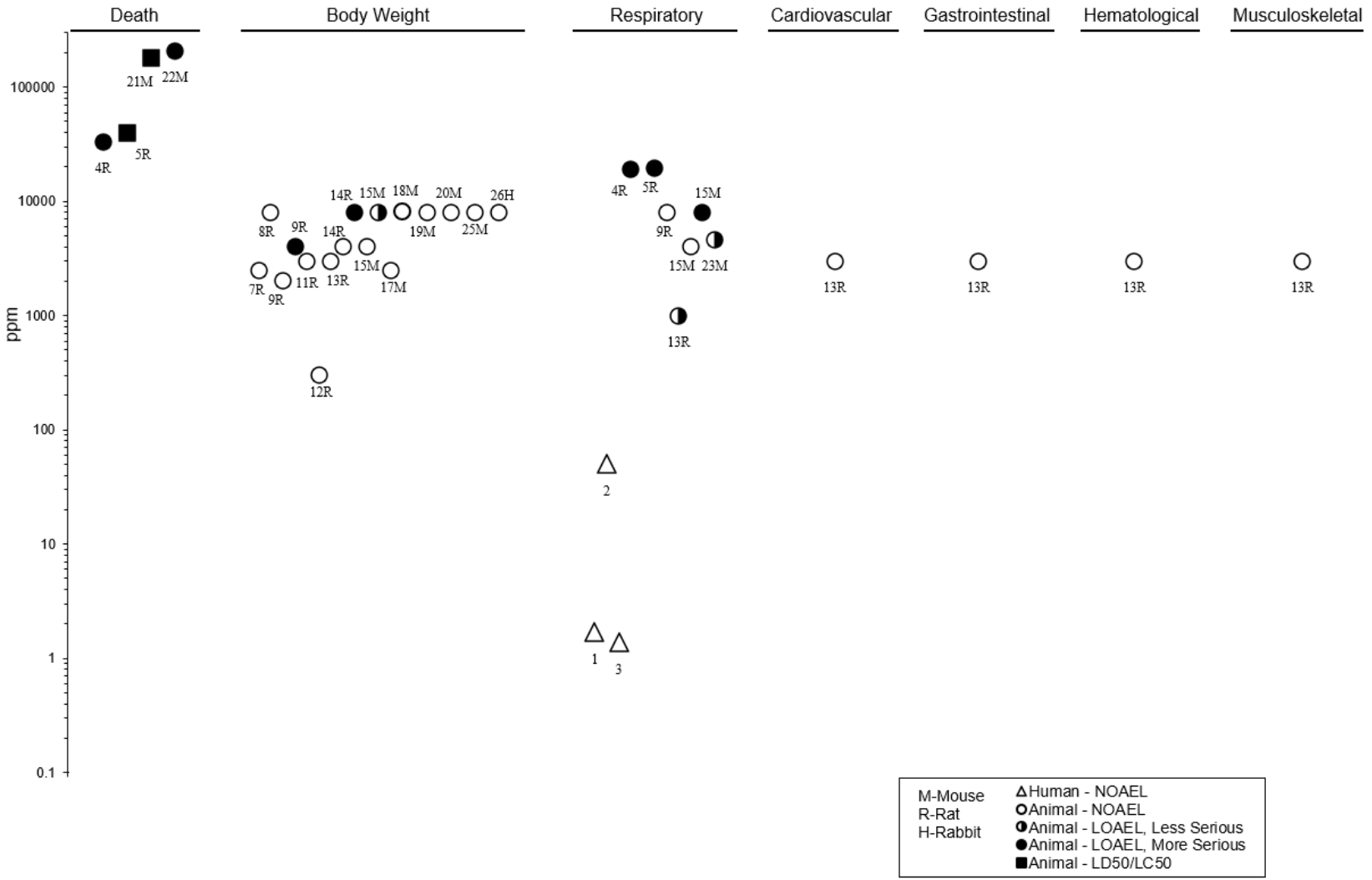


Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation
Acute (≤14 days)

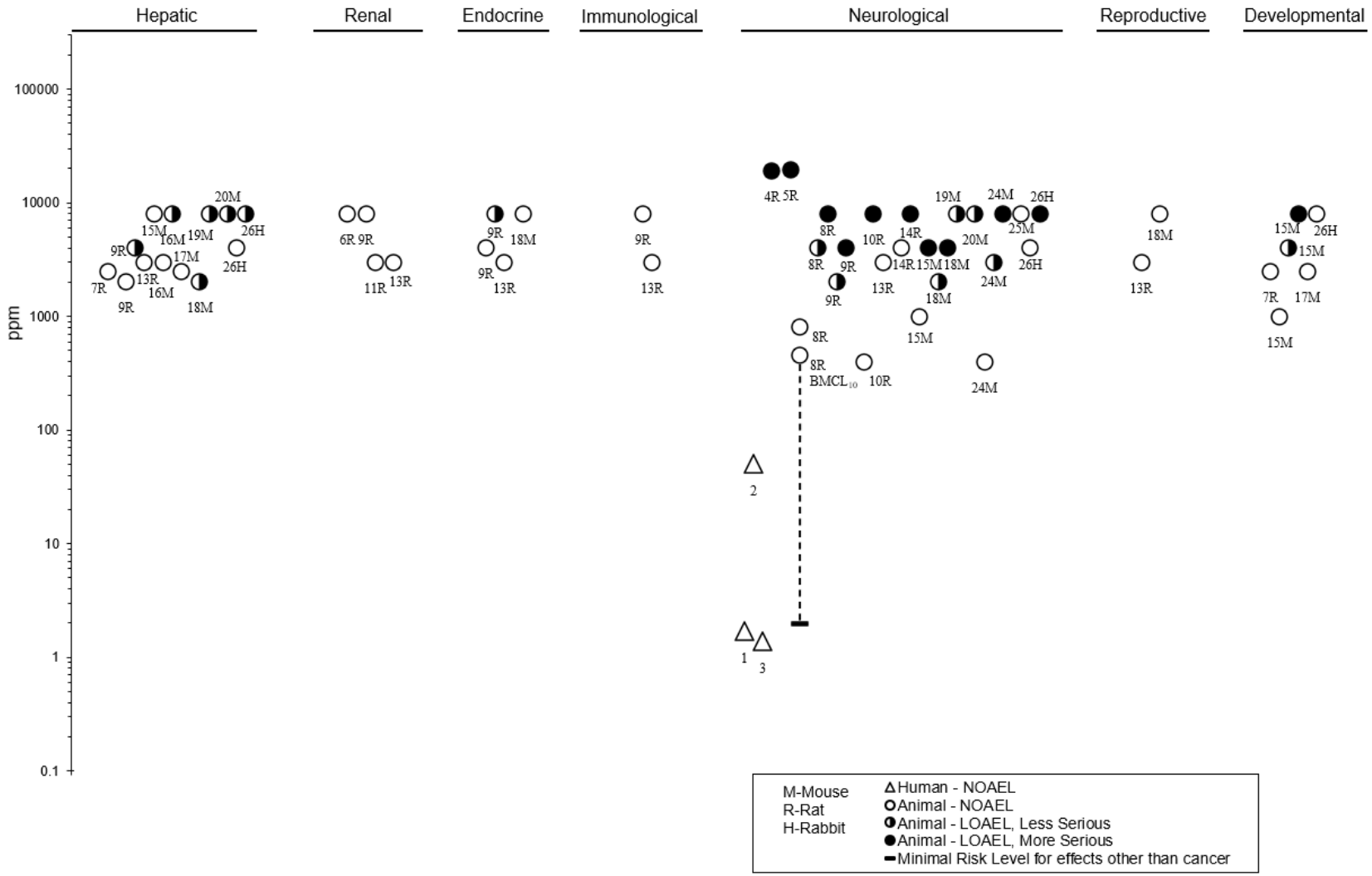


Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation
Intermediate (15–364 days)

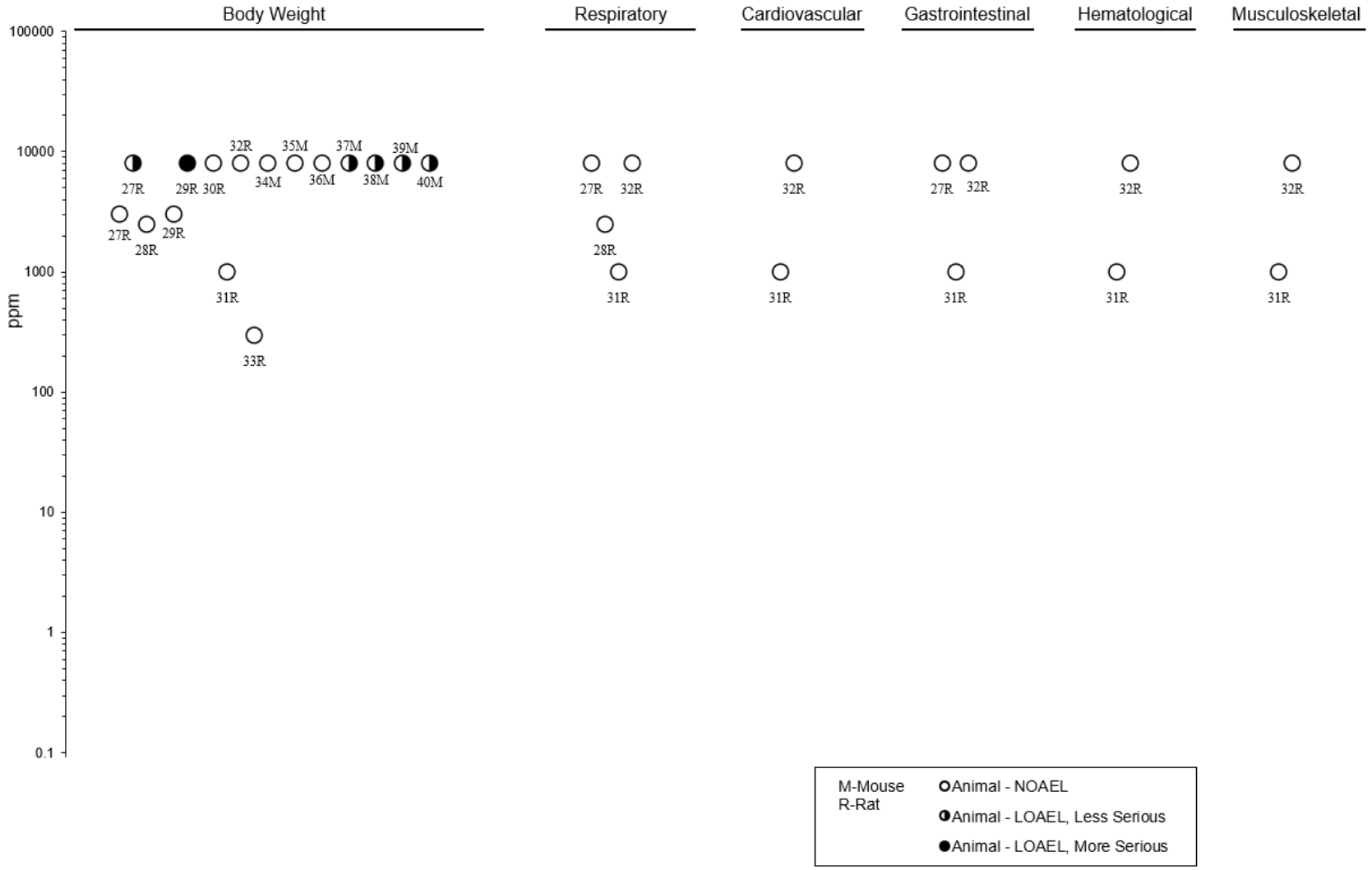


Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation
Intermediate (15–364 days)

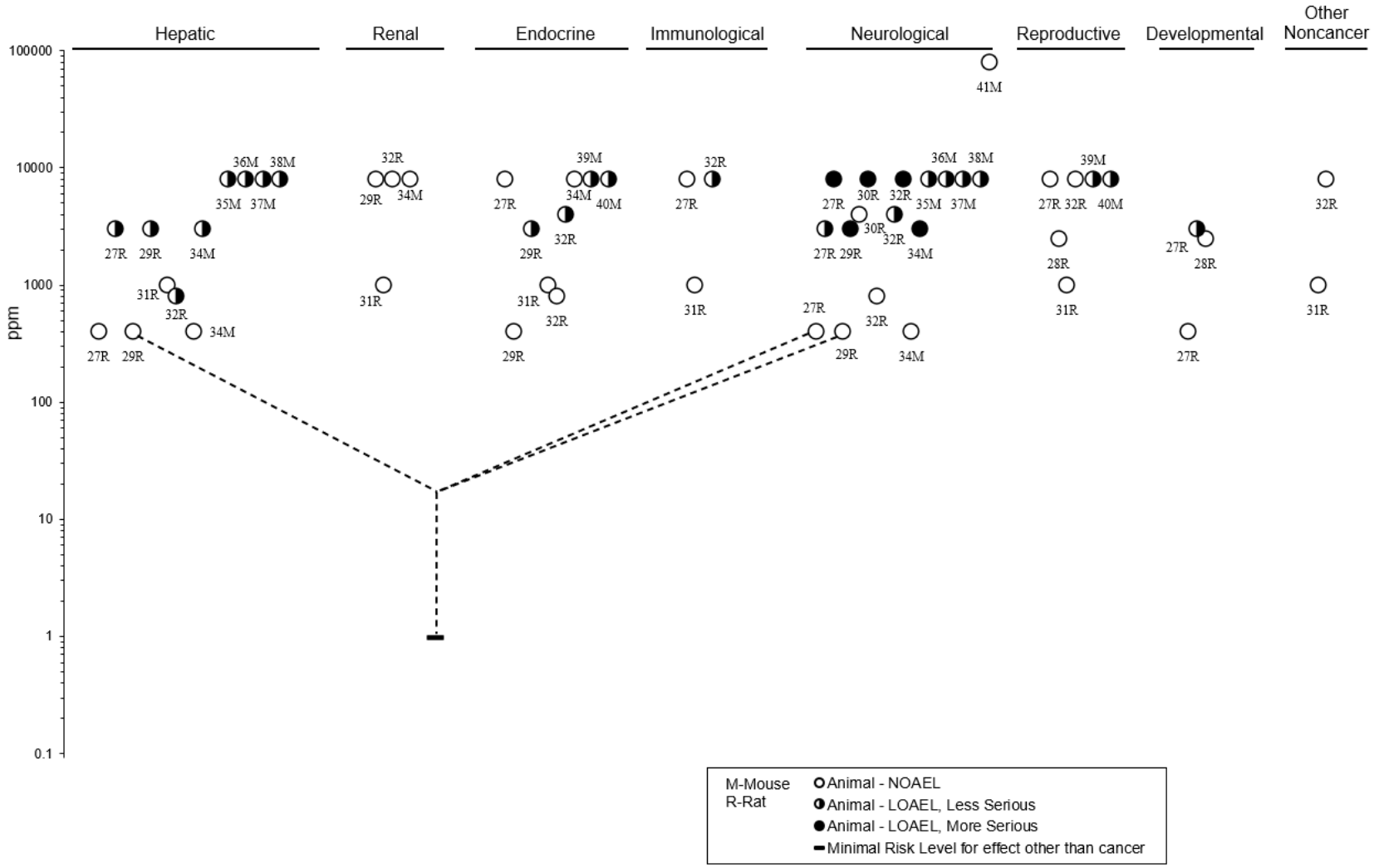


Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation
Chronic (≥365 days)

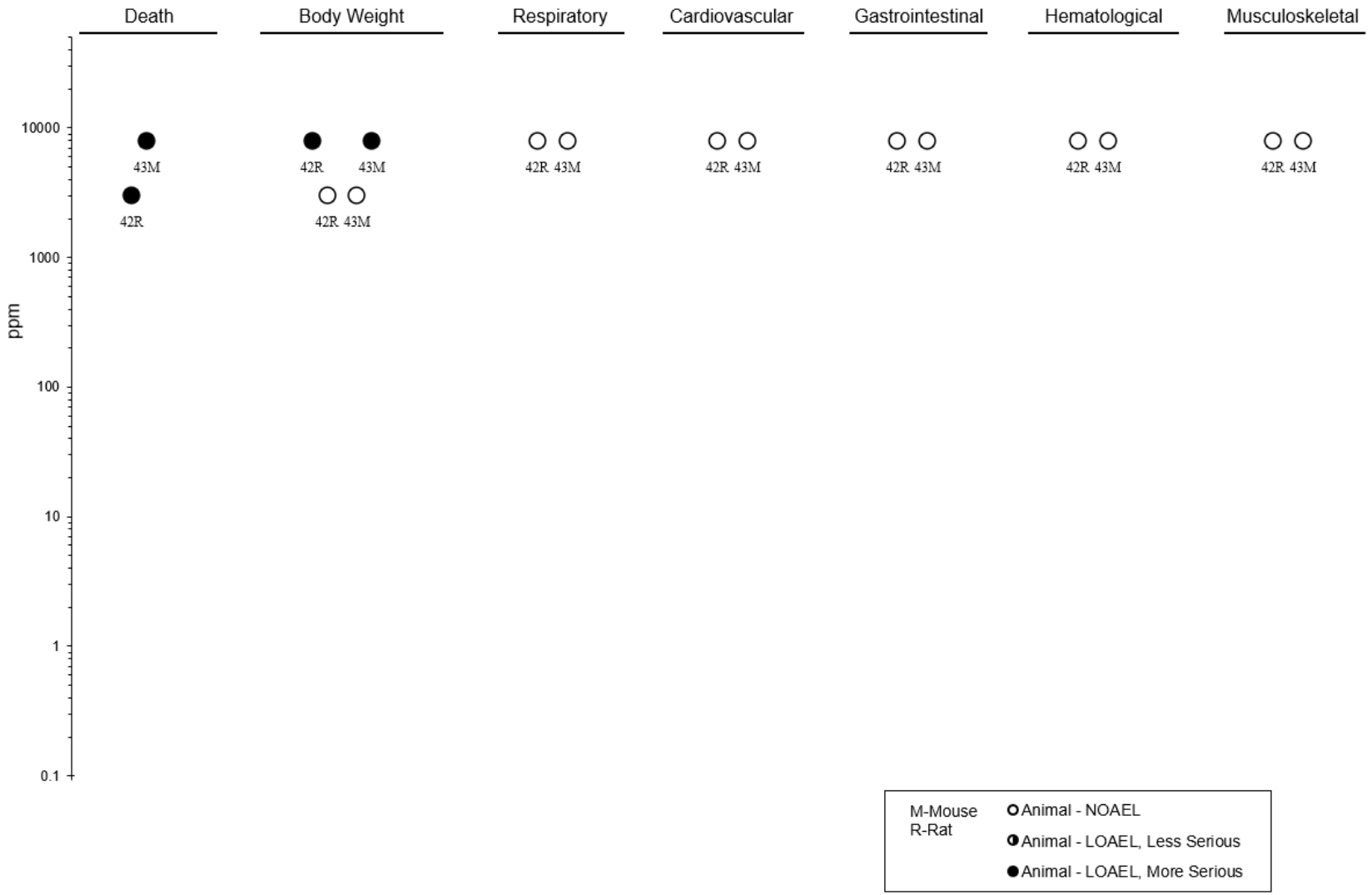


Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Inhalation
Chronic (≥365 days)

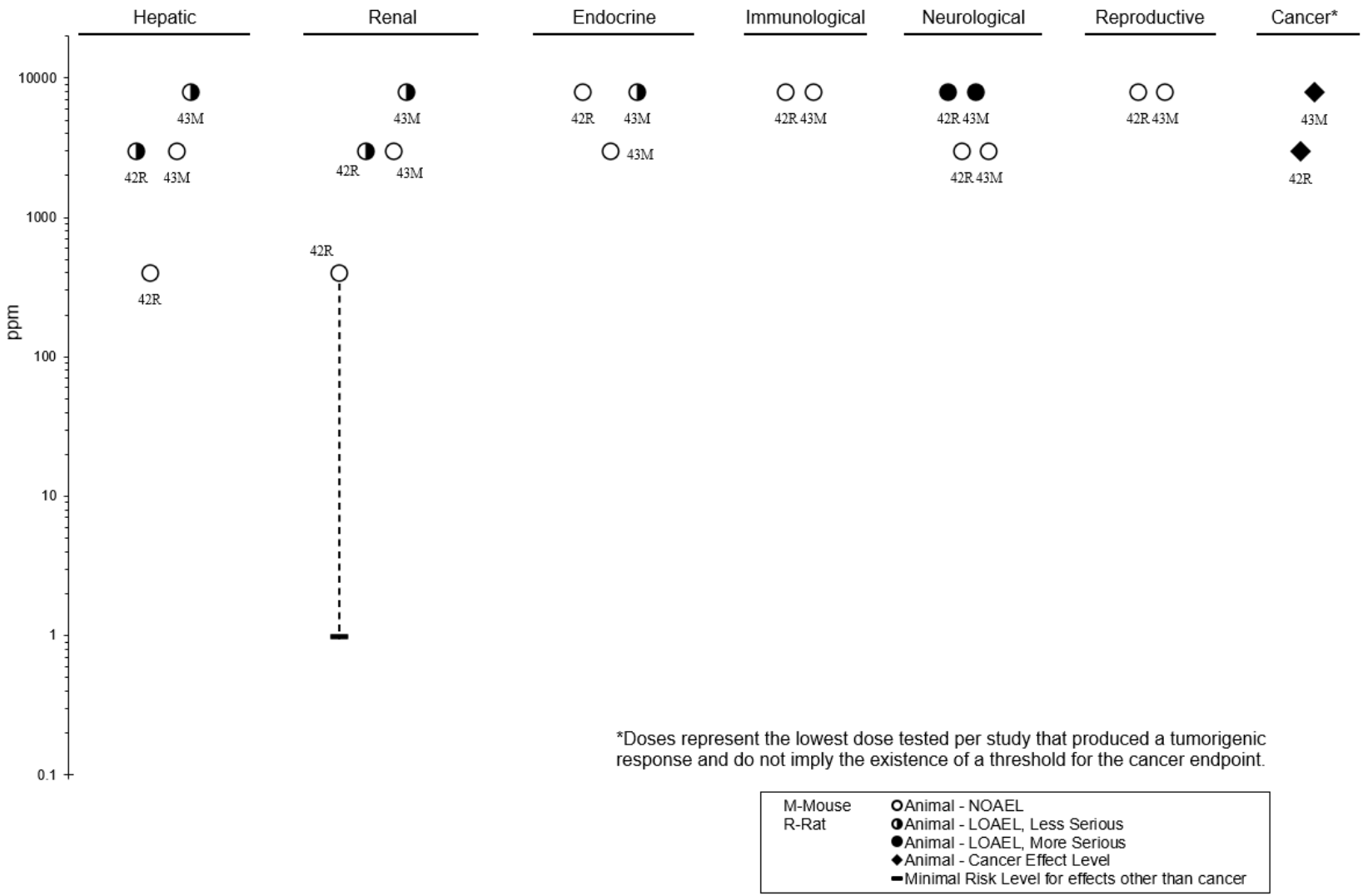


Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
ARCO 1980									
1	Rat (NS) 5 M, 5 F	Once (G)	1,900, 2,450, 3,160, 4,080, 5,270, 6,810	CS, LE, GN	Death			3,866	LD_{50}
					Resp	1,900	2,450	4,080	Gross evidence of respiratory tract irritation at 2,450 mg/kg/day; labored respiration at 4,080 mg/kg/day
					Neuro		1,900	2,450	Slight to marked CNS depression; ataxia at ≥2,450 mg/kg/day
Berger and Horner 2003									
2	Rat (Sprague-Dawley) 6 F	2 weeks (W)	0, 520	BW, RX	Bd wt	520			
					Repro	520			
Bermudez et al. 2012									
3	Rat (Wistar) 5 M, 5 F	1 week (W)	M: 0, 37, 209, 972 F: 0, 50, 272, 1,153	BI, HP	Renal	1,153 F 209 M	972 M		Hyaline droplets, elevated α2u-globulin
					Repro	972 M			
de Peyster et al. 2003									
4	Rat (Sprague-Dawley) 10 M	14 days (GO)	0, 1,200	BW, BC, BI, OW	Bd wt	1,200			
					Hepatic		1,200		18% increase in relative liver weight
					Repro		1,200		51% decrease in serum testosterone, 10% decrease in serum LH, 36% increase in serum estradiol

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
de Peyster et al. 2003									
5	Rat (Sprague-Dawley) 12 M	12 days; every other day (GO)	0 (naïve), 0 (vehicle), 1,000, 1,500	CS, BW, BC	Bd wt	1,000		1,500	Body weight loss
					Neuro			1,000	Sedation, ataxia
					Repro	1,000	1,500		74% decrease in serum testosterone (4–5 hours after initial dose)
de Peyster et al. 2014									
6	Rat (Sprague-Dawley) 10 M	2 weeks 6–7 days/week (GO)	0, 600, 1,200	LE, CS, BW, BC, BI, OW	Death			1,200	3/10 died
					Bd wt	600	1,200		10% decrease in body weight
					Hepatic	1,200			
					Renal	1,200			
					Endocr		600		2-fold increase in serum corticosterone; 10–12% increase in relative adrenal weight
					Neuro		600		Lethargy; transient ataxia in some animals
					Repro	1,200			
de Peyster et al. 2014									
7	Rat (Sprague-Dawley) 10 M	2 weeks (GO)	0, 400/500, 800/1,000, 1,200/1,500	CS, BW, BC, BI, OW	Bd wt	1,350			
					Hepatic	900	1,350		13% increase in relative liver weight
					Renal	900	1,350		12% increase in relative kidney weight
					Neuro		450		Lethargy; transient ataxia in some animals

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	450	900		≥75% decrease in serum testosterone
(doses listed for week 1/week 2; TWA doses = 0, 450, 900, 1,350 mg/kg/day)									
de Peyster et al. 2014									
8	Rat (Sprague-Dawley) 5 M	2 weeks (GO)	0, 1,200	CS, BW, BC, BI, OW	Bd wt	1,200			
					Hepatic	1,200			
					Renal	1,200			
					Endocr		1,200		21% increase in relative adrenal weight
					Neuro		1,200		Lethargy; transient ataxia in some animals
Dong-mei et al. 2009									
9	Rat (Sprague-Dawley) 10 M	2 weeks (GO)	0, 400, 800, 1,600	CS, BW, FI, LE, BC, HE, OW	Bd wt	1,600			
					Hemato	800	1,600		Transient changes in hematology (2-fold increase in total WBC count; 2–5-fold increase in percentage of lymphocytes, granulocytes, and eosinophils)
					Hepatic	800	1,600		Transient increases in serum cholesterol and relative liver weight
					Renal	1,600			
					Neuro		1,600		Transient signs of CNS depression
					Repro		400		Transient decreases in relative testes weight

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Li et al. 2008									
10	Rat (Sprague-Dawley) 10 M	2 weeks (GO)	0, 400, 800, 1,600	BC, HP	Repro		400		40–50% increase in LH at ≥400 mg/kg/day; 60–70% decrease in testosterone and 40–70% increase in FSH at ≥800 mg/kg/day; altered testicular histology at 1,600 mg/kg/day
MTBE Committee 1990b									
11	Rat (Fischer 344) 6 M, 6 F	Once (GW)	0, 40, 400	LE, CS	Neuro	40	400		Drowsiness
Robinson et al. 1990									
12	Rat (Sprague-Dawley) 10 M, 10 F	14 days (GO)	0, 357, 714, 1,071, 1,428	LE, CS, BW, HE, BC, FI, WI, OW, GN, HP	Bd wt	1,428			
					Resp	1,428			
					Cardio	1,428			
					Gastro		357		Diarrhea
					Hemato	1,428 F 714 M	1,071 M		33% decrease in percent monocytes in males
					Hepatic	357 F 714 M	714 F 1,071 M		29% increase in serum cholesterol in females; 74% increase in AST and 4-fold increase in LDH at 1,071 mg/kg/day and 59% increase in serum cholesterol at 1,428 mg/kg/day in males
					Renal	1,428 F 1,071 M	1,428 M		Renal tubule nephropathy characterized by increased hyaline droplets
					Endocr	1,428			
					Immuno	1,428			

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	1,071		1,428	Transient anesthesia
					Repro	1,428			
					Other noncancer	1,071 F 1,428 M	1,428 F		17% increase in serum glucose in females
Billitti et al. 2005									
13	Mouse (CD-1) 5 M	1 week, 3 days/week (GO)	0, 400, 1,000, 2,000	BW, BC, OW, HP	Bd wt	2,000			
					Repro	2,000			
de Peyster et al. 2008									
14	Mouse (CD-1) 6 M	1 week 3 days/week (GO)	0, 400, 1,000, 2,000	BC, BW, OW, HP	Bd wt	2,000			
					Neuro	1,000		2,000	Ataxia, lethargy
					Repro	2,000			
Little et al. 1979									
15	Mouse (NS) NS	once (G)		LE	Death			4,000	LD_{50}
INTERMEDIATE EXPOSURE									
Amoco 1992									
16	Rat (Sprague-Dawley) 10 M, 10 F	4 weeks 5 days/week (G)	0, 90, 440, 1,750	LE, CS, BW, BC, HE, OW, GN, HP	Bd wt	1,750			
					Resp	1,750			
					Cardio	1,750			
					Gastro	440	1,750		Submucosal edema
					Hemato	1,750			
					Musc/skel	1,750			
					Hepatic	440	1,750		9–13% increase in relative liver weight and increased serum cholesterol
					Renal	440 F 90 M	1,750 F 440 M		8–9% increase in relative kidney weight; hyaline droplets in proximal convoluted tubules in males

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Dermal	1,750			
					Ocular	1,750			
					Endocr	1,750 F 440 M	1,750 M		23% increase in relative adrenal gland weight in males
					Immuno	1,750			
					Neuro	440 F 90 M	440 M	1,750	Hypoactivity in males at ≥440 mg/kg/day; ataxia in both sexes at 1,750 mg/kg/day
					Repro	1,750			
Bermudez et al. 2012									
17	Rat (Wistar) 10 M, 10 F	13 weeks (W)	M: 0, 37, 209, 514, 972 F: 0, 50, 272, 650, 1,153	CS, BW, FI, WI, UR, OW, HP	Bd wt	1,153 F 972 M			
					Resp	1,153 F 972 M			
					Cardio	1,153 F 972 M			
					Gastro	1,153 F 972 M			
					Musc/skel	1,153 F 972 M			
					Hepatic	1,153 F 972 M			
					Renal	272 F	650 F		

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
						209 M	514 M		19–21% increase in relative kidney weights in both sexes and increased incidence of hyaline droplets in males; increased tubular epithelial regeneration in males at 972 mg/kg/day
					Ocular	1,153 F			
						972 M			
					Endocr	1,153 F			
						972 M			
					Immuno	1,153 F			
						972 M			
					Neuro	1,153 F			
						972 M			
					Repro	1,153 F			
						972 M			
Bermudez et al. 2012									
18	Rat (Wistar) 5 M, 5 F	4 weeks (W)	M: 0, 37, 209, 972 F: 0, 50, 272, 1,153	BI, HP	Renal	1,153 F			Elevated cell replication of cortical proximal tubule cells, elevated α2u-globulin in males
						209 M	972 M		
					Repro	972 M			
Bermudez et al. 2012									
19	Rat (Wistar) 5 M, 5 F	13 weeks (W)	M: 0, 37, 209, 972 F: 0, 50, 272, 1,153	BI, HP	Renal	1,153 F			Hyaline droplets in males
						37 M	209 M		
					Repro	972 M			

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bermudez et al. 2012									
20	Rat (Wistar) 5 M	6 months (W)	0, 29, 166, 384	CS, BW, FI, WI, UR, OW, HP	Bd wt	384			
					Resp	384			
					Cardio	384			
					Gastro	384			
					Musc/skel	384			
					Hepatic	384			
					Renal	384			
					Ocular	384			
					Endocr	384			
					Immuno	384			
					Neuro	384			
					Repro	384			
de Peyster et al. 2003									
21	Rat (Sprague-Dawley) 12 M	28 days; every other day (GO)	0 (naïve), 0 (vehicle), 1,000/500, 1,500/750	CS, BW, BC, BI, OW	Hepatic	357	536		Induction of hepatic enzymes
					Repro	536			
(doses reduced on day 13, after six treatments; TWA doses over 28-day exposure period were 357 and 536 mg/kg/day)									
de Peyster et al. 2003									
22	Rat (Sprague-Dawley) 12–13 M	28 days (GO)	0, 40, 400, 800	BW, BC, OW	Bd wt	400	800		11% decrease in body weight
					Endocr	400	800		2-fold increase in serum corticosterone; 26% increase in relative adrenal gland weight
					Repro	400	800		42% decrease in serum testosterone

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
de Peyster et al. 2003									
23	Rat (Sprague-Dawley) 12 M	28 days (GO)	0, 1,000	BC, BI, BW, OW, HP	Hepatic		1,000		11% increase in relative liver weight, increased AST and ALT
Dong-mei et al. 2009									
24	Rat (Sprague-Dawley) 10 M	4 weeks (GO)	0, 400, 800, 1,600	LE, CS, BW, FI, HE, OW, BC	Bd wt	1,600			
					Hemato	1,600			
					Hepatic	1,600			
					Renal	1,600			
					Neuro		1,600		Transient CNS depression
Gholami et al. 2015									
25	Rat (Sprague-Dawley) 5 M	30 days (GO)	0, 400, 800, 1,600	BW, FI, OW, HP	Bd wt	1,600			
					Repro		400		Pyknosis, decreased cell layers, increased cell distance, and vacuoles in seminiferous tubules; increased seminiferous tubule diameter and decreased spermatocytes and spermatids at ≥800 mg/kg/day; decreased interstitial cells at 1,600 mg/kg/day
Khalili et al. 2015									
26	Rat (Sprague-Dawley) 5 M	30 days (GO)	0, 400, 800, 1,600	CS, BW, FI, BC, DX, RX	Bd wt	1,600			
					Repro	800	1,600		40% decrease in fertility; 58% decrease in serum testosterone
					Develop	1,600			
(offspring evaluated for number, sex ratio, and gross anomalies only)									

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Li et al. 2008									
27	Rat (Sprague-Dawley) 10 M	4 weeks (GO)	0, 400, 800, 1,600	CS, BW, FI, BC, OW, HP	Bd wt	1,600			
					Repro		400	1,600	~1.6-fold increase in percent abnormal sperm at 400 mg/kg/day; 2.4-fold increase in percent abnormal sperm at 1,600 mg/kg/day; altered serum testosterone and testicular histology at ≥800 mg/kg/day
Robinson et al. 1990									
28	Rat Sprague-Dawley 10 M, 10 F	90 days (GO)	0, 100, 300, 900, 1,200	CS, BW, OW, FI, WI, HE, BC, OW, GN, HP, NX	Bd wt	1,200			
					Resp	1,200			
					Cardio	1,200			
					Gastro		100		Diarrhea
					Hemato	900	1,200		55% increase in percent monocytes in males; 40% decrease in WBC count in females
					Hepatic		100 F		Females: 15% increase in serum cholesterol
						100 M	300 M		Males: 52% increase in AST; increase in relative liver weight at ≥900 mg/kg/day
					Renal	1,200 F			16% increase in relative kidney weight; hyaline droplets and granular casts at 1,200 mg/kg/day
						300 M	900 M		
					Endocr	1,200			
					Immuno	1,200			
					Neuro	900		1,200	Transient anesthesia

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	1,200			
					Other noncancer	100 F 1,200 M	300 F		17% decrease in serum glucose in females
Williams et al. 2000									
29	Rat (Sprague-Dawley) 11–15 M	28 days (GO)	0, 250, 500, 1,000, 1,500	CS, BW, BC, OW, HP	Bd wt	1,000	1,500		7–12% decrease in body weight from day 15 to 28
					Hepatic	250	500		Centrilobular hypertrophy; 11–16% increase in relative liver weight at ≥1,000 mg/kg/day
					Renal		250		10–22% increase in relative kidney weight, increased incidence and severity of protein droplet nephropathy
					Endocr	1,500			
					Repro	1,000	1,500		17% increase in relative testes weight, 20% decrease in serum LH, 45% decrease in serum dihydrotestosterone
Williams et al. 2000									
30	Rat (Sprague-Dawley) 15 M	15 days (GO)	0, 1,500	CS, BW, BC, OW, HP	Bd wt	1,500			
					Hepatic		1,500		Centrilobular hypertrophy
					Renal		1,500		12% increase in relative kidney weights; increased incidence and severity of protein droplet nephropathy
					Endocr	1,500			
					Repro		1,500		>50% decrease in serum testosterone, serum prolactin, and testicular testosterone

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Williams et al. 2000									
31	Rat (Sprague-Dawley) 12–15 M	15 days (GO)	0, 250, 500, 1,000	CS, BW, BC, OW	Bd wt	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Endocr	1,000			
					Repro	1,000			
Zhu et al. 2022									
32	Rat (Sprague-Dawley) 6 M	21 days PNDs 35–56	0, 300, 600, 1,200	DX	Develop		300[b]		≥50% reduction in serum testosterone; decrease Leydig cell number at 1,200 mg/kg/day ($BMCL_{1SD}$ for decreased serum testosterone in male offspring = 36 mg/kg/day)
de Peyster et al. 2008									
33	Mouse (BALB/c) 6 M	28 days (W)	0, 0.01, 0.1, 1	BW, WI, BC, BI, OW, HP	Bd wt	1			
					Repro	1			
de Peyster et al. 2008									
34	Mouse (BALB/c) 10 M	51 days PNDs 25/26–76/77 (W)	0, 0.02, 0.2, 2	DX	Develop	2			
(endpoints evaluated at PNDs 76–77 were primarily reproductive toxicity)									
Tang et al. 2019									
35	Mouse (C57BL/6J) 5 M, F	14 weeks (G)	0, 0.1, 1, 100	BW, BC, OW, HP, OF	Bd wt	100			
					Hepatic	100			
					Other noncancer	100			

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ward et al. 1994									
36	Mouse (CD-1) 10 M, 10 F	3 weeks 5 days/week (GO)	0, 1, 10, 100, 1,000	LE, CS, BW, HP	Bd wt	1,000			
					Repro	1,000			
CHRONIC EXPOSURE									
Belpoggi et al. 1995, 1997									
37	Rat (Sprague-Dawley) 60 M, 60 F	104 weeks 4 days/week (GO)	0, 250, 1,000	CS, BW, FI, WI, GN, HP	Death			250 F	20–30% decrease in survival
					Bd wt	1,000			
					Resp	1,000			
					Cardio	1,000			
					Gastro	1,000			
					Musc/skel	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Dermal	1,000			
					Endocr	1,000			
					Immuno	1,000 M	250 F		Dysplastic proliferation of lymphoreticular tissue (possibly preneoplastic) in females
					Neuro	1,000			
					Repro	1,000			
					Cancer			250 F 1,000 M	CEL: Leukemia and lymphoma in females, Leydig cell tumors in males

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bermudez et al. 2012									
38	Rat (Wistar) 10 M, 10 F	1 year (W)	M: 0, 29, 166, 384 F: 0, 54, 258, 1,119	CS, BW, FI, WI, UR, OW, HP	Bd wt	1,119 F			
						384 M			
					Resp	1,119 F			
						384 M			
					Cardio	1,119 F			
						384 M			
					Gastro	1,119 F			
						384 M			
					Musc/skel	1,119 F			
						384 M			
					Hepatic	1,119 F			
						384 M			
					Renal	1,119 F			
							29 M		9–19% increase in relative kidney weights, increased incidence and severity of chronic progressive nephropathy in males
					Ocular	1,119 F			
						384 M			
					Endocr	1,119 F			
						384 M			
					Immuno	1,119 F			
						384 M			
					Neuro	1,119 F			
						384 M			
					Repro	1,119 F			
						384 M			

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Dodd et al. 2013									
39	Rat (Wistar) 50 M, 50 F	2 years (W)	M: 0, 25, 140, 330 F: 0, 49, 232, 1,042	CS, BW, WI, FI, OW, GN, HP	Bd wt	1,042 F 330 M			
					Resp	1,042 F 330 M			
					Cardio	1,042 F 330 M			
					Gastro	1,042 F 330 M			
					Musc/skel	1,042 F 330 M			
					Hepatic	1,042 F 330 M			
					Renal	232 F 140 M	1,042 F 330 M		>10% increase in relative kidney weights and increased severity of chronic progressive nephropathy
					Ocular	1,042 F 330 M			
					Endocr	1,042 F 330 M			
					Immuno	1,042 F 330 M			
					Neuro	1,042 F 330 M			

Table 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	1,042 F 330 M			

Shaded row indicates the MRL principal study.

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.4 mg/kg/day for MTBE. The $BMDL_{1SD}$ of 36 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability); see Appendix A for more detailed information regarding the MRL.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; BMDL = 95% lower confidence limit on the benchmark dose; Cardio = cardiovascular; CEL = cancer effect level; CNS = central nervous system; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; F = female(s); FI = food intake; FSH = follicle-stimulating hormone; (G) = gavage; (GO) = gavage in oil; (GW) = gavage in water; Gastro = gastrointestinal; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = median lethal dose; LDH = lactate dehydrogenase; LE = lethality; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; SD = standard deviation; TWA = time-weighted average; UR = urinalysis; (W) = water; WBC = white blood cell; WI = water intake

Figure 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral
Acute (≤14 days)

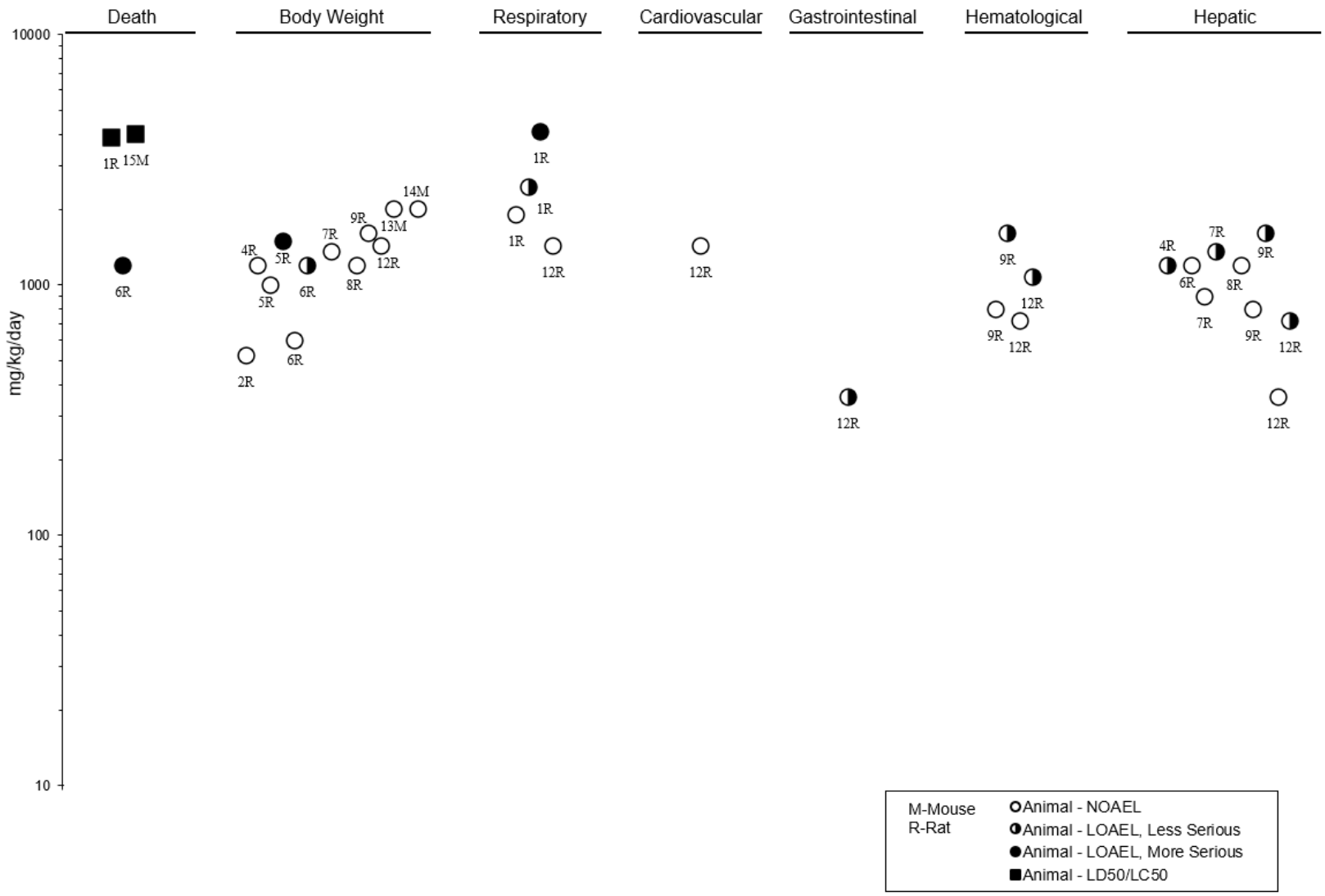


Figure 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral
Acute (≤14 days)

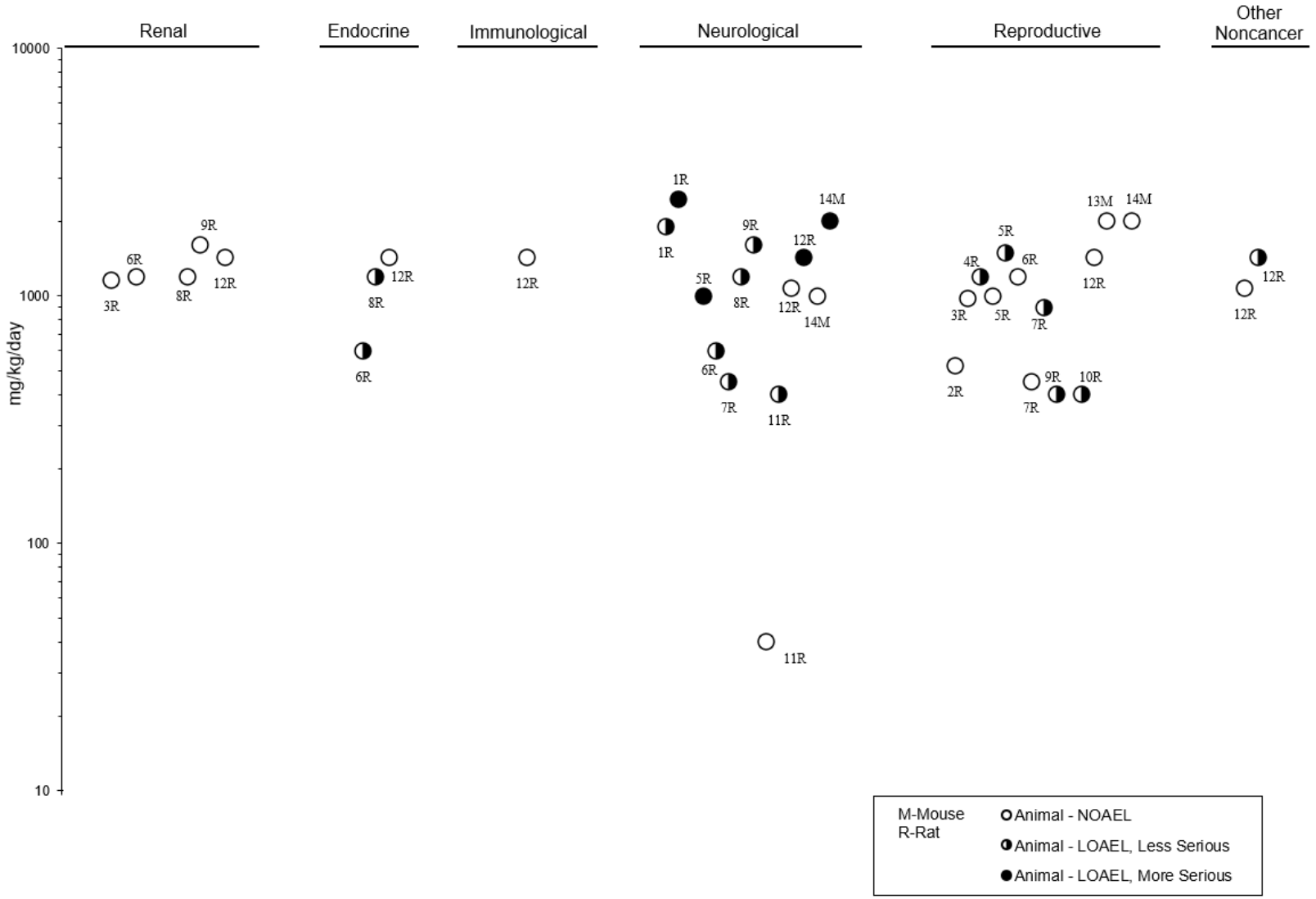


Figure 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral
Intermediate (15–364 days)

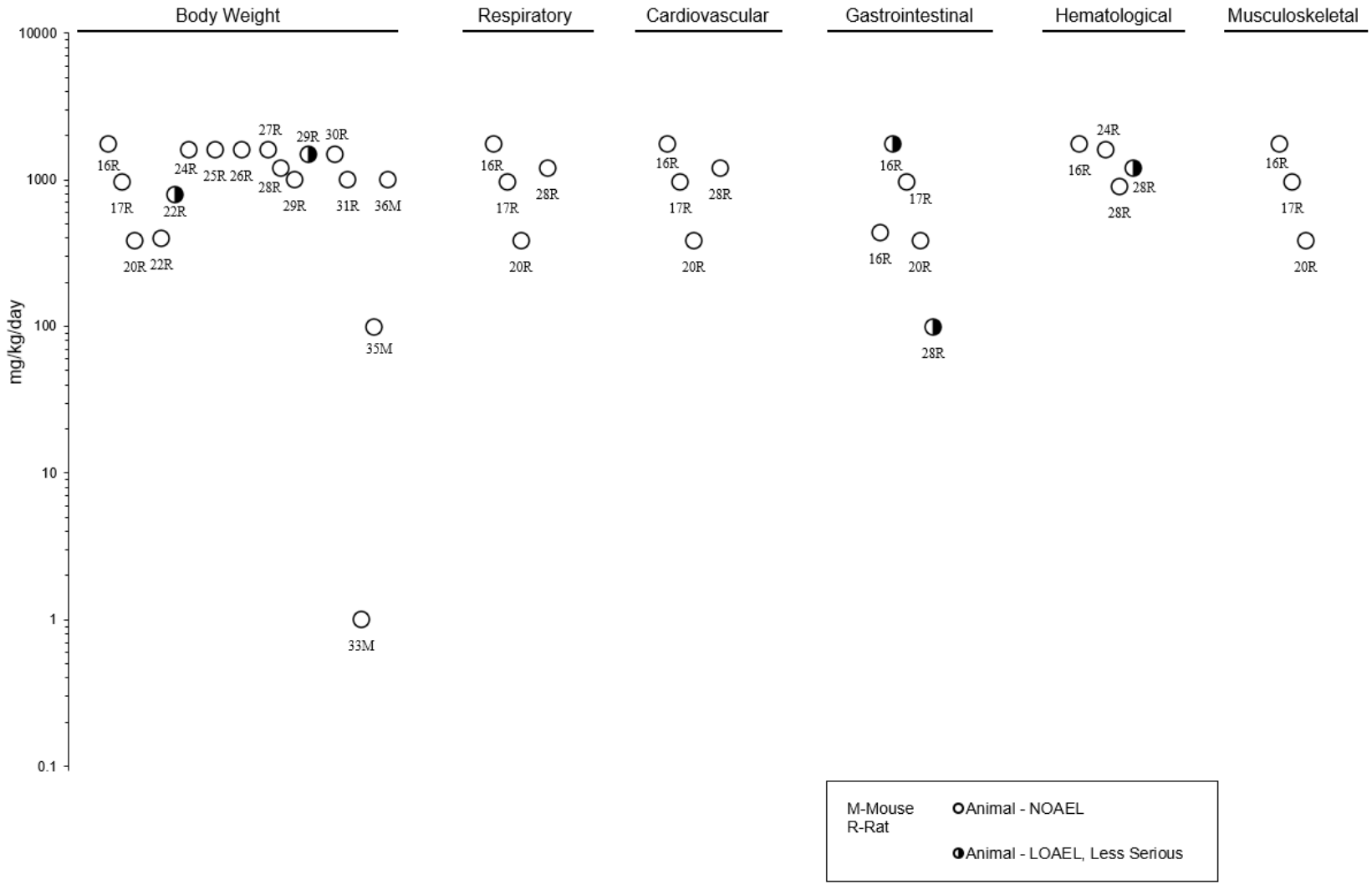


Figure 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral
Intermediate (15–364 days)

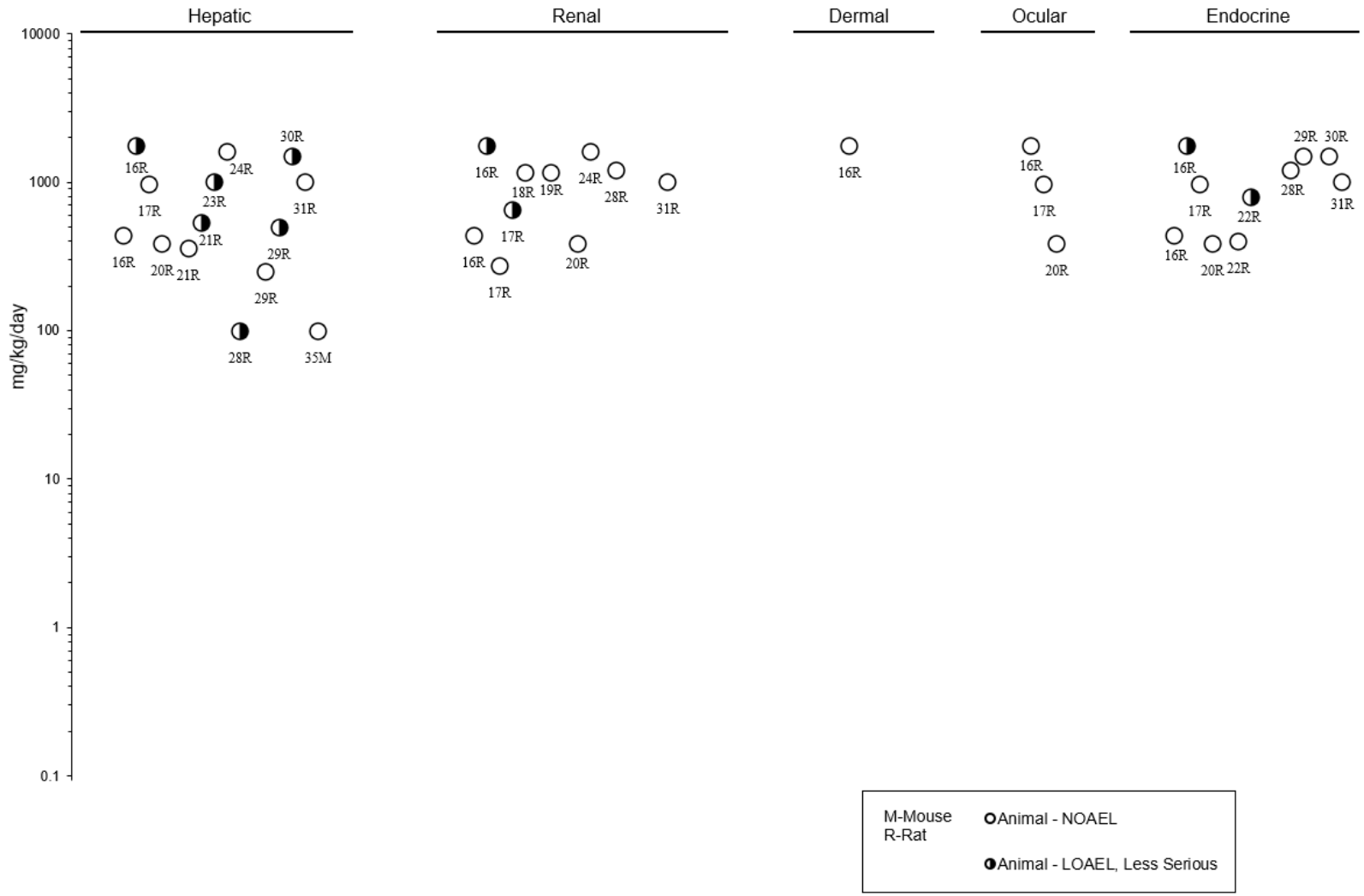


Figure 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral
Intermediate (15–364 days)

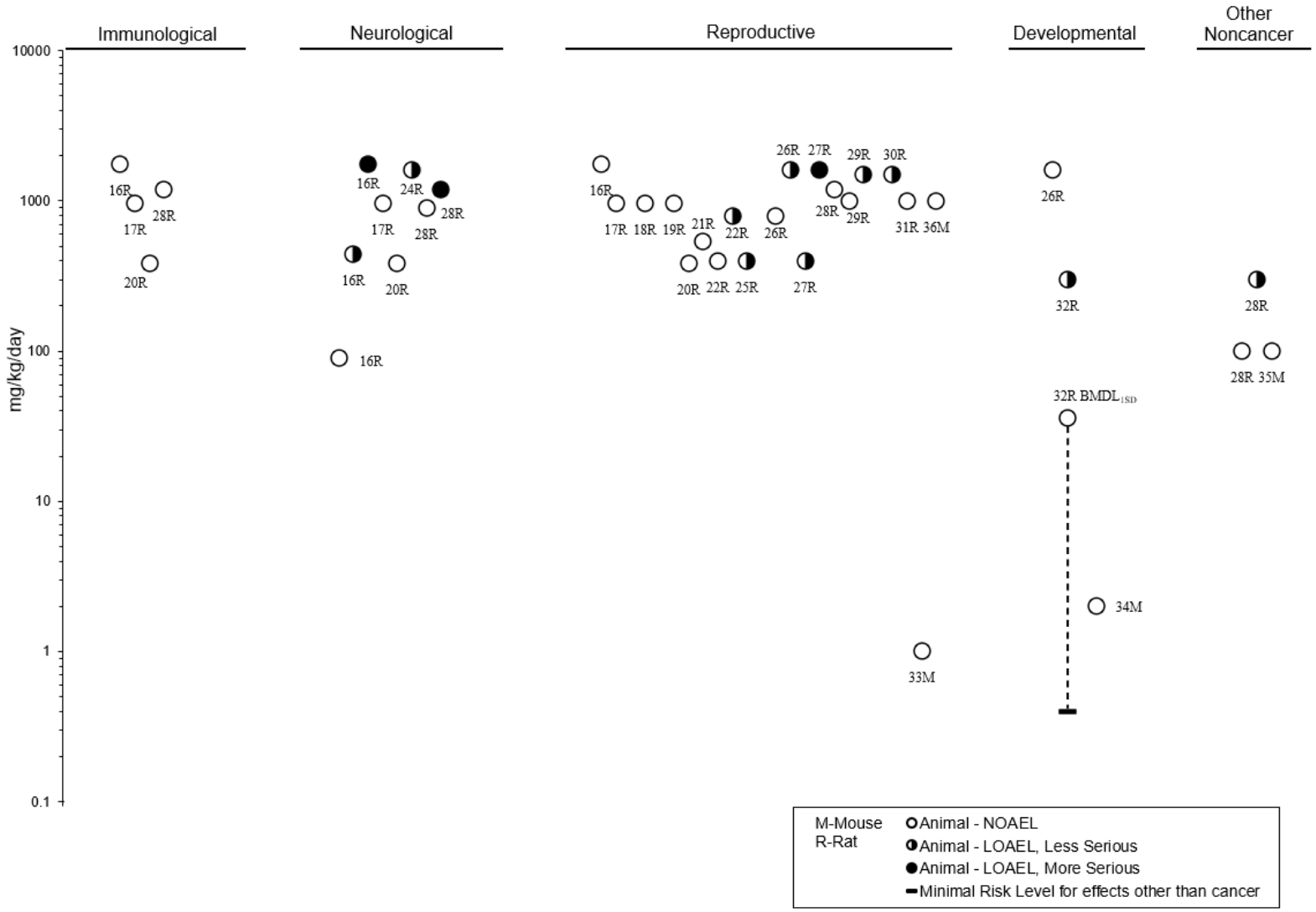


Figure 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral
Chronic (≥365 days)

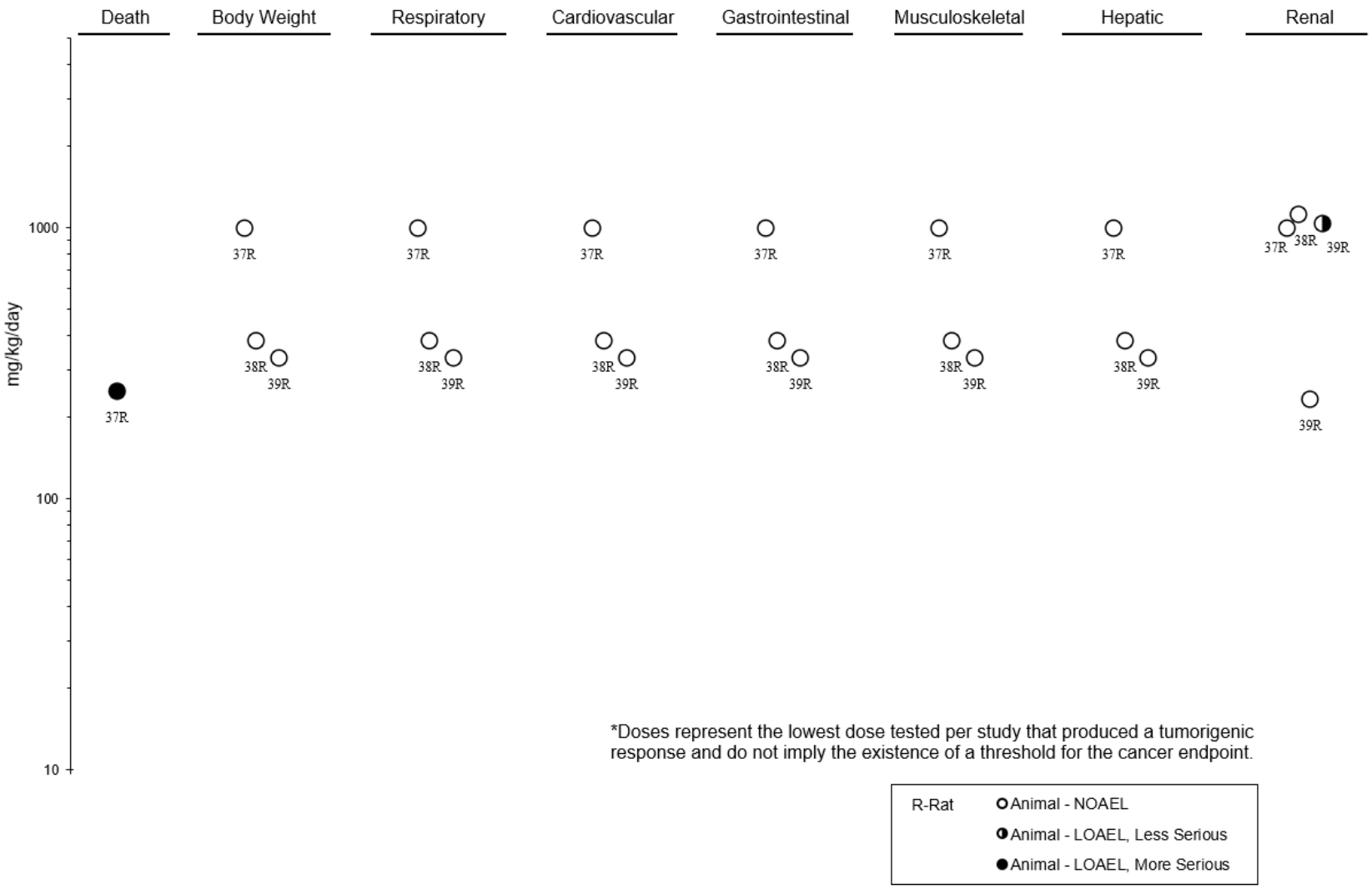


Figure 2-3. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Oral
Chronic (≥365 days)

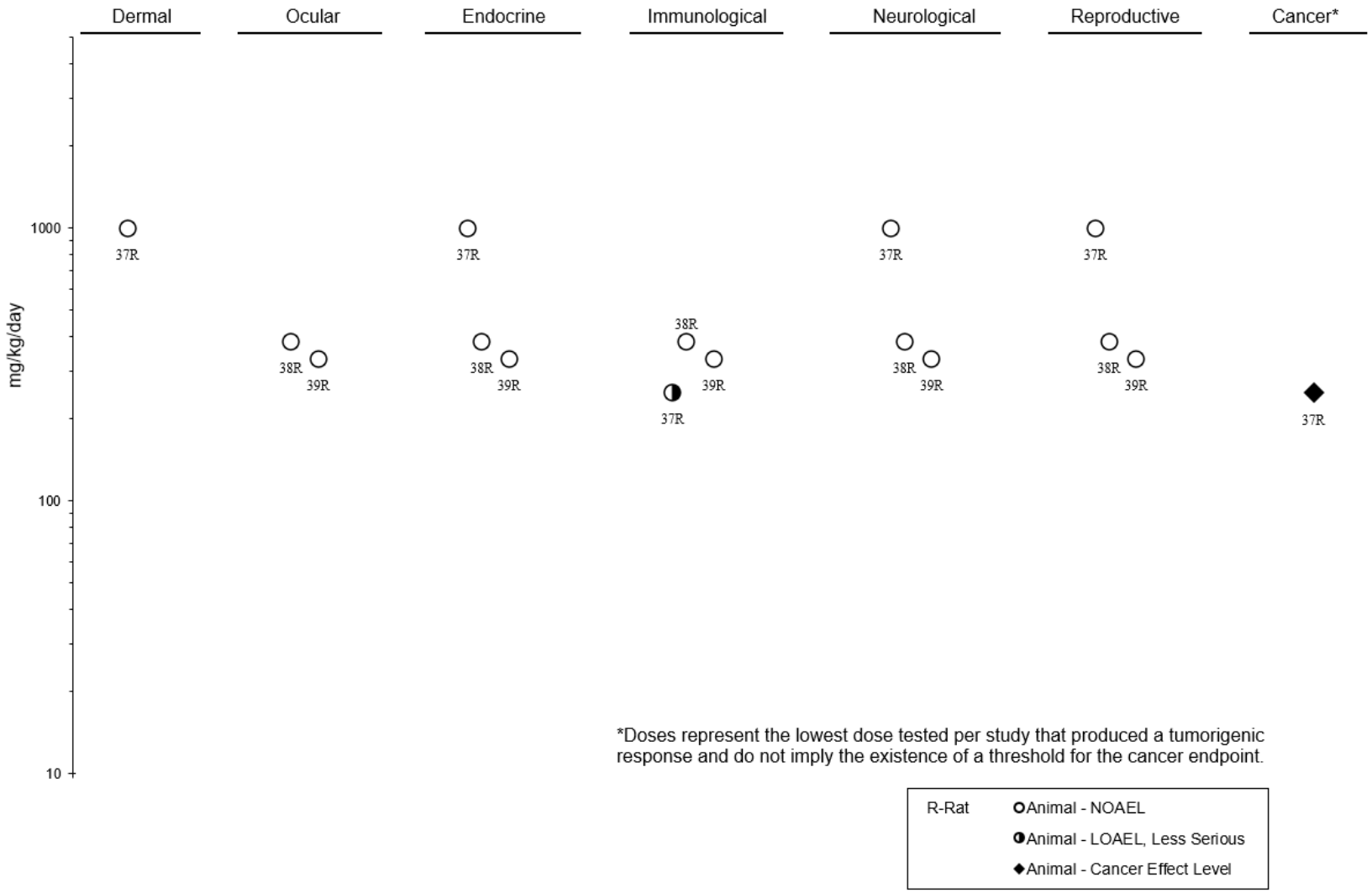


Table 2-4. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Cain et al. 1996								
Human 22 M, 21 F	1 hour	0, 1.7 ppm	CS	Dermal	1.7			
				Ocular	1.7			
				Immuno	1.7			
(data also available in unpublished report by Cain et al. 1994; exposure via MTBE in air; immune cells measured in tear fluid and nasal lavage)								
Johanson et al. 1995; Nihlén et al. 1998a								
Human 10 M	2 hours	5, 25, 50 ppm	CS	Ocular	50			
				Immuno	50			
(exposure via MTBE in air; immune cell composition and inflammatory markers measured in nasal lavage)								
Prah et al. 1994								
Human 19 M, 18 F	1 hour	0, 1.39 ppm	CS	Dermal	1.39			
				Ocular	1.39			
				Immuno	1.39			
(exposure via MTBE in air; inflammatory markers measured in nasal lavage)								
ARCO 1980								
Rat (NS) NS	4 hours	18,892, 34,127, 38,657, 41,860, 63,953 ppm	CS	Ocular		18,892		Eye discharge, irritation
(exposure to commercial MTBE [99.1% purity] via air)								
ARCO 1980								
Rat (NS) NS	4 hours	19,621, 29,681, 39,406, 55,784 ppm	CS	Ocular		19,621		Irritation, lacrimation
(exposure to ARCO MTBE (96.2% purity) via air)								
Daughtrey et al. 1997								
Rat (Fischer-344) 22 M, 22 F	6 hours	0, 800, 4,000, 8,000 ppm	CS	Ocular	4,000	8,000		Lacrimation
(data also available in unpublished report by Gill 1989; exposure via MTBE in air)								

Table 2-4. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Dodd and Kintigh 1989								
Rat (Fischer 344) 5 M, 5 F	13 days 6 hours/day	0, 2,000, 4,000, 8,000 ppm	CS, GN	Dermal Ocular	8,000 8,000			
(exposure via MTBE in air)								
Texaco Inc. 1981								
Rat (Sprague-Dawley) 20 M, 20 F	9 days 5 days/week 6 hours/day	0, 100, 300, 1,000, 3,000 ppm	CS	Ocular		100		Lacrimation, conjunctival swelling
(exposure via MTBE in air)								
Bevan et al. 1997a								
Mouse (CD-1) 30 F	10 days (GDs 6–15) 6 hours/day	0, 1,000, 4,000, 8,000 ppm	CS	Ocular	4,000	8,000		Lacrimation, periocular encrustation
(data also available in unpublished report by Tyl and Neeper-Bradley 1989; exposure via MTBE in air)								
Conaway et al. 1985								
Mouse (CD-1) 30 F	10 days GDs 6–15 6 hours/day	0, 250, 1,000, 2,500 ppm	CS	Ocular		250		Slightly increased lacrimation
(exposure via MTBE in air)								
ARCO 1980								
Rabbit (New Zealand) 6 NS	24 hours	0.5 mL	HP, CS	Dermal		0.5		Slight to severe erythema, acanthosis, focal necrosis
ARCO 1980								
Rabbit (New Zealand) 5 NS	24 hours	10,000 mg/kg	LE, CS, BW, GN, HP	Dermal		10,000		Erythema, skin thickening, edema, blanching

Table 2-4. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ARCO 1980								
Rabbit (New Zealand) 9 NS	Once	0.1 mL	CS	Ocular		0.1		Corneal opacities, chemosis, conjunctival redness, discharge
Snamprogetti 1980								
Rabbit (NS) NS B	Once	0.05 mL	CS	Ocular		0.05 M		Congestion of conjunctival, palpebral thickening, and hypersecretion
INTERMEDIATE EXPOSURE								
Bevan et al. 1997b								
Rat (Sprague-Dawley) 25 M, 25 F	14–19 weeks 5 days/week 6 hours/day	0, 400, 3,000, 8,000 ppm	CS	Ocular	3,000	8,000		Periocular encrustation and ocular discharge
(data also available in unpublished report by Neeper-Bradley 1991; exposure to MTBE via air)								
Biles et al. 1987								
Rat (Sprague-Dawley) 15 M, 30 F	16–28 weeks 5–7 days/week 6 hours/day	0, 250, 1,000, 2,500 ppm	CS	Dermal Ocular	2,500 2,500			
(exposure via MTBE in air)								
Greenough et al. 1980								
Rat (Sprague-Dawley) 10 M, 10 F	13 weeks 5 days/week 6 hours/day	0, 250, 500, 1,000 ppm	CS	Dermal Ocular	1,000 1,000			
(exposure via MTBE in air)								
Lington et al. 1997								
Rat (Fischer-344) 25 M, 25 F	13 weeks 5 days/week 6 hours/day	0, 800, 4,000, 8,000 ppm	CS	Dermal Ocular	8,000 8,000			
(data also available in unpublished report by Dodd and Kintigh 1989; exposure via MTBE in air)								

Table 2-4. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ARCO 1980								
Guinea pig (Hartley) 10 M	3 weeks every other day	0.5 mL of 1% solution initially, then 0.1 mL	CS	Dermal		0.5		Local irritation and increased erythema
				Immuno	0.5			
CHRONIC EXPOSURE								
Bird et al. 1997								
Rat (Fischer-344) 50 M, 50 F	24 months 5 days/week 6 hours/day	0, 400, 3,000, 8,000 ppm	CS	Dermal	8,000			
				Ocular	8,000 F 400 M	3,000 M		Swollen periocular tissue
(data also available in unpublished report by Chun et al. 1992; exposure via MTBE in air)								
Bird et al. 1997								
Mouse (CD-1) 50 M, 50 F	18 months 5 days/week 6 hours/day	0, 400, 3,000, 8,000 ppm	CS	Dermal	8,000			
				Ocular	8,000			
(data also available in unpublished report by Burleigh-Flayer et al. 1992; exposure via MTBE in air)								

BI = biochemical changes; BW = body weight; CS = clinical signs; DX = developmental toxicity; F = female(s); GD = gestation day; GN = gross necropsy; HP = histopathology; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OP = ophthalmology; OW = organ weight

2.2 DEATH

No studies were located regarding death in humans following exposure to MTBE. Exposure-related deaths have been reported in laboratory animals following acute- and chronic-duration inhalation and oral exposures.

Information regarding death in animals following inhalation exposure was located for rats, mice, and rabbits. Acute-duration inhalation 4-hour LC_{50} (lethal concentration, 50% kill) values in rats for two grades of MTBE were determined to be 39,395 ppm for ARCO MTBE (96.2% MTBE) and 33,370 ppm for commercial MTBE (99.1% MTBE) (ARCO 1980). An acute-duration LC_{50} in mice following inhalation of MTBE for 10 minutes was determined to be 180,000 ppm; the LT_{50} (time at which death occurs in 50% of exposed animals) in mice exposed to 209,300 ppm was 5.6 minutes (Snamprogetti 1980). Repeated brief exposures to high inhalation concentrations of MTBE (5–10 minutes/day, 5 days/week for 30 days) did not result in mortalities in rats at up to 80,000 ppm (Snamprogetti 1980) or mice at 50,000 ppm; 1/30 mice died at 80,000 ppm. No deaths occurred in rats or mice exposed to concentrations up to 8,000 ppm in acute-duration studies (Bevan et al. 1997a; Bird et al. 1997; Conaway et al. 1985; Daughtrey et al. 1997; Dodd and Kintigh 1989; Moser et al. 1996; MTBE Committee 1990a; Prescott-Mathews et al. 1997; Texaco Inc. 1981; Vergnes and Chun 1994; Vergnes and Kintigh 1993; Vergnes and Morabit 1989) or intermediate-duration studies (Bevan et al. 1997b; Biles et al. 1987; Bird et al. 1997; Daughtrey et al. 1997; Greenough et al. 1980; Lington et al. 1997; Moser et al. 1996, 1998). In a 24-month inhalation study, increased mortality and decreased mean survival time occurred in male rats exposed to ≥3,000 ppm (Bird et al. 1997). Slight, but not statistically significant, increases in mortality and decreases in mean survival time also occurred in the male rats exposed to 400 ppm (lowest concentration tested) and females exposed to ≥3,000 ppm. The early mortality was attributed to chronic progressive nephropathy in both males and females. In an 18-month inhalation study, increased mortality and decreased mean survival time were observed in male mice at 8,000 ppm; female survival was comparable to controls (Bird et al. 1997). The early mortality in male mice was attributed to obstructive uropathy.

Oral LD_{50} (lethal dose, 50% kill) values for rats and mice were determined to be 3,866 mg/kg and 4,000 mg/kg, respectively (ARCO 1980; Little et al. 1979). In a series of 2-week experiments, 3/10 rats died following exposure to 1,200 mg/kg/day via gavage in the first experiment (6–7 days/week), but not in three additional experiments by the same study authors at doses ranging from 1,200 to 1,500 mg/kg/day for 12–14 days (de Peyster et al. 2003, 2014). No treatment-related deaths were

observed in rats or mice orally exposed to MTBE via gavage at doses up to 2,000 mg/kg for acute-duration studies (Billitti et al. 2005; de Peyster et al. 2008; Dong-mei et al. 2009; Li et al. 2008; MTBE Committee 1990c; Robinson et al. 1990) or intermediate-duration studies (de Peyster et al. 2003, 2008; Dong-mei et al. 2009; Gholami et al. 2015; Amoco 1992; Khalili et al. 2015; Li et al. 2008; Robinson et al. 1990; Ward et al. 1994; Williams et al. 2000). Similarly, no deaths were observed in rats or mice exposed via drinking water to doses as high as 1,153 mg/kg/day in acute-duration studies (Berger and Horner 2003; Bermudez et al. 2012) or intermediate-duration studies (Bermudez et al. 2012). Dose-related decreases in survival were observed in female rats beginning at week 16 following exposure to gavage doses of ≥250 mg/kg/day, 4 days/week, for 104 weeks; male survival was comparable to controls at doses up to 1,000 mg/kg/day (Belpoggi et al. 1995, 1997). Chronic-duration exposure via drinking water did not result in treatment-related mortalities in rats at doses up to 384 mg/kg/day in males or 1,119 mg/kg/day in females (Bermudez et al. 2012; Dodd et al. 2013).

No deaths occurred in rats dermally exposed to doses up to 400 mg/kg for 6 hours (MTBE Committee 1990b) or in rabbits dermally exposed to 10,000 mg/kg for 24 hours (ARCO 1980).

Significant numbers of rats died after a dose of 148 mg/kg was administered intravenously or intrahepatically, but no rats died after the same dose was administered intraperitoneally (Akimoto et al. 1992). In other intraperitoneal studies, one study determined intraperitoneal LD_{50} values of 1,249 mg/kg in rats and 1,010 mg/kg in mice (Snamprogetti 1980), while another study reported death in two of five rats after dosing with 3,705 mg/kg (Brady et al. 1990). The intravenous LD_{50} in rats was 415 mg/kg; subcutaneous LD_{50} values were much higher (4,946 mg/kg for rats and 2,646 mg/kg for mice). Two of six rabbits that received 1,782 mg/kg through the bile duct died (Adam et al. 1990).

2.3 BODY WEIGHT

No studies were located regarding body weight effects in humans following exposure to MTBE. Decreased body weight following exposure to MTBE has been reported in laboratory animals following acute-, intermediate-, and chronic-duration inhalation exposures and inconsistently reported in acute- and intermediate-duration oral exposure studies.

Body weight effects following acute-duration inhalation exposure were only observed at high concentrations (≥4,000 ppm). Single exposures up to 8,000 ppm for 6 hours had no effect on body weight in rats (Daughtrey et al. 1997). However, exposure to 8,000 ppm for 5 days (6 hours/day) resulted in a

significant 12% decrease in terminal body weights associated with a 3% weight loss over the treatment period (compared to an 8% body weight gain in controls) (Vergnes and Morabit 1989). At lower exposure levels (800 and 4,000 ppm), terminal body weights in males at these two levels did not significantly differ from controls despite significant decreases in body weight gain of ~20% compared to control. In the same study, female body weight effects were only observed at 8,000 ppm, with a 73% decrease in body weight gain compared to controls in female rats. Similarly, a decrease in body weight gain of 65–66% occurred in male rats during the first 1–3 and 1–14 days of exposure to 4,000 and 8,000 ppm, respectively, and a 36% decrease in body weight gain occurred in female rats during the first 1–7 days of exposure to 8,000 ppm in a preliminary 13-day range-finding study for a 13-week study (Dodd and Kintigh 1989). In other acute-duration studies, no body weight effects were noted in rats following intermittent acute-duration exposure to concentrations up to 3,000 ppm for 9–14 days (Prescott-Mathews et al. 1997; Savolainen et al. 1985; Texaco Inc. 1981) or in mice following intermittent acute-duration exposure to concentrations up to 8,000 ppm for 1–13 days (Dodd and Kintigh 1989; Moser et al. 1996; Vergnes and Kintigh 1993).

In longer-duration inhalation studies, body weight effects were inconsistently observed at 8,000 ppm; no body weight effects were noted at lower doses. In a 4–5-week study in rats, a 2% loss of body weight was observed in male rats intermittently exposed to 8,000 ppm during the first week, and body weight gain across the exposure period was decreased by 24–35%, compared with controls (Bird et al. 1997). In similarly exposed females, a transient 24% decrease in body weight gain was observed at 8,000 ppm during the first 2 weeks of exposure. Decreased body weight and/or body weight gain were reported at 8,000 ppm in several other studies with longer exposure durations, including 2-generation and 24-month studies in rats (Bevan et al. 1997b; Bird et al. 1997) and 16-week, 32-week, and 18-month studies in mice (Bird et al. 1997; Moser et al. 1996, 1998). In other intermediate-duration studies, no exposure-related body weight effects were noted at concentrations up to 8,000 ppm in rats and mice exposed for 3–13 weeks (Bird et al. 1997; Daughtrey et al. 1997; Greenough et al. 1980; Lington et al. 1997; Moser et al. 1996), up to 2,500 ppm in rats exposed for 16–28 weeks (Biles et al. 1987), or up to 300 ppm in rats exposed for 6–15 weeks (Savolainen et al. 1985).

In pregnant mice, a >10% reduction in maternal weight and a >25% reduction in maternal body weight gain were observed both during and after exposure to 8,000 ppm on gestational days (GDs) 6–15 (Bevan et al. 1997a). Body weight effects were accompanied by an approximate 30% reduction in food consumption during exposure only, which may be secondary to observed hypoactivity in exposed dams. In other gestational exposure studies, no maternal body weight effects were noted in rats or mice exposed

to concentrations up to 2,500 ppm (Conaway et al. 1985) or in rabbits exposed to concentrations up to 8,000 ppm (Bevan et al. 1997a). In a 2-generation study, no maternal body weight effects were noted in F0 or F1 rat dams intermittently exposed to concentrations up to 8,000 ppm (Bevan et al. 1997b).

Body weight effects following acute-duration oral exposure were only observed at high doses and were likely due to decreased food intake secondary to sedative effects of MTBE at high doses (see Section 2.15). Daily gavage exposure to MTBE for 14 days caused significant decreases in body weight gains of male and female rats at doses ≥714 and ≥1,071 mg/kg/day, respectively (magnitude not specified); however, final body weights remained within 10% of controls at doses up to 1,428 mg/kg/day (highest dose tested) (Robinson et al. 1990). Observed changes in body weight gain are likely secondary to the significant decrease in food intake in treated rats, which may be due to the hypoactivity induced by MTBE. In a series of 2-week experiments, a 10% decrease in body weight was observed in male rats exposed to 1,200 mg/kg/day via gavage in the first experiment (6–7 days/week), but not in three additional experiments by the same study authors at doses ranging from 1,200 to 1,500 mg/kg/day (de Peyster et al. 2014). In a similar experiment, body weight loss was observed in male rats exposed to 1,500 mg/kg/day via gavage every other day for 12 days (de Peyster et al. 2003). Food consumption was not reported in the studies by de Peyster et al. (2003, 2014); however, doses associated with decreased body weight reportedly caused lethargy, which may have decreased food intake. Another 2-week gavage study reported no significant changes in body weights or food consumption in male rats exposed daily to doses up to 1,600 mg/kg/day (Dong-mei et al. 2009). In an acute-duration drinking water study in rats, no body weight effects were noted in females exposed to 520 mg/kg/day for 2 weeks (Berger and Horner 2003). No body weight effects were observed in mice exposed to gavage doses up to 2,000 mg/kg/day for 3 days (Billitti et al. 2005; de Peyster et al. 2008).

There is no consistent evidence for body weight effects in intermediate-duration oral studies in rats and mice, or chronic-duration oral studies in rats. In 28-day gavage studies, male rats showed an 11% decrease in body weight following daily exposure to 800 mg/kg/day (de Peyster et al. 2003), and a 7–12% decrease in body weight from day 15 to 28 following daily exposure to 1,500 mg/kg/day, but not at doses ≤1,000 mg/kg/day (Williams et al. 2000). In contrast, no body weight effects were noted in rats in other intermediate-duration studies at daily gavage doses of up to 1,500 mg/kg/day for 15 days (Williams et al. 2000), 1,750 mg/kg/day for 28–30 days (Amoco 1992; Dong-mei et al. 2009; Gholami et al. 2015; Khalili et al. 2015; Li et al. 2008), 1,200 mg/kg/day for 90 days (Robinson et al. 1990), or 100 mg/kg/day for 14 weeks (Tang et al. 2019). Similarly, no body weight effects were noted following chronic-duration exposure to gavage doses up to 1,000 mg/kg/day (Belpoggi et al. 1995, 1997). In drinking water studies,

no body weight effects were reported in rats following intermediate-duration exposure to doses ≤1,153 mg/kg/day (Bermudez et al. 2012), or chronic-duration doses ≤1,119 mg/kg/day (Bermudez et al. 2012; Dodd et al. 2013). In addition, no changes in body weight were found in mice exposed to gavage doses up to 1,000 mg/kg/day for 3 weeks (Ward et al. 1994) or to drinking water doses up to 1 mg/kg/day for 28 days (de Peyster et al. 2008).

2.4 RESPIRATORY

There is some evidence that workers exposed to fuel containing MTBE in the early 1990s during the oxyfuel program experienced increased respiratory symptoms (e.g., irritation, coughing) compared to workers exposed to fuels that did not contain MTBE; however, clear conclusions cannot be drawn due to confounding factors and study limitations. In controlled exposure studies, self-reported respiratory symptoms were not increased in healthy volunteers exposed to MTBE at concentrations up to 50 ppm. In animal studies, evidence of respiratory irritation was observed at high exposure levels in both inhalation and oral studies.

Following several anecdotal reports of respiratory symptoms associated with introduction of MTBE into gasoline in the early 1990s during the oxyfuel program in the United States, the Centers for Disease Control and Prevention (CDC) conducted several studies evaluating potential associations between MTBE exposure and respiratory symptoms (see Table 2-1). Several of these studies reported an increase in self-reported respiratory symptoms (burning sensation in the nose, mouth, or throat or cough) in Alaskan workers during the oxyfuel program following occupational exposure to gasoline containing MTBE (e.g., taxi drivers or health-care workers that travelled routinely in cars), compared either with individuals with low exposure (e.g., noncommuter students) or with workers exposed to gasoline that did not contain MTBE (Alaska DHSS 1992a, 1992b; Moolenaar et al. 1994). However, these studies only provide suggestive evidence due to several limitations, including lack of a referent group (Alaska DHSS 1992b only), small to modest subject numbers, lack of statistical analysis of incidence data, potential influence of widespread media coverage and public opposition to the oxyfuel program, and/or lack of consideration for other confounding factors (e.g., other exposures, including potential combustion products of MTBE). No evidence of increased respiratory symptoms was observed in occupationally exposed workers, compared with unexposed referents, in similar studies during the oxyfuel program conducted in New York (CDC 1993a), Connecticut (CDC 1993b; White et al. 1995), or New Jersey (Mohr et al. 1994).

Results from population-based studies evaluating the potential association between respiratory symptoms and MTBE exposure due to the oxyfuel program are mixed (see Table 2-1). In a population-based telephone survey, a statistically significant increased risk of respiratory symptoms (throat irritation, sinus congestion) was observed in residents of metropolitan Milwaukee, Wisconsin, in which oxyfuel was used, compared to residents of non-metropolitan Wisconsin (MTBE-free gasoline); however, the risk was not increased for any respiratory symptom for residents of metropolitan Chicago, Illinois (oxyfuel) compared to non-metropolitan Wisconsin (Wisconsin DHSS 1995). The results in Milwaukee may have been biased by knowledge of the oxyfuel program and potential health effects due to increased media coverage in Milwaukee because, in the telephone survey, familiarity with MTBE as a reformulated gasoline additive was reported by 54% of the Milwaukee residents, 23% of Chicago residents, and 40% of the Wisconsin residents. Additionally, non-metropolitan Wisconsin may not have been an appropriate referent group for the Chicago residents. One ecological study also reported a statistically significant increase in doctor's visits with diagnostic codes pertaining to respiratory symptoms, including burning throat, burning nose, cough, wheezing, and upper respiratory infection, in Philadelphia, Pennsylvania during 1997 (during the 6th year of the oxyfuel program), compared to 1992 (at the initiation of the oxyfuel program) based on medical record review (Joseph and Weiner 2002). The study authors concluded that "some environmental factor" in the mid-1990s was associated with immune system findings and suggested that this factor is MTBE. However, this study has several major limitations, primarily a study design that does not allow for control for confounders (other chemical exposures, occupation, concomitant diseases, sex, age, race, etc.), that preclude meaningful conclusions. In contrast, another ecological study in Fairbanks and Anchorage, Alaska (Gordian et al. 1995) did not find an increased rate of treatment for respiratory symptoms (based on medical insurance records) over the winter of 1992–1993 (during the oxyfuel program), compared with the number of claims during the two preceding winters (prior to the oxyfuel program). However, this study would miss mild and/or transient respiratory effects associated with MTBE exposure that would not result in seeking medical attention.

In controlled human inhalation experiments, no increases in self-reported respiratory symptoms (nose or throat irritation, dry or sore throat, stuffy or runny nose, sinus congestion, cough, wheezing, chest tightness, and/or shortness of breath) were observed in volunteers during or after exposure to MTBE at 1.39 or 1.7 ppm for 1 hour (Cain et al. 1996; Prah et al. 1994), or 50 ppm for 2 hours during light exercise (Johanson et al. 1995; Nihlén et al. 1998a). The odor threshold was determined to be approximately 0.18 ppm (Prah et al. 1994).

Respiratory effects have been observed in animals following acute-duration inhalation exposure to MTBE. A 4-hour exposure of rats to concentrations ≥19,621 ppm ARCO MTBE (96.2% MTBE) caused hyperpnea, while a 4-hour exposure of rats to ≥18,892 ppm commercial MTBE (99.1% MTBE) caused tachypnea and nasal discharge, with respiration gradually slowing until the rats died (ARCO 1980). In a mouse study to determine the RD_{50} (the concentration that results in 50% decrease in respiratory rate) for respiratory irritancy of MTBE, a threshold irritant response (13%) in respiratory rate occurred at 83 ppm and a 52% decrease in breathing frequency occurred at 8,321 ppm (Tepper et al. 1994). No pulmonary irritation was observed at concentrations ≤2,774 ppm, but a mixed pattern of irritant response, indicating both sensory and pulmonary irritation, occurred at 8,321 ppm. The RD_{50}, indicative of sensory irritation, was determined to be 4,604 ppm. Intermittent exposure of rats for 9 days to concentrations of 1,000 or 3,000 ppm resulted in high incidence and increased severity of inflammation of the nasal mucosa and trachea (Texaco Inc. 1981). In contrast, no clinical signs of respiratory irritation or gross or microscopic lesions of the respiratory tract were observed in rats intermittently exposed to concentrations up to 8,000 ppm for 13 days (Dodd and Kintigh 1989).

In pregnant mice, labored breathing was observed in dams exposed via inhalation to 8,000 ppm, but not 4,000 ppm, from GD 6 to 15 (Bevan et al. 1997a). In similarly exposed rabbits, no clinical signs of respiratory irritation were observed (Bevan et al. 1997a). No exposure-related clinical signs of respiratory irritation or histological alterations of the respiratory tract were observed in intermediate- or chronic-duration inhalation studies in rats or mice exposed to concentrations as high as 8,000 ppm (Bevan et al. 1997b; Biles et al. 1987; Bird et al. 1997; Greenough et al. 1980; Lington et al. 1997). Decreases in absolute and relative lung weights were reported in an intermediate-duration inhalation study in rats at 1,000 ppm (Greenough et al. 1980); these changes were not associated with histological alterations and were therefore not considered biologically relevant.

A single high oral dose (≥4,080 mg/kg) of MTBE caused labored respiration in rats, and gross pathological changes consistent with the irritating nature of MTBE were observed at ≥2,450 mg/kg (ARCO 1980). Alterations in absolute and/or relative lung weights were reported in one acute-duration (decreased weight) and one intermediate-duration (increased weight) oral study in rats (Robinson et al. 1990), but not others (Bermudez et al. 2012; Dong-mei et al. 2009); these changes were not associated with histological alterations and were therefore not considered biologically relevant (not included in LSE table). Acute-, intermediate-, and chronic-duration studies have not found histopathological alterations in the lungs or other respiratory tract tissues in rats and mice exposed to doses as high as 1,428 mg/kg/day (Amoco 1992; Belpoggi et al. 1995, 1997; Bermudez et al. 2012; Dodd et al. 2013; Robinson et al. 1990).

A number of studies have been conducted in animals to determine possible side effects of MTBE therapy for gallstone dissolution. Pulmonary hemorrhage was observed in rats given 148 mg/kg MTBE intrahepatically, intraperitoneally, or intravenously, with intravenous dosing producing the greatest damage (Akimoto et al. 1992). Intravenous injection of rats, rabbits, and cats with ≥7.4 mg/kg resulted in increased respiratory rates, which paralleled decreases in blood pressure and bradycardia, and intraperitoneal injection of rats with 185 mg/kg/day for 15 days resulted in pneumonia (Snamprogetti 1980). Transient dyspnea occurred in rabbits injected with 740.5 mg/kg MTBE through a catheter to the cystic duct (Tritapepe et al. 1989), and lung congestion with pneumonia occurred in pigs infused with 4,255 mg/kg MTBE through a catheter to the gallbladder (McGahan et al. 1988). However, no histopathological lung lesions were found in dogs injected with 635 mg/kg MTBE through a catheter to the gallbladder (Allen et al. 1985b).

2.5 CARDIOVASCULAR

Available human studies are too limited to determine if MTBE exposure affects cardiovascular health or function. Based on inhalation and oral studies in animals, MTBE does not appear to have adverse effects on the cardiovascular system.

One ecological study reported a statistically significant increase in doctor's visits with diagnostic codes pertaining to cardiac symptoms (tachycardia, palpitations, murmurs) in medical records from the General Medicine Division of the Clinical Practices at the University of Pennsylvania in 1997, 6 years after introduction of oxyfuel containing MTBE to the surrounding Philadelphia region, compared to 1992, the year that the oxyfuel program was initiated (Joseph and Weiner 2002; see Table 2-1). The study authors concluded that "some environmental factor" in the mid-1990s was associated with increased cardiac symptoms and suggested that this factor is MTBE. However, this study has several major limitations, primarily a study design that does not allow for control for potential confounders (other chemical exposures, occupation, concomitant diseases, sex, age, race, etc.), that preclude meaningful conclusions.

A retrospective cohort study of pregnant women from Utah and Idaho did not observe an association between estimated environmental MTBE levels (based on Community Multiscale Air Quality modeling) and risk of hypertension during pregnancy (Nobles et al. 2019a, 2019b; see Table 2-1). However, the highest quartile of ambient MTBE exposure levels during the 3 months prior to conception and during pregnancy was associated with increased risk of pre-eclampsia. While some potential cofounders were

controlled for (e.g., age, race/ethnicity, body mass index, smoking and alcohol use), the study did not control for other key confounders, such as other chemical exposures, occupation, or concomitant cardiovascular diseases.

A number of clinical studies of patients receiving MTBE therapy intracystically for the dissolution of gallstones have recorded side effects. Infrequently reported cardiac findings include transient hypertension in 1/10 patients (Murray et al. 1988) and hypotension in 2/29 patients, palpitations in 1/29 patients, and angina in 1/29 patients (Neoptolemos et al. 1990) given MTBE via nasobiliary catheter. Vasovagal reactions were found in 4/24 patients given MTBE via the percutaneous transhepatic route to the gallbladder (Eidsvoll et al. 1993). In another study, a vasovagal reaction was only observed in 1/75 patients given MTBE via percutaneously placed cholecystostomy catheter (Williams et al. 1990).

No treatment-related changes in heart weight or gross or histopathological lesions of the heart were found in rats intermittently exposed to inhalation concentrations up to 3,000 ppm for 9 days (Texaco Inc. 1981) or up to 8,000 ppm for 13 weeks (Greenough et al. 1980; Lington et al. 1997). Similarly, no treatment-related changes in heart weight or gross or histopathological lesions of the heart were observed in rats or mice exposed to concentrations up to 8,000 ppm for 24 or 18 months, respectively (Bird et al. 1997).

Based on histological examination of the heart and aorta of rats, oral exposure to MTBE appears to be without effects on the cardiovascular system. Gavage administration of MTBE did not result in alterations in heart weight or heart or aorta histology of rats at ≤1,428 mg/kg/day for 14 days (Robinson et al. 1990), ≤1,750 for 4 weeks (Amoco 1992), or ≤1,000 mg/kg/day for 104 weeks (Belpoggi et al. 1995, 1997). In drinking water studies, no exposure-related changes in heart weight or histology were observed in rats at doses up 972 mg/kg/day in males or 1,153 mg/kg/day in females for 13 weeks (Bermudez et al. 2012), 384 mg/kg/day in males for 6 months (Bermudez et al. 2012), 1,119 mg/kg/day in females for 1 year (Bermudez et al. 2012), or 330 mg/kg/day in males or 1,042 mg/kg/day in females for 2 years (Dodd et al. 2013). In males exposed to doses ≥29 mg/kg/day via drinking water for 1 year, absolute, but not relative, weight of the heart was significantly less in all treated groups, compared to controls; however, these findings were attributed to observed decreases in male body weights and no histopathological changes were observed in the heart at doses up to 384 mg/kg/day (Bermudez et al. 2012).

Effects noted in animals after administration of MTBE by other routes include decreased blood pressure in rabbits and decreased blood pressure, heart rate changes, and electrocardiographic variations in cats

given 7.4 mg/kg intravenously and decreased blood pressure and bradycardia in rats given 7.4 mg/kg intravenously or 370 mg/kg intraperitoneally (Snamprogetti 1980).

2.6 GASTROINTESTINAL

Several epidemiology studies report nausea and/or vomiting with inhalation exposure to gasoline containing MTBE; however, these symptoms are likely related to neurological effects associated with MTBE exposure. Therefore, these studies are discussed in Section 2.15 (Neurological). Other human studies are limited to one occupational study that evaluated self-reported diarrhea and studies in patients receiving MTBE therapy for gallstone dissolution, which reported gastrointestinal side effects. In animals, the gastrointestinal tract only appears to be a target of toxicity following exposure to high gavage doses. Observed effects in humans and animals are consistent with irritative effects on the gastrointestinal mucosa.

No associations between diarrhea and inhalation exposure to MTBE in gasoline were observed in a cross-sectional occupational study conducted by the CDC in Albany, New York (CDC 1993a; see Table 2-1). A number of clinical studies of patients receiving MTBE therapy for gallstone dissolution have recorded gastrointestinal side effects, indicative of irritation. These include vomiting, nausea, anorexia, emesis, duodenitis, retching, upper abdominal burning sensation during infusion, gas, and duodenal ulcer in patients receiving MTBE via percutaneous intracystic infusion, gallbladder catheter, or nasobiliary catheter (Allen et al. 1985a; Bonardi et al. 1986; Brandon et al. 1988; Di Padova et al. 1986; Eidsvoll et al. 1993; Hellstern et al. 1990; Holl et al. 1991; Janowitz et al. 1993; Kaye et al. 1990; Leuschner et al. 1988, 1991; McNulty et al. 1991; Murray et al. 1988; Neoptolemos et al. 1990; Saraya et al. 1990; Thistle et al. 1989; Tobio-Calo et al. 1992; Uchida et al. 1994; Williams et al. 1990). The irritation occurs due to leakage of MTBE from the gallbladder into the gastrointestinal tract.

The gastrointestinal tract was not affected in rats exposed to MTBE via inhalation exposure. Intermittent exposure of rats for 9 days to concentrations up to 3,000 ppm caused no gross or histological changes of the stomach, duodenum, jejunum, ileum, colon, or rectum (Texaco Inc. 1981). Similarly, no exposure-related gastrointestinal tract lesions were observed in intermediate-duration studies that exposed rats to MTBE at concentrations up to 8,000 ppm for 13–19 weeks (Bevan et al. 1997b; Greenough et al. 1980; Lington et al. 1997). In chronic-duration inhalation studies, no treatment-related changes in the gastrointestinal tract were observed in rats or mice exposed to concentrations up to 8,000 ppm for 24 or 18 months, respectively (Bird et al. 1997).

MTBE appears to be irritating to the gastrointestinal tract of rats following gavage exposure as evidenced by diarrhea and histological lesions, which may be due to irritative portal-of-entry effects associated with bolus gavage. Rats treated with daily oral doses ≥357 mg/kg/day for 14 days had diarrhea by the third day of dosing, which continued throughout the remaining treatment period (Robinson et al. 1990). Similarly, diarrhea was observed in rats during a 90-day study immediately after daily gavage exposure to doses ≥100 mg/kg/day throughout the entire exposure period (Robinson et al. 1990). Daily oral administration of 1,750 mg/kg/day MTBE via gavage for 4 weeks to rats resulted in submucosal edema in the squamous portion of the stomach; no gross lesions were observed in the duodenum, jejunum, ileum, cecum, colon, rectum, salivary glands, stomach, or tongue (Amoco 1992). In a 104-week study, in which male and female rats were given gavage doses ≤1,000 mg/kg/day, 4 days/week, histological examination of the oral cavity, salivary glands, tongue, esophagus, stomach (fore and glandular), and intestines (four levels) revealed no treatment-related lesions (Belpoggi et al. 1995, 1997).

In drinking water studies, no exposure-related clinical signs or gross or histopathological changes in the gastrointestinal tract were reported in rats following intermediate-duration exposure to doses ≤1,153 mg/kg/day (Bermudez et al. 2012), or chronic-duration exposure to doses ≤1,119 mg/kg/day (Bermudez et al. 2012; Dodd et al. 2013).

In a pharmacokinetic study, in which MTBE was applied dermally to the dorsal flank of rats via an occluded chamber, some rats developed slight diarrhea at a dermal dose of 40 mg/kg but not 400 mg/kg (MTBE Committee 1990b). Since the dermally applied MTBE was protected by occlusion, it is unlikely that oral uptake via grooming contributed to the absorbed dose. This study is not included in the dermal LSE table due to the lack of controls and dose-response.

Effects noted in animals after administration of MTBE by other routes include light diarrhea in rats injected intravenously (MTBE Committee 1990b), necrosis of the duodenum in rabbits infused intraductally (Adam et al. 1990), and vomiting and/or duodenitis in rabbits (Tritapepe et al. 1989) and pigs (McGahan et al. 1988; Vergunst et al. 1994) treated intraductally, and dogs infused via gallbladder catheter (Allen et al. 1985b).

In a study in which jejunal segments were cannulated in rats, filled with 2–3 mL of MTBE, and perfused with α-aminoisobutyric acid (an actively absorbed nonmetabolizable amino acid) and polyethylene glycol 4000 (a nonabsorbable reference marker), or with mannitol (a passively absorbed hexone) and

polyethylene glycol, MTBE caused reduction in active transport, increased passive permeability, and loss of mucosal weight (Zakko et al. 1995).

2.7 HEMATOLOGICAL

No studies were located regarding hematological effects in humans following inhalation, oral, or dermal exposure to MTBE; however, transient hematological effects have been noted as a side effect in some humans treated with MTBE intracystically for the dissolution of gallstones. In laboratory animals, no biologically relevant changes in hematological parameters were noted in inhalation studies, and findings in oral studies were limited to effects in white blood cell parameters at high gavage doses.

A number of clinical studies of patients receiving MTBE therapy have recorded hematological findings. These include a transient leukocytosis (Allen et al. 1985a; Hellstern et al. 1990, 1998; Holl et al. 1991; Janowitz et al. 1993; Leuschner et al. 1991, 1994; Neubrand et al. 1994; Thistle et al. 1989) and decreased hemoglobin levels (Kaye et al. 1990). The transient leukocytosis has been attributed to a slight leakage of bile after removal of the catheter used during MTBE therapy (Thistle et al. 1989). In two studies of 75 patients (Thistle et al. 1989) and 8 patients (Ponchon et al. 1988), hemolysis and/or hematuria occurred in 1 patient in each study. In both cases, excessive overflow of MTBE from the gallbladder occurred, leading to systemic absorption or direct contact of MTBE with the vascular structure. Most clinical studies in which hematological parameters were monitored did not find changes except in a few patients, and some found none at all (Di Padova et al. 1986; Eidsvoll et al. 1993; McNulty et al. 1991; Uchida et al. 1994).

No biologically relevant changes in hematological parameters were reported in animals following exposure to MTBE via inhalation. In the only reliable acute-duration study evaluating hematological parameters, no exposure-related changes in hematological values were observed in rats exposed intermittently for 9 days at concentrations up to 3,000 ppm (Texaco Inc. 1981). Badr (2019) reported a significant reduction in white blood cells (WBCs) after exposure to 1 ppm for 10 days; however, this was not observed after exposure to 10 ppm for 28 days. Additionally, while the study authors indicated that concurrent control groups were utilized, only one set of control data were reported; it is unclear if control data were combined or if controls were only sacrificed at a single time-point. Due to potential invalidity of controls (i.e., comparison of 10-day treated animals to older rats sacrificed after 28-day experimental period), the results of this study cannot be adequately assessed. Therefore, this study is not included in the LSE table. In other intermediate-duration inhalation studies in rats, several mild, but statistically

significant, changes were observed in exposed rats; however, none of the findings were considered biologically or toxicologically relevant. In one 13-week study in rats, reported hematological findings following intermittent exposure to 1,000 ppm included a slight increase in white blood cell counts in males and females, and a slight increase in hemoglobin levels in males (Greenough et al. 1980). In another 13-week study in rats, most observed effects were ≤5% different from controls and included decreased red blood cell (RBC) hemoglobin and increased hematocrit, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), reticulocytes, and leukopenia in males at 4,000 and/or 8,000 ppm and increased hematocrit and segmented neutrophil count in females at 8,000 ppm (Lington et al. 1997). In chronic-duration inhalation studies, no treatment-related changes in hematological parameters were observed in rats or mice exposed to concentrations up to 8,000 ppm for 24 or 18 months, respectively (Bird et al. 1997). In addition, histological examination of the bone marrow revealed no treatment-related lesions.

Oral studies do not provide consistent evidence of adverse hematological effects in rats following gavage exposure to MTBE. The percent of monocytes was significantly increased in male rats exposed to ≥357 mg/kg/day for 14 days or 1,200 mg/kg/day for 90 days via gavage (Robinson et al. 1990). In female rats, a significant 40% decrease in WBC count was observed after exposure to 1,200 mg/kg/day for 90 days via gavage; no adverse hematological effects were noted at doses up to 1,428 mg/kg/day for 14 days (Robinson et al. 1990). Additional statistically significant findings were noted in exposed animals, including alterations in the RBC compartment, but findings were small in magnitude (<10%), predominantly in males, and likely secondary to dehydration associated with observed diarrhea (see Section 2.6). Another 2-week gavage study in male rats reported alterations in WBC parameters at 1,600 mg/kg/day, but not ≤800 mg/kg/day, including a 2-fold increase in total WBC count, 2-fold increase in the percentage of lymphocytes, 3-fold increase in the percentage of granulocytes, and 5–fold increase in the percentage of eosinophils (Dong-mei et al. 2009). Males exposed to 1,600 mg/kg/day also showed a small, but significant, 20% reduction in the RBC volume distribution width. When animals were exposed to the same doses for 4 weeks, hematological findings were limited to an 8% increase in hemoglobin at 1,600 mg/kg/day and a 50% reduction in eosinophils at 800 mg/kg/day, but not 1,600 mg/kg/day (Dong-mei et al. 2009). The findings at 4 weeks were not considered biologically relevant due to small magnitude or lack of dose-response. Similarly, no biologically relevant hematological effects were observed in another 4-week study that exposed male or female rats to gavage doses up to 1,750 mg/kg/day (Amoco 1992). Statistically significant changes included increased mean RBC counts in males at 440 mg/kg/day, but not higher doses, and increased mean corpuscular hemoglobin (MCH) in female rats at 90 and 1,750 mg/kg/day only. The toxicological significance of

these changes is not certain since they are not dose-related, and no other hematological effects were noted. No pathology was seen in the femur, sternum, or bone marrow.

A number of studies have been conducted in animals to determine possible side effects of MTBE therapy for gallstone dissolution, but only three studies were located that monitored hematological parameters. In these studies, dogs (Allen et al. 1985b; Peine et al. 1990) or pigs (Vergunst et al. 1994) received MTBE via gallbladder catheter, and no hematological effects were found.

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans following exposure to MTBE. In animal studies, the only observed musculoskeletal effect was osteodystrophy secondary to chronic progressive nephropathy in male rats chronically exposed to MTBE vapors. No primary musculoskeletal effects were observed following inhalation or oral exposure.

The musculoskeletal system was not directly affected in rats or mice exposed to MTBE via inhalation. Gross and histological examination of bone and skeletal muscle of rats revealed no treatment-related lesions following intermittent exposure to concentrations up to 3,000 ppm for 9 days (Texaco Inc. 1981), or up to 1,000 ppm for 13 weeks (Greenough et al. 1980). Similarly, an intermittent exposure for 13 weeks to concentrations up to 8,000 ppm produced no microscopic lesions in bones of treated rats (Lington et al. 1997). In chronic-duration inhalation studies, no treatment-related changes in gross and histological examination of the gastrocnemius muscle were observed in rats or mice exposed to concentrations up to 8,000 ppm for 24 or 18 months, respectively (Bird et al. 1997). No treatment-related histopathological lesions were found in the bone tissue of mice (Bird et al. 1997); however, fibrous osteodystrophy, which was secondary to chronic progressive nephropathy, was observed in male rats at all concentrations (≥400 ppm) (Bird et al. 1997). As discussed for Renal Effects in Section 2.10, the higher incidence and greater severity of chronic progressive nephropathy at lower exposure concentrations in male rats compared with female rats may be due to the exacerbation of this syndrome by the accumulation of α2u,-globulin or another unknown protein unique to male rats.

In a study evaluating enzyme activities in muscle, intermittent exposure to 300 ppm for 2–15 weeks did not affect muscle succinate dehydrogenase or acetylcholinesterase activities in rats (Savolainen et al. 1985). Muscle creatine kinase activity decreased at 2 weeks, returned to normal levels at week 10, and then significantly increased at 15 weeks in rats exposed to 100 or 300 ppm. These changes in creatine

kinase activity were attributed to adaptation at the muscle level to MTBE exposure and were not considered adverse. Musculoskeletal endpoints from this study were considered too limited to establish NOAEL/LOAEL values; therefore, this study was not included in the LSE table.

Based on histological examination of the muscle and skeletal tissues, oral exposure to MTBE appears to be without effects on the musculoskeletal system. Histological examination of the skeletal muscle and sternum of rats given gavage doses up to 1,750 mg/kg/day, 5 days/week, for 4 weeks revealed no treatment-related lesions (Amoco 1992). In drinking water studies, no pathological lesions were reported in "skeletomuscular tissues" in rats in intermediate-duration studies at doses up to 1,153 mg/kg/day (Bermudez et al. 2012), or chronic-duration studies at doses up to 1,119 mg/kg/day (Bermudez et al. 2012; Dodd et al. 2013). In another chronic-duration rat study, histological examination of the cranium (five levels) revealed no treatment-related lesions at gavage doses up to 1,000 ppm, 4 days/week, for 104 weeks (Belpoggi et al. 1995, 1997).

2.9 HEPATIC

Human occupational studies evaluating potential effects of MTBE exposure on hepatic endpoints are limited to a single cross-sectional study reporting no association between non-alcoholic fatty liver disease (NAFLD) and low MTBE exposures in Chinese gas station workers. Additional human studies have reported hepatic side effects in patients treated with MTBE intracystically for the dissolution of gallstones. Inhalation and oral exposure studies in rats, mice, and rabbits provide suggestive evidence that the liver is a target following acute-, intermediate-, or chronic-duration exposure.

Potential associations between MTBE exposure and NAFLD were evaluated in a cross-sectional study of gas station attendants employed in Southern China (Yang et al. 2016). Among the study participants, 11/71 were diagnosed with NAFLD (10/41 males, 1/30 females). The average MTBE exposure did not differ significantly between workers diagnosed with NAFLD and workers without NAFLD; the average exposure concentration was 0.081 ppm in workers with NAFLD and 0.079 ppm in workers without NAFLD. No associations between the prevalence of NAFLD and MTBE exposure were found (see Table 2-1 for odds ratios). Limitations of this study are the relatively low exposure levels (did not exceed the American Conference of Governmental Industrial Hygienists [ACGIH] Threshold Limit Value [TLV] of 50 ppm), small sample size, and cross-sectional design.

A number of clinical studies of patients receiving MTBE therapy have recorded side effects in liver, bile duct, and gallbladder, due perhaps to leakage or overflow of MTBE. For example, clinical studies reported transient or slight elevations of serum aminotransaminases, hematobilia, or increases in serum bilirubin (Allen et al. 1985a, Bonardi et al. 1986; Holl et al. 1991; Janowitz et al. 1993; Kaye et al. 1990; Leuschner et al. 1991, 1994; Neubrand et al. 1994; Thistle et al. 1989; Uchida et al. 1994). Other effects reported in patients exposed to MTBE by these procedures include cholangitis in patients with elevated serum aminotransaminase levels (Hellstern et al. 1998; Kaye et al. 1990), cholecystitis and pericholecystitis (Schumacher et al. 1990), persistent dilatation of the common bile duct (Tritapepe et al. 1989), transient, reversible edema and inflammation of the gallbladder mucosa (Eidsvoll et al. 1993; Uchida et al. 1994; van Sonnenberg et al. 1991), and bile leak after therapy (Hellstern et al. 1998).

The liver effects observed in laboratory animals include increases in liver weight, alterations in serum clinical chemistry parameters, and histological alterations. In general, the liver effects are minimal to mild in severity and have not been consistently found across studies.

Increases in liver weight have been observed in rats, mice, and rabbits following inhalation and oral exposure to MTBE. Acute-duration inhalation exposure resulted in relative liver weight increases of ≥10% in rats exposed ≥4,000 ppm for 13 days (Dodd and Kintigh 1989), mice exposed to 8,000 ppm for 3 days (Moser et al. 1996), mice exposed to ≥2,000 ppm for 13 days (Dodd and Kintigh 1989), and rabbits exposed to 8,000 ppm on GDs 6–15 (Bevan et al. 1997a). No biologically relevant alterations in liver weight were observed in rats exposed to MTBE via inhalation at concentrations up to 3,000 ppm for 9–10 days (Conaway et al. 1985; Texaco Inc. 1981), or in mice exposed to concentrations up to 8,000 ppm on GDs 6–15 (Bevan et al. 1997a; Conaway et al. 1985). Intermediate- and chronic-duration inhalation exposures resulted in an 8–45% increase in relative liver weight in rats exposed to 800–8,000 ppm for ≥4 weeks (Bevan et al. 1997b; Bird et al. 1997; Lington et al. 1997); however, no liver weight alterations were observed in rats after 13 weeks of exposure to 1,000 ppm MTBE (Greenough et al. 1980). Several mouse studies conducted by Moser et al. (1996) reported >10% increases in relative liver weight resulting from exposure to 8,000 ppm MTBE for 3–32 weeks. A 9–13% increase in relative liver weight was observed in mice exposed to ≥3,000 ppm for 28 days, and a 39% increase in relative liver weight was observed in mice exposed to 8,000 ppm for 18 months (Bird et al. 1997).

Several acute-duration oral studies in rats reported alterations in liver weight. A small, but statistically significant, 7% increase in relative liver weight was observed in male rats following gavage exposure to 1,428 mg/kg/day for 14 days; no exposure-related liver weight changes were observed in females

(Robinson et al. 1990). In other 14-day gavage studies in male rats, significant 13–18% increases in relative liver weight were observed following exposure to ≥1,200 mg/kg/day (Dong-mei et al. 2009; de Peyster et al. 2003, 2014). However, two additional 14-day studies in rats reported no effect on liver weight at doses up to 1,200 mg/kg/day (de Peyster et al. 2014). Longer-term studies have reported increases in liver weight when high doses of MTBE were administered via gavage, but not following drinking water exposure. In 28-day studies, reported LOAELs for elevated relative liver weights in rats following daily gavage exposure range from 1,000 mg/kg/day (de Peyster et al. 2003; Williams et al. 2000) to 1,750 mg/kg/day (Amoco 1992); relative liver weights were also significantly increased by 13–15% in rats administered ≥900 mg/kg/day for 90 days (Robinson et al. 1990). No changes in liver weight were observed in mice following exposure to gavage doses up to 100 mg/kg/day for 14 weeks (Tang et al. 2019). In drinking water studies, no changes in liver weight were reported in rats at doses up to 1,153 mg/kg/day for 13 weeks (Bermudez et al. 2012), 384 mg/kg/day for 6 months (Bermudez et al. 2012), 1,119 mg/kg/day for 1 year (Bermudez et al. 2012), or 1,042 mg/kg/day for 2 years (Dodd et al. 2013).

Acute- and intermediate-duration inhalation studies have not found biologically relevant alterations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and/or bilirubin levels at concentrations of 3,000 ppm for 9 days (Texaco Inc. 1981), or up to 8,000 ppm for 4–13 weeks (Bird et al. 1997; Lington et al. 1997; Greenough et al. 1980). Oral exposure studies have reported some alterations in serum clinical chemistry parameters. The observed alterations included increased serum AST and LDH levels in male rats administered via gavage ≥1,071 mg/kg/day for 14 days (Robinson et al. 1990), increased serum AST and ALT in rats administered via gavage 1,000 mg/kg/day for 28 days (de Peyster et al. 2003), and increased serum AST levels in males administered ≥ 300 mg/kg/day via gavage for 90 days (Robinson et al. 1990). No adverse alterations in these serum clinical chemistry parameters were observed after exposure to doses up to 1,600 mg/kg/day for 2 weeks or 1,750 mg/kg/day for 2 or 4 weeks (Amoco 1992; Dong-mei et al. 2009; Williams et al. 2000). Several oral exposure studies reported increases in serum cholesterol levels in rats. Significant increases in serum cholesterol were observed in male rats exposed to 1,428 mg/kg/day and in female rats exposed to 714 or 1,071 mg/kg/day for 14 days, but not 1,428 mg/kg/day (Robinson et al. 1990); in male rats exposed to 1,600 mg/kg/day for 2 weeks (Dong-mei et al. 2009); and in females rats exposed to ≥100 mg/kg/day and male rats at 900 mg/kg/day, but not at 1,200 mg/kg/day, for 90 days (Robinson et al. 1990). In a 4-week study, several measures of cholesterol were significantly altered at doses from 400 to 1,600 mg/kg/day, but no clear pattern was observed (Dong-mei et al. 2009). One 4-week study reported a decrease in serum cholesterol at 1,750 mg/kg/day in male and female rats

(Amoco 1992). In mice, no exposure-related changes in serum cholesterol or triglycerides were observed at gavage doses up to 100 mg/kg/day for 14 weeks (Tang et al. 2019). Another study evaluated serum cholesterol and triglycerides in rats following a 3-month exposure to very low doses of MTBE (0.006, 0.03, or 0.15 mg/kg/day) (Saeedi et al. 2017). Serum triglycerides and cholesterol were decreased at all tested doses, serum high density lipoprotein (HDL) cholesterol was decreased at all tested doses, serum low density lipoprotein (LDL) cholesterol was increased at ≥0.03 mg/kg/day; however, findings were not clearly dose related. Due to lack of clear dose-response and limited endpoints evaluated in this low-dose study, a NOAEL/LOAEL determination was not made based on alterations in serum cholesterol.

Hepatocellular hypertrophy has been reported in rats following oral exposure and in mice following inhalation and oral exposure. Mild hepatocellular hypertrophy was observed in mice exposed to 8,000 ppm for acute-, intermediate-, or chronic-durations (Bird et al. 1997; Moser et al. 1996). In contrast, no histological alterations were observed in the livers of rats following inhalation exposure to 1,000 ppm for 13 weeks (Greenough et al. 1980), or 8,000 ppm for 28 days (Bird et al. 1997), 13 weeks (Lington et al. 1997), 14–19 weeks (Bevan et al. 1997b), or 24 months (Bird et al. 1997). Most oral studies did not report exposure-related hepatic lesions; however, Williams et al. (2000) reported centrilobular hypertrophy in rats exposed to gavage doses ≥500 mg/kg/day for 28 days or 1,500 mg/kg/day for 15 days. In other gavage studies, no exposure-related hepatic lesions were observed in rats at doses up to 1,428 mg/kg/day for 14 days (Robinson et al. 1990), 1,200 mg/kg/day for 90 days (Robinson et al. 1990), or 1,000 mg/kg/day for 104 weeks (Belpoggi et al. 1995, 1997). In drinking water studies, no changes in liver histopathology were reported in rats at doses up to 1,153 mg/kg/day for 13 weeks (Bermudez et al. 2012), 384 mg/kg/day for 6 months (Bermudez et al. 2012), 1,119 mg/kg/day for 1 year (Bermudez et al. 2012), or 1,042 mg/kg/day for 2 years (Dodd et al. 2013).

A number of studies have been conducted in animals to determine possible hepatic side effects of MTBE therapy for gallstone dissolution. These treatments of animals have also resulted in increases in serum levels of liver enzymes, such as serum ALP in dogs (Allen et al. 1985b) and increased serum ALP, ALT, and AST in rabbits (Tritapepe et al. 1989). Lobular necrosis of hepatocytes and mild portal inflammation in the liver was found in pigs given MTBE via the percutaneous transhepatic route to the gallbladder (Chen et al. 1995). Effects on the gallbladder after administration of MTBE via catheter to the gallbladder or the liver include necrosis of the gallbladder and bile ducts, fibrosis of the gallbladder, hyperplastic cholecystitis, inflammation and focal ulceration of the gallbladder mucosa, and edema in rabbits and pigs (Adam et al. 1990; Chen et al. 1995; Dai et al. 1989; Esch et al. 1992; Griffith et al. 1990;

McGahan et al. 1988; Vergunst et al. 1994). That these effects were not due to the surgical procedure was demonstrated using sham-treated saline controls or solvent controls.

Mechanisms of Hepatotoxicity. Observed findings of increased liver weight and centrilobular hypertrophy in mice are likely due to an initial increase in metabolic demand on liver cells with exposure to MTBE, resulting in a compensatory increase in hepatocellular hypertrophy that progresses to hepatocellular proliferation with repeated exposure (Chun and Kintigh 1993; Clary 1997; McGregor 2006). Intraperitoneal injection of rats with MTBE resulted in a 47-fold induction of pentoxyresorufin O-dealkylase activity, an activity associated with cytochrome P4502B1 (Brady et al. 1990). Cytochrome P4502B1 is also involved in the demethylation of MTBE; thus, MTBE appears to induce its own metabolism. In support, liver enzyme induction in female mice intermittently exposed to MTBE at air concentrations of 8,000 ppm for 3 days, 3 weeks, 16 weeks, or 32 weeks was accompanied by increased hepatic deoxyribonucleic acid (DNA) syntheses and/or hepatic hypertrophy (Moser et al. 1996). In a special experiment for cell proliferation evaluations of hepatocytes, male and female mice were exposed to 0, 400, 3,000, or 8,000 for 5 or 23 exposures (Bird et al. 1997). Significantly increased uptake of 5-bromo-2'-deoxyuridine in the nuclei of hepatocytes of female mice, but not male mice, was found at an exposure level of 8,000 ppm, but not ≤3,000 ppm, for 5 days. No increase in hepatocellular proliferation was found when mice were similarly exposed for 23 exposures.

In a 4-week gavage study in rats, liver cytochrome P450 enzyme levels were significantly elevated by ~1.5-fold following exposure to 1,500 mg/kg/day from days 1 to 12 (every other day) and 750 mg/kg/day from days 13 to 28 (every other day); time-weighted average (TWA) dose was calculated to be 536 mg/kg/day (de Peyster et al. 2003). Another 2-week study in rats reported dose-related increases in liver microsomal uridine diphosphate glucuronosyl transferase (UDPGT) activity following intermittent exposure to 50, 100, and 300 ppm for 2 weeks, but not at 6, 10, or 15 weeks (Savolainen et al. 1985). The biological significance of these findings is not clear. Exposure to these levels for up to 15 weeks did not affect rat liver microsomal cytochrome P450 content or the enzymatic activities of NADP-cytochrome c reductase or 7-ethoxycoumarin 0-deethylase. Although induction of liver microsomal enzymes may be potentially adverse, other studies in rats (Bevan et al. 1997b; Bird et al. 1997; Dodd and Kintigh 1989; Lington et al. 1997; Texaco Inc. 1981) indicate that hepatic effects associated with enzyme induction (e.g., increased liver weight) occur in rats only at much higher exposure levels.

Mechanisms for elevated serum cholesterol noted in some oral studies are unknown, and the adversity of this finding is unclear due to the lack of associated hepatic lesions (e.g., fatty liver).

2.10 RENAL

No studies were located regarding renal effects in humans following inhalation, oral, or dermal exposure to MTBE. Data from patients treated with MTBE intracystically for the dissolution of gallstones do not consistently report renal side effects. Based on inhalation and oral studies in animals, the male rat, and to a lesser extent the female rat, are susceptible to kidney damage following exposure to MTBE. Findings in male rats are likely due, in part, to α2u-globulin accumulation, which is not relevant to human health.

A number of clinical studies of patients receiving MTBE therapy have recorded side effects. In two such studies, urinalysis in 1 patient suggested that MTBE did not cause abnormal renal function (Allen et al. 1985a), and no renal failure was found in 12 patients (Uchida et al. 1994). Renal failure and anuria, however, were reported in a patient who experienced severe complications due to extravasation of MTBE from the gallbladder lumen (Ponchon et al. 1988).

Studies in rats have identified sex-related differences in the renal toxicity of MTBE. In male rats, renal alterations have been reported following acute-, intermediate-, and chronic-duration inhalation and oral exposures. The observed effects include alterations in kidney weight and histopathology.

Increases in absolute and/or relative kidney weights have been inconsistently reported in male rats. Increases in kidney weight were observed following inhalation exposure to 8,000 ppm for 28 days (Bird et al. 1997), inhalation exposure to ≥800 ppm for 13 weeks (Lington et al. 1997), oral exposure to a TWA gavage dose of 1,350 mg/kg/day for 2 weeks (de Peyster et al. 2014), gavage doses of 1,500 mg/kg/day for 15 days or ≥250 mg/kg/day for 28 days (Williams et al. 2000), gavage doses of 1,750 mg/kg/day for 4 weeks (Amoco 1992), gavage doses ≥900 mg/kg/day for 90 days (Robinson et al. 1990), drinking water doses of ≥514 mg/kg/day for 13 weeks or 29 mg/kg/day for 1 year (Bermudez et al. 2012), and drinking water doses of 330 mg/kg/day for 2 years (Dodd et al. 2013). However, other studies have not found alterations in male rat kidney weight following acute- or chronic-duration inhalation exposure to concentrations as high as 8,000 ppm (Bird et al. 1997; Dodd and Kintigh 1989), oral acute- or intermediate-duration exposure to gavage doses as high as 1,600 mg/kg/day (de Peyster et al. 2014; Dong-mei et al. 2009; Robinson et al. 1990), or chronic-duration exposure to drinking water doses as high as 384 mg/kg/day (Bermudez et al. 2012).

Two studies found increases in serum blood urea nitrogen (BUN) in male rats exposed via inhalation to 1,000 ppm for 13 weeks (Greenough et al. 1980), or via gavage to 1,428 mg/kg/day for 14 days (Robinson et al. 1990). However, most studies did not find biologically relevant alterations in BUN and/or creatinine following acute- or intermediate-duration inhalation exposure to up to 8,000 ppm (Bird et al. 1997; Lington et al. 1997; Texaco Inc. 1981), or oral exposure to up to 1,750 mg/kg/day (Amoco 1992; de Peyster et al. 2014; Dong-mei et al. 2009; Robinson et al. 1990). Bird et al. (1997) reported increased urine volume and decreased urinary pH in male rats exposed to 8,000 ppm for 28 days, but there was no other indication of renal damage in serum clinical chemistry or urinalysis measures.

Histological alterations have been observed in the kidney of male rats following inhalation and oral exposure to MTBE. In male rats, increased cell proliferation in the epithelial cells of the renal proximal convoluted tubules were observed in male rats following inhalation exposures to ≥1,500 ppm for 10 days (Prescott-Mathews et al. 1997), with increased cell proliferation following inhalation exposure to ≥3,000 ppm for 5–10 days (Bird et al. 1997; Prescott-Mathews et al. 1997) or 28 days (Bird et al. 1997). Several studies have reported hyaline droplets in the renal proximal convoluted tubules of male rats following inhalation exposure to 8,000 ppm for 13 weeks (Lington et al. 1997), acute-duration oral exposure to ≥972 mg/kg/day (Bermudez et al. 2012; Robinson et al. 1990), and intermediate-duration oral exposure to ≥250 mg/kg/day (Amoco 1992; Bermudez et al. 2012; Robinson et al. 1990; Williams et al. 2000). Chronic-duration exposure resulted in increases in the incidence and severity of chronic progressive nephropathy in male rats following inhalation exposure to ≥400 ppm for 24 months (Bird et al. 1997), oral exposure to ≥29 mg/kg/day for 1 year (Bermudez et al. 2012), or oral exposure to 330 mg/kg/day for 2 years (Dodd et al. 2013). No histological alterations were observed in the kidneys of male rats following inhalation exposure to 3,000 ppm for 9 days (Texaco Inc. 1981) or 1,000 ppm for 13 weeks (Greenough et al. 1980) or following oral exposure to 384 mg/kg/day for 6 months (Bermudez et al. 2012) or 1,000 mg/kg/day for 2 years (Belpoggi et al. 1995, 1997).

In contrast to the findings in male rats, renal effects in female rats have been limited to alterations in kidney weight following acute-, intermediate-, and chronic-duration exposure and histological alterations after chronic-duration exposure. Although some acute- and intermediate-duration studies reported increases in absolute and/or relative kidney weights in female rats (Bermudez et al. 2012; Dodd and Kintigh 1989; Lington et al. 1997; Robinson et al. 1990), these increases were not associated with histological alterations and were not considered biologically relevant. Inhalation or oral exposure to MTBE for ≤1 year did not result in histological alterations in female rats. The highest NOAEL values for each exposure duration were 3,000 ppm for acute-duration inhalation exposure (Texaco Inc. 1981),

8,000 ppm for intermediate-duration inhalation exposure (Bird et al. 1997; Greenough et al. 1980; Lington et al. 1997), 1,428 mg/kg/day for acute-duration oral exposure (Bermudez et al. 2012; Robinson et al. 1990), 1,750 mg/kg/day for intermediate-duration oral exposure (Amoco 1992; Bermudez et al. 2012: Robinson et al. 1990), and 1,119 mg/kg/day for a 1-year oral exposure (Bermudez et al. 2012). In chronic-duration studies, elevated kidney weight observed in female rats exposed to ≥3,000 ppm via inhalation and 1,042 mg/kg/day via drinking water for 2 years was considered relevant, as it was accompanied by increased incidence and severity of chronic progressive nephropathy (Bird et al. 1997; Dodd et al. 2013). A third 2-year study did not find altered renal weights or histological alterations at a gavage dose of 1,000 mg/kg/day (Belpoggi et al. 1995, 1997).

Data on renal effects in mice are limited to one intermediate-duration and one chronic-duration inhalation study. No exposure-related changes in renal weight or histology were observed in mice exposed to concentrations up to 8,000 ppm for 28 days (Bird et al. 1997). Following inhalation exposure for 18 months, elevated kidney weights were observed in male mice at ≥400 ppm and female mice at 8,000 ppm (Bird et al. 1997). A slight increase in the pH of the urine was observed in males and females exposed to 8,000 ppm; a slight increase in the gamma globulin fraction was also observed in males at 8,000 ppm. Male mice exposed to 8,000 ppm were reported to have increased mortality and decreased mean survival time due to a slight, but not statistically significant, increase in incidence of obstructive uropathy, which may be related to the increases in pH and gamma globulin fraction.

The urinary bladder does not appear to be affected by MTBE as evidenced by the lack of histological alterations in rats exposed by inhalation to 3,000 ppm for 9 days (Texaco Inc. 1981) or 1,000 ppm for 13 weeks (Greenough et al. 1980), or by oral exposure to 1,000 ppm for 2 years (Belpoggi et al. 1995, 1997).

In one study conducted in rabbits to determine possible side effects of MTBE therapy for gallstone dissolution, histological examination revealed no renal damage (Dai et al. 1989).

Mechanisms of Renal Toxicity. Male rats appear more sensitive to renal toxicity following exposure to MTBE than female rats. The histological findings of protein accumulation and large hyaline droplets in the renal tubules of male rats exposed via inhalation (Bird et al. 1997; Lington et a. 1997) or oral exposure (Bermudez et al. 2012) suggest the involvement of α2u-globulin accumulation. α2u-Globulin is a low molecular weight protein synthesized in large quantities in the male rat liver, secreted into the blood under the influence of testosterone (Alden 1986), and filtered through the glomerulus. Renal tubule cells

reabsorb α2u-globulin and sequester it into lysosomes, where it is catabolized into amino acids and peptides. In the normal rat kidney, the rate of catabolism of α2u-globulin is relatively slow compared with other proteins (Swenberg et al. 1989). Chemicals that bind to α2u-globulin yield a complex that is more resistant to the proteolytic enzymes in the lysosomes, which leads to the accumulation of the complex in the tubule cells. Accumulation of the chemical α2u-globulin complex causes lysosomal overload and necrosis of the tubule cells, with subsequent proliferative regeneration. If exposure to the chemical is chronic, then accumulation, necrosis, and subsequent cellular proliferation continues, and can lead to a carcinogenic response. α2u-Globulin nephropathy is a condition specific to male rats; that is, it has not been found in female rats or males or females of any other species, including humans (Ahmed 2001; Bogen and Heilman 2015; McGregor 2006; Phillips et al. 2008).

A 10-day inhalation study by Prescott-Mathews et al. (1997) specifically reported α2u-globulin droplet accumulation in association with proximal tubule necrosis and cell proliferation in male rats following intermittent exposure to ≥1,500 ppm MTBE (Prescott-Mathews et al. 1997). Similarly, hyaline droplets were associated with α2u-globulin accumulation in male rats exposed to 972 mg/kg/day via drinking water for 1 or 4 weeks (Bermudez et al. 2012). However, α2u-globulin levels were not significantly elevated in kidneys of male rats exposed to 972 mg/kg/day via drinking water for 13 weeks (Bermudez et al. 2012). Additionally, while Bird et al. (1997) reported evidence of protein accumulation in association with increased cell proliferation in the epithelial cells of the proximal convoluted tubules at ≥3,000 ppm in male rats exposed for 28 days, immunostaining for α2u-globulin accumulation was negative. This suggests that a mechanism other than a α2u-globulin accumulation (perhaps the accumulation of another protein unique to male rats) may be responsible for the observed increased cell proliferation. Similarly, while male rats exposed to ≥800 ppm for 13 weeks showed a treatment-related increase in area and intensity of α2u -globulin positive staining, the α2u-globulin positive staining was not dose-related and α2u-globulin positive proteinaceous casts were not observed at the junction of the proximal tubules and thin limb of Henle, which are the classical lesions of other α2u-globulin inducing agents (Swenberg and Dietrich 1991). Taken together, available data suggest that while α2u-globulin induction may contribute to renal effects observed in male rats, other mechanisms (potentially another protein specific to male rats) may also play a role in renal pathogenesis following exposure to MTBE. Furthermore, an additional unknown mechanism may also be involved in the enhancement of chronic progressive nephropathy by MTBE due to observed effects, albeit to a lesser degree, in female rats (Bird et al. 1997).

Metabolism of MTBE to *tert*-butanol (Section 3.1.1) likely underlies, or at least contributes to, any α2u-globulin-mediated effects, as α2u-globulin accumulation, protein droplet accumulation, and renal cell

proliferation have been reported in male rats exposed to *tert*-butanol at roughly the same potency as MTBE (Ahmed 2001; Bogen and Heilman 2015; McGregor 2006), although modeling predicts that MTBE has a greater binding affinity for α2u-globulin than *tert*-butanol (Leavens and Borghoff 2009).

The effect of intermittent inhalation exposure to 50–300 ppm for 2–15 weeks on rat kidney microsomal enzymes has been studied (Savolainen et al. 1985). Cytochrome P450 content was reported to be significantly increased only after 15 weeks of exposure to 100–300 ppm, while UDPGT and NADP-cytochrome c reductase activities were significantly increased only after 2 weeks of exposure. The enzymatic activity of 2-ethoxycoumarin 0-deethylase was not affected. Although induction of kidney microsomal enzymes may be potentially adverse, other studies (Bird et al. 1997; Greenough et al. 1980; Lington et al. 1997; Texaco Inc. 1981) indicate that microscopic renal lesions and increased kidney weight due to enzyme induction occur in animals only at much higher exposure levels.

2.11 DERMAL

Skin exposure to MTBE vapors in inhalation studies did not result in dermal effects in humans or animals. However, direct exposure to liquid MTBE resulted in skin irritation and damage in rabbits and guinea pigs. No adverse dermal effects were noted in oral studies in animals.

No associations between skin irritation or rash and exposure to MTBE in gasoline were observed in cross-sectional occupational (CDC 1993a) or population-based studies (Wisconsin DHSS 1995) (see Table 2-1). In controlled human inhalation experiments, no differences in incidence of skin rash or dry skin were observed in volunteers during or after exposure to MTBE at ≤1.7 ppm for 1 hour (Cain et al. 1996; Prah et al. 1994). Because any observed changes would likely be attributed to direct vapor contact, dermal endpoints from these studies are included in the dermal LSE table (Table 2-4).

No gross or histopathological lesions were found on the skin of rats exposed to gavage doses up to 1,750 mg/kg/day, 5 days/week, for 4 weeks (Amoco 1992). Similarly, no gross or histopathological lesions of the skin or subcutaneous tissues were observed in rats exposed to gavage doses up to 1,000 mg/kg/day, 4 days/week, for 104 weeks (Belpoggi et al. 1995, 1997).

Direct exposure of the skin to MTBE vapors in air during inhalation studies did not result in dermal effects. No irritation or treatment-related skin lesions were observed during clinical, gross, and/or histological examination in rats exposed to airborne MTBE at concentrations up to 8,000 ppm for 13 days

(Dodd and Kintigh 1989) or 13 weeks (Greenough et al. 1980; Lington et al. 1997). Alopecia was commonly observed in rats exposed to 250, 1,000 or 2,500 ppm for 16–28 weeks, but it was not considered to be related to MTBE exposure because the incidence was similar in the exposed and control groups (Biles et al. 1987). In chronic-duration studies, histological examination of the skin revealed no treatment-related lesions in rats or mice exposed to airborne MTBE at concentrations up to 8,000 ppm for 24 or 18 months, respectively (Bird et al. 1997). Because inhalation exposure to chemicals may result in dermal effects due to direct contact of the vapor on the skin, skin irritation effects in animals following inhalation exposure are included in the dermal LSE table (Table 2-4).

Application of 0.5 mL or 10,000 mg/kg ARCO MTBE (96.2% MTBE) or commercial MTBE (99.1% MTBE) to the intact or abraded skin of rabbits resulted in slight to severe erythema, blanching, epidermal thickening, acanthosis, or focal necrosis (ARCO 1980). In a dermal sensitization test in guinea pigs (see Section 2.14), local irritation and increased erythema developed at the site after the initial intradermal injection of 0.5 mL of a 1% MTBE solution (ARCO 1980).

2.12 OCULAR

Eye irritation has been noted in workers and motorists exposed to fumes from gasoline containing MTBE. However, eye irritation has not been reported in healthy volunteers exposed to pure MTBE at concentrations up to 50 ppm. In laboratory animals, eye irritation has also been observed in animals following exposure to high MTBE concentrations during inhalation studies. Direct exposure to liquid MTBE resulted in eye irritation and damage in rabbits. No adverse ocular effects were noted in oral studies in animals.

Several occupational studies conducted in the 1990s evaluated potential associations between eye irritation and MTBE exposure following the introduction of MTBE into gasoline during the oxyfuel program in United States (see Table 2-1). Several of these studies report an increase in self-reported eye irritation during the oxyfuel program in Alaskan workers with occupational exposure to gasoline containing MTBE (e.g., taxi drivers or health-care workers who travelled routinely in cars), compared either with individuals with low exposure (e.g., noncommuter students) or workers exposed to gasoline that did not contain MTBE (Alaska DHSS 1992a, 1992b; Moolenaar et al. 1994). However, these studies only provide suggestive evidence due to several limitations, including lack of a referent group (Alaska DHSS 1992b only), small to modest subject numbers, lack of statistical analysis of incidence data, potential influence of widespread media coverage and public opposition to the oxyfuel program, and/or

lack of consideration for other confounding factors (e.g., other exposures, including potential combustion products of MTBE). No clear evidence of increased eye irritation was observed in occupationally exposed workers, compared with unexposed referents, in similar studies during the oxyfuel program conducted in Connecticut (CDC 1993b; White et al. 1995) or New Jersey (Mohr et al. 1994).

Results from population-based studies evaluating the potential association between eye irritation and MTBE exposure due to the oxyfuel program are mixed (see Table 2-1). In a population-based telephone survey, a statistically significant increase in risk of eye irritation was observed in residents of metropolitan Milwaukee, Wisconsin, in which oxyfuel was used, compared to residents of non-metropolitan Wisconsin (MTBE-free gasoline); however, the risk for eye irritation was not increased for residents of metropolitan Chicago, Illinois (oxyfuel) compared to non-metropolitan Wisconsin (Wisconsin DHSS 1995). The results in Milwaukee may have been biased by knowledge of the oxyfuel program and potential health effects due to increased media coverage in Milwaukee because, in the telephone survey, familiarity with MTBE as a reformulated gasoline additive was reported by 54% of the Milwaukee residents, 23% of Chicago residents, and 40% of the Wisconsin residents. Additionally, non-metropolitan Wisconsin may not have been an appropriate referent group for the Chicago residents. One ecological study also reported a statistically significant increase in doctor's visits with diagnostic codes pertaining to eye irritation in Philadelphia, Pennsylvania in 1997 (during the sixth year of the oxyfuel program), compared to 1992 (at the initiation of the oxyfuel program) based on medical record review (Joseph and Weiner 2002). The study authors concluded that "some environmental factor" in the mid-1990s was associated with immune system findings and suggested that this factor is MTBE. However, this study has several major limitations, primarily a study design that does not allow for control for confounders (other chemical exposures, occupation, concomitant diseases, sex, age, race, etc.), that preclude meaningful conclusions.

In controlled human inhalation experiments, no differences in self-reported symptoms of eye irritation (e.g., dry, itching, or irritated eyes; tired or strained eyes; burning eyes) were observed in volunteers during or after exposure to MTBE at air levels ≤1.7 ppm for 1 hour (Cain et al. 1996; Prah et al. 1994), or ≤50 ppm for 2 hours (Johanson et al. 1995; Nihlén et al. 1998a). Additionally, no exposure-related effects were observed in quantitative measures of ocular irritation (blinking frequency, eye redness score, tear film break-up time, or conjunctival epithelial damage) in subjects exposed to ≤50 ppm for 2 hours (Johanson et al. 1995; Nihlén et al. 1998a). Because any observed changes would likely be attributed to direct vapor contact, ocular endpoints from these studies are included in the dermal LSE table (Table 2-4).

No gross lesions were found in the eyes, exorbital lacrimal glands, or Harderian glands and no histopathological lesions were found in the eyes (glands not examined microscopically) in rats exposed to gavage doses up to 1,750 mg/kg/day MTBE for 4 weeks (Amoco 1992). In drinking water studies, no gross or histopathological lesions of the eye were reported in rats in intermediate-duration studies at doses up to 1,153 mg/kg/day (Bermudez et al. 2012), or chronic-duration studies at doses up to 1,119 mg/kg/day (Bermudez et al. 2012; Dodd et al. 2013).

Direct instillation of ARCO MTBE (96.2% MTBE) or commercial MTBE (99.1% MTBE) into the eyes of rabbits resulted in ocular irritation regardless of whether or not the eyes were washed after exposure; however, ARCO MTBE was more irritating than commercial MTBE (ARCO 1980). ARCO MTBE induced corneal opacities, chemosis, and conjunctival redness, while commercial MTBE caused slight conjunctival redness and some discharge, but no corneal opacities. In a similar study, delayed and reversible congestion of the conjunctivae, palpebral thickening, and hypersecretion were observed in the eyes of rabbits following direct instillation of MTBE (Snamprogetti 1980).

Direct exposure of the eyes to MTBE vapors during inhalation exposure has also resulted in ocular effects in animals. Single 4–6-hour exposures to MTBE vapors resulted in lacrimation at air levels ≥8,000 ppm, with irritation and ocular discharge at concentrations ≥18,892 ppm (ARCO 1980; Daughtrey et al. 1997). Inhalation exposure for 9 days to concentrations ≥100 ppm caused higher incidences of lacrimation and conjunctival swelling in exposed rats than in controls; however, gross and histological examination of the eyes revealed no lesions (Texaco Inc. 1981). Pregnant mice exposed to 250–2,500 ppm on GDs 6–15 had a slight increase in the incidence of lacrimation during exposure (Conaway et al. 1985). In another inhalation developmental study in mice, lacrimation with periocular encrustations was observed in mouse dams at 8,000 ppm (Bevan et al. 1997a). Similarly, in a 2-generation study in rats, ocular discharges and periorbital encrustation were observed in F1 adults exposed to 8,000 ppm for 10 weeks before breeding and throughout mating and gestation (Bevan et al. 1997b). However, eye irritation and other ocular effects, as determined histologically or by ophthalmoscopy, were not found in rats exposed to concentrations up to 8,000 ppm for 13 days (Dodd and Kintigh 1989) or for 13 weeks (Greenough et al. 1980; Lington et al. 1997). No exposure-related clinical signs of eye irritation were noted in rats exposed to concentrations up to 2,500 ppm for 16–28 weeks; lacrimation was among the most common effects observed, but incidences were similar between controls and exposed animals (Biles et al. 1987). In chronic-duration inhalation studies, swollen periocular tissue was observed in male rats intermittently exposed to concentrations ≥3,000 ppm for up to 24 months (Bird et al. 1997); however, no exposure-related lesions were observed in the eye. No treatment-related clinical signs of irritation or ocular lesions

were observed in mice exposed to concentrations up to 8,000 ppm for 18 months (Bird et al. 1997). Because inhalation exposure to chemicals may result in ocular effects due to direct eye contact with vapor in the air, eye irritation effects in animals following inhalation exposure are included in the dermal LSE table (Table 2-4).

Blepharospasm was also reported in F0 rats from the 2-generation study and rats and mice from chronic-duration inhalation studies at concentrations ≥3,000 ppm (Bevan et al. 1997b; Bird et al. 1997); however, this is likely a neurological effect associated with inhalation of MTBE rather than evidence of eye irritation from direct vapor exposure.

2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans following inhalation, oral, or dermal exposure to MTBE. Studies in patients treated with MTBE intracystically for the dissolution of gallstones do not indicate pancreatic damage following treatment. Based on inhalation and oral studies in laboratory animals, the adrenal gland may be a target of toxicity following exposure to high levels of MTBE. There is no clear evidence of toxic effects on other non-reproductive endocrine organs/systems following MTBE exposure in animals. Effects to reproductive endocrine glands and hormones are discussed in Section 2.16 (Reproductive).

A number of clinical studies of patients receiving MTBE therapy have investigated effects on the pancreas. In one study of 75 patients administered MTBE via percutaneous intracystic infusion, follow-up examination 6–42 months after therapy revealed that none had pancreatitis (Thistle et al. 1989). Furthermore, pancreatic function tests administered to eight patients 1 year after intraductal administration of MTBE revealed no pancreatic abnormalities (Tritapepe et al. 1989).

Inhalation and oral exposure studies in laboratory animals have not found alterations in organ weight or histology of the pancreas. No pancreatic alterations were observed in rats following inhalation exposure to ≤3,000 ppm (Texaco Inc. 1981), ≤8,000 ppm (Bevan et al. 1997b; Greenough et al. 1980; Lington et al. 1997), or ≤8,000 ppm (Bird et al. 1997) in acute-, intermediate-, or chronic- duration studies, respectively, or in mice exposed to ≤8,000 ppm in a chronic-duration study (Bird et al. 1997). Similarly, no histopathological alterations were observed in the pancreas of rats following oral exposure ≤1,750 mg/kg/day in an intermediate-duration study (Amoco 1992), or ≤1,042 mg/kg/day in a chronic-duration study (Belpoggi et al. 1995, 1997; Dodd et al. 2013). Several studies conducted in animals to

determine possible side effects of MTBE therapy for gallstone dissolution have examined the pancreas histologically. No treatment-related histological lesions were found in the pancreas of rabbits (Adam et al. 1990; Dai et al. 1989), dogs (Allen et al. 1985b), or pigs (McGahan et al. 1988; Vergunst et al. 1994).

A number of inhalation and oral exposure studies in rats and mice evaluated the potential of MTBE to affect the adrenal gland. The results of these studies provide some suggestive evidence that the adrenal gland is a target; however, the results are inconsistent and additional data are needed. Increases in absolute and/or relative adrenal weights have been inconsistently found following acute-, intermediate-, or chronic-duration exposure. Increases in adrenal weight were reported in rats following acute-duration inhalation exposure to 8,000 ppm (Dodd and Kintigh 1989), intermediate-duration inhalation exposure to ≥3,000 ppm (Bird et al. 1997; Lington et al. 1997), acute-duration oral exposure to ≥600 mg/kg/day (de Peyster et al. 2014), and intermediate-duration oral exposure to ≥800 mg/kg/day (de Peyster et al. 2003, 2014; Amoco 1992; Williams et al. 2000). Adrenal weight increases have also been observed in mice following chronic-duration inhalation exposure to 8,000 ppm (Bird et al. 1997). Several inhalation and oral exposure studies have not found significant increases in adrenal weight in rats following acute-duration inhalation exposure to ≤3,000 ppm (Texaco Inc. 1981), chronic-duration inhalation exposure to ≤8,000 ppm (Bird et al. 1997), acute-duration oral exposure to ≤1,428 mg/kg/day (de Peyster et al. 2014; Robinson et al. 1990), intermediate-duration oral exposure to ≤1,500 mg/kg/day (Bermudez et al. 2012; Williams et al. 2000), or chronic-duration oral exposure to ≤1,119 mg/kg/day (Bermudez et al. 2012; Dodd et al. 2013); no alterations in adrenal weight were reported in mice following exposure to 8,000 ppm for 13 days, 4 months, or 8 months (Dodd and Kintigh 1989; Moser et al. 1998). Histological alterations consisting of a loss of zona reticularis tissue was found in mice following inhalation exposure to 8,000 ppm for 4 or 8 months (Moser et al. 1998). Interpretation of this finding is limited by the lack of incidence data and other studies confirming this finding. No histological alterations were observed in the adrenal gland of rats following inhalation exposure to 3,000 ppm for 9 days (Texaco Inc. 1981), intermediate- or chronic-duration inhalation exposure to 8,000 ppm (Bird et al. 1997; Lington et al. 1997), intermediate-duration oral exposure to ≤1,750 mg/kg/day (Amoco 1992; Bermudez et al. 2012; Robinson et al. 1990; Williams et al. 2000), or chronic-duration oral exposure to ≤1,119 mg/kg/day (Belpoggi et al. 1995, 1997; Bermudez et al. 2012; Dodd et al. 2013). Similarly, no histological alterations were observed in the adrenal gland of mice exposed via inhalation to 8,000 ppm for 18 months (Bird et al. 1997).

Altered corticosterone levels have also been reported in rats following inhalation and oral exposure to MTBE. In a 13-week study, a 3-fold increase in corticosterone was observed in male and female rats at 8,000 ppm; no exposure-related changes were observed in aldosterone or adrenocorticotropic hormone

levels (Lington et al. 1997). In a 24-month study in which rats were exposed intermittently to 400, 3,000, or 8,000 ppm, decreased levels of corticosterone were found at 81 weeks in male rats exposed to 8,000 ppm (this group was terminated at week 82 because of high mortality from chronic progressive nephropathy) (Bird et al. 1997). However, corticosterone levels were increased in male and female mice after exposure to 8,000 ppm at week 79 of an 18-month study (Bird et al. 1997). In oral studies, a 2-fold increase in serum corticosterone was observed in male rats exposed to gavage doses ≥600 mg/kg/day for 2 weeks (de Peyster et al. 2014), or 800 mg/kg/day for 4 weeks (de Peyster et al. 2003). However, no changes in serum corticosterone were observed in male rats exposed to doses up to 1,500 mg/kg/day every other day for 12 days followed by exposure to doses up to 750 mg/kg/day for an additional 16 days (TWA doses up to 536 mg/kg/day) (de Peyster et al. 2003). The toxicological significance of these transient and inconsistent changes in corticosterone levels in rats and mice is questionable. One potential reason for inconsistent results between studies may be due to time-of-day-dependent variations in corticosterone levels; however, studies did not report at what time of day blood was collected.

Laboratory animal studies examining the thyroid gland have not found alterations in organ weight or histology following inhalation exposure to ≤3,000 ppm in acute-duration studies (Texaco Inc. 1981), inhalation exposure to ≤8,000 ppm in intermediate- or chronic-duration studies (Bird et al. 1997; Greenough et al. 1980), oral exposure to 1,750 mg/kg/day in intermediate-duration studies (Amoco 1992; Bermudez et al. 2012), or oral exposure to ≤1,119 mg/kg/day in chronic-duration studies (Belpoggi et al. 1995, 1997; Bermudez et al. 2012; Dodd et al. 2013).

Special clinical chemistry analysis was conducted in mice intermittently exposed to MTBE for 28 days followed by a 16-day recovery period (Chun and Kintigh 1993). Serum total triiodothyronine (T3), total thyroxine (T4), and thyroid stimulating hormone (TSH) were evaluated. Total T4 and TSH were significantly elevated by 30 and 26%, respectively, in male mice at 8,000 ppm at the end of the 28-day exposure period. In contrast, no changes were observed in similarly exposed females at the end of the exposure period, but total T4 was significantly decreased after 3 days of recovery. These alterations were not considered to be biologically significant because they were transient and not associated with histopathological thyroid lesions. A second study reported a decrease in serum total T3 in male rats exposed to daily gavage doses of ≥1,000 mg/kg/day for 28 days (Williams et al. 2000). No changes were observed in serum total T3 at doses up to 1,500 mg/kg/day for 15 days or serum total T4 or TSH at doses up to 1,500 mg/kg/day for 15 or 28 days. The thyroid was not examined for weight or histopathological changes in this study. This finding was not considered biologically relevant due to the small magnitude of change (18%) and lack of evidence for thyroid damage from other oral studies.

Evidence for altered weight or histology in other endocrine organs is limited. In a 24-month study in rats, hyperplasia of the parathyroid glands was observed in males at ≥400 ppm; however, this lesion was secondary to chronic progressive nephropathy, which occurred at the same exposure concentrations (Bird et al. 1997). No treatment-related histopathological lesions were found in the pituitary in this study. Other studies have not reported effects on the parathyroid glands following inhalation or oral exposure (Amoco 1992; Belpoggi et al. 1995, 1997; Bird et al. 1997; Dodd et al. 2013; Greenough et al. 1980; Texaco Inc. 1981). In female mice, a 20–44% decrease in absolute and relative pituitary weight was observed following exposure to 8,000 ppm for 4 or 8 months; this finding was accompanied by hyaline droplets containing adrenal corticotrophin hormone (ACTH) in the pars intermedia of the pituitary gland (Moser et al. 1998). In other studies in rats and mice, no histopathological lesions were observed in the pituitary gland (Amoco 1992; Belpoggi et al. 1995, 1997; Bermudez et al. 2012; Bevan et al. 1997b; Bird et al. 1997; Dodd et al. 2013; Greenough et al. 1980; Lington et al. 1997).

2.14 IMMUNOLOGICAL

Human studies are too limited to determine if MTBE exposure alters immune function. Immune function tests in laboratory animals were limited to a negative skin sensitization study. Data from inhalation and oral studies in laboratory animals that evaluated immune organ weight and histology, but not immune function, provide limited evidence of proliferation of lymphoreticular tissues in rats. These lesions may be preneoplastic in nature (see Section 2.19, Cancer).

Two ecological studies evaluated potential associations between exposure to MTBE in gasoline during the oxyfuel program and immune endpoints (see Table 2-1). The rate of treatment for asthma (based on medical insurance records) was not increased in Fairbanks or Anchorage, Alaska over the winter of 1992–1993 (during the oxyfuel program), compared with the number of claims during the two preceding winters (prior to the oxyfuel program) (Gordian et al. 1995). Similarly, asthma diagnosis was not increased in Philadelphia, Pennsylvania in 1997 (during the 6th year of the oxyfuel program) compared with 1992 (at the initiation of the oxyfuel program) based on medical record review, although a diagnosis of wheezing was statistically increased in 1997 compared with 1992 (Joseph and Weiner 2002). Additionally, diagnostic codes pertaining to immune function (upper respiratory infection, middle ear infection) and allergies (wheezing, skin rash, allergic rhinitis, general allergy) were significantly increased in 1997 compared with 1992 (Joseph and Weiner 2002). The study authors concluded that "some environmental factor" in the mid-1990s was associated with immune system findings and

suggested that this factor is MTBE. However, this study has several major limitations, primarily a study design that does not allow for control for confounders (other chemical exposures, occupation, concomitant diseases, sex, age, race, etc.), that preclude meaningful conclusions.

A small occupational study in Fairbanks, Alaska did not find any differences in pre- and post-shift plasma interleukin-6 levels in 22 mechanics exposed to automobile emissions derived from oxyfuels containing MTBE; interleukin-1 levels were below the level of detection at both time points (Duffy 1994). In controlled human inhalation experiments, MTBE exposure did not result in increased inflammatory markers in nasal lavage fluid and/or alterations in immune cell counts in tear fluid or nasal lavage fluid at ≤1.7 ppm for 1 hour (Cain et al. 1996; Prah et al. 1994), or at ≤50 ppm for 2 hours (Johanson et al. 1995; Nihlén et al. 1998a). No concentration-related increases in nasal swelling, as determined via blocking index, were observed at concentrations ranging from ≤50 ppm for 2 hours (Johanson et al. 1995; Nihlén et al. 1998a). Because any observed changes would likely be attributed to direct vapor contact, immune endpoints from these studies are included in the dermal LSE table (Table 2-4).

No animal inhalation studies evaluating the function of the immune system were identified. However, several inhalation studies evaluated immune organ weight and/or histology. Acute-duration intermittent exposure of rats for 9 days to concentrations up to 3,000 ppm did not produce gross or histological lesions in bone marrow, lymph nodes, or spleen (Texaco Inc. 1981). Similarly, no gross or histopathological lesions were observed in lymph nodes of rats after exposure to concentrations up to 8,000 ppm for 13 days (Dodd and Kintigh 1989). One intermediate-duration study reported a higher incidence of lymphoid hyperplasia in submandibular lymph nodes in male rats exposed to 8,000 ppm for 13 weeks; incidence of hemosiderosis in the spleen was non-significantly elevated in males at this concentration, but interpretation of results is unclear due to high background rate (Lington et al. 1997). However, other intermediate- and chronic-duration studies in rats did not report organ weight changes or gross or histopathological lesions in the spleen, thymus, or lymph nodes at exposures up to 8,000 ppm (Bevan et al. 1997b; Bird et al. 1997; Greenough et al. 1980). In mice exposed to MTBE for 18 months, absolute spleen weights were decreased in male and female mice at 8,000 ppm, but no treatment-related gross or histopathological lesions accompanied the decreased spleen weights (Bird et al. 1997). Furthermore, no treatment-related lesions were found in the lymph nodes, thymus, or bone marrow.

In 28-day studies in rats and mice, decreased absolute and relative spleen weights were found in both sexes of rats and in female, but not male, mice following exposure to 8,000 ppm; however, no histological examination of the spleen was performed, so adversity could not be determined (Bird et al. 1997).

No animal oral studies evaluating the function of the immune system were identified; several studies evaluated immune organ weight and/or histology. Oral administration of 1,428 mg/kg/day MTBE for 14 days significantly reduced absolute spleen weight and absolute and relative thymus weights in female rats, but not male rats; however, these findings were not associated with histopathological changes in either organ in either sex (Robinson et al. 1990). Similar results were obtained following 90 days of treatment with daily gavage doses of 100–1,200 mg/kg MTBE (Robinson et al. 1990). In a 4-week gavage study, rats given doses up to 1,750 mg/kg/day had no gross pathological changes in the bone marrow, mesenteric lymph nodes, mandibular lymph nodes, spleen, or thymus (Amoco 1992). This treatment did not produce microscopic histopathological changes in all tissues examined (i.e., mesenteric lymph nodes, spleen, or thymus). In intermediate-duration drinking water studies, no changes in spleen or thymus weight or histology were reported in rats at doses up to 1,153 mg/kg/day for 13 weeks or 384 mg/kg/day for 6 months (Bermudez et al. 2012).

In a 104-week study, in which male and female rats were given gavage doses of 250 or 1,000 mg/kg/day, 4 days/week, an increased incidence of dysplastic proliferation of lymphoreticular tissues (hyperplastic lymphoid tissues, at various body sites, consisting of atypical lymphoid cells, usually lympho-immunoblasts) was observed in females at both doses (Belpoggi et al. 1995, 1997). The increase was greater at the low dose than at the high dose. Since dose-related increased incidences of lymphomas and leukemia were observed in the female rats (see Section 2.19), more of the dysplastic proliferation lesions might have developed into the lymphomas and leukemias in the high-dose female, suggesting that the dysplastic proliferation represents a preneoplastic lesion. No histopathological lesions were found in the spleen or thymus. In a 1-year drinking water study, no changes in spleen or thymus weight or histology were reported in male or female rats at doses up to 384 or 1,119 mg/kg/day, respectively (Bermudez et al. 2012). Similarly, no exposure-related changes in spleen weight or spleen, thymus, or lymph node histology were observed in male or female rats at drinking water doses up to 330 or 1,042 mg/kg/day, respectively, for 2 years (Dodd et al. 2013).

In a dermal sensitization test, guinea pigs received an initial intradermal injection of 0.5 mL of a 1% MTBE solution, followed by intradermal injection of 0.1 mL every other day for 3 weeks for a total of 10 injections (ARCO 1980). Two weeks after the 10^{th} injection, a challenge dose of 0.05 mL was injected. The injection sites were evaluated at 24 and 48 hours after treatment and scored for erythema, edema, and color. MTBE produced no significant increase in response to the challenge compared with the initial sensitizing or inducing injection.

2.15 NEUROLOGICAL

Effects consistent with transient CNS depression have been reported in humans exposed to MTBE in fuel or via MTBE therapy for gallstone dissolution, including headache, nausea or vomiting, dizziness, drowsiness, confusion, and a feeling of spaciness or disorientation. No subjective symptoms or alterations in performance on neurobehavioral tests were observed in volunteers following acute-duration exposure to low air levels of MTBE (≤50 ppm). In laboratory animals, MTBE is a CNS depressant following high-concentration inhalation exposure or high-dose gavage. Effects are transient, generally subsiding within hours of exposure, and do not increase in severity with duration of study. Exposure to MTBE via drinking water, as opposed to bolus gavage doses, does not appear to cause CNS depressive effects. There is no evidence of structural damage to the central or peripheral nervous systems via inhalation or oral exposure.

Following several anecdotal reports of neurological symptoms in the early 1990s associated with introduction of MTBE into gasoline during the oxyfuel program in the United States, the CDC conducted several studies evaluating potential associations between MTBE exposure and neurological symptoms (see Table 2-1). Several of these studies report an increase in self-reported neurological symptoms (headache, nausea or vomiting, dizziness, or spaciness) during the oxyfuel program in workers from Alaska with occupational exposure to gasoline containing MTBE (e.g., taxi drivers; policemen, toll booth workers, and parking garage attendants exposed to automobile exhaust; health-care workers who travelled routinely in cars), compared either with individuals with low level exposure (e.g., noncommuter students) or workers exposed to gasoline that did not contain MTBE (Alaska DHSS 1992a, 1992b; Moolenaar et al. 1994). A similar study in New York reported increased headache and dizziness in one group of individuals occupationally exposed to MTBE (policemen, toll booth workers, parking garage attendants), but not another group with higher exposure levels (automobile repair shop and service station attendants) (CDC 1993a), compared to individuals with low exposure (office workers, college students). However, these studies only provide suggestive evidence due to several limitations, including the lack of a referent group (Alaska DHSS 1992b only), small to modest subject numbers, lack of statistical analysis of incidence data, potential influence of widespread media coverage and public opposition to the oxyfuel program, and/or lack of consideration for other confounding factors (e.g., other exposures, including potential combustion products of MTBE). No evidence of increased neurological symptoms was observed in occupationally exposed workers, compared with unexposed referents, in similar studies

during the oxyfuel program conducted in Connecticut (CDC 1993b; White et al. 1995) or New Jersey (Mohr et al. 1994).

Results from population-based studies evaluating the potential association between self-reported neurological symptoms and MTBE exposure due to the oxyfuel program are mixed (see Table 2-1). In a population-based telephone survey, a statistically significant increase in risk of headache, but not nausea, dizziness, or spaciness, was observed in residents of metropolitan Milwaukee, Wisconsin, in which oxyfuel was used, compared to residents of non-metropolitan Wisconsin (MTBE-free gasoline); however, the risk was not increased for any neurological symptom for residents of metropolitan Chicago, Illinois (oxyfuel) compared to non-metropolitan Wisconsin (Wisconsin DHSS 1995). The results in Milwaukee may have been biased by knowledge of the oxyfuel program and potential health effects due to increased media coverage in Milwaukee because, in the telephone survey, familiarity with MTBE as a reformulated gasoline additive was reported by 54% of the Milwaukee residents, 23% of Chicago residents, and 40% of the Wisconsin residents. Additionally, non-metropolitan Wisconsin may not have been an appropriate referent group for the Chicago residents. One ecological study also reported a statistically significant increase in doctor's visits with diagnostic codes pertaining to neurological symptoms (headache, nausea, dizziness) and an increase in visits with diagnostic codes for spaciness in Philadelphia, Pennsylvania in 1997, during the 6th year of the oxyfuel program, compared to 1992, the year that the oxyfuel program was initiated, based on medical record review (Joseph and Weiner 2002). The study authors concluded that "some environmental factor" in the mid-1990s was associated with neurological findings and suggested that this factor is MTBE. However, this study has several major limitations, primarily a study design that does not allow for control for confounders (other chemical exposures, occupation, concomitant diseases, sex, age, race, etc.), that preclude meaningful conclusions. Another ecological study in Fairbanks and Anchorage, Alaska (Gordian et al. 1995) did not find an increased rate of treatment for headaches (based on medical insurance records) over the winter of 1992–1993 (during the oxyfuel program), compared with the number of claims during the two preceding winters (prior to the oxyfuel program). However, this study would miss mild and/or transient neurological effects associated with MTBE exposure that would not result in seeking medical attention.

In controlled human inhalation experiments, no increases in self-reported neurological symptoms (headache, difficulty in memory or concentration, depressed feelings, unusual tiredness, fatigue, drowsiness, tension, irritability, nervousness, dizziness, lightheadedness, mental fatigue, "fuzziness," or pain, stiffness, or numbness of the back, shoulders, neck, hands, or wrists) were observed in volunteers during or after exposure to MTBE at ≤1.7 ppm for 1 hour (Cain et al. 1996; Prah et al. 1994). Similarly,

no increases in headache, fatigue, feeling of sickness, dizziness, or intoxication were observed in volunteers following exposure to ≤50 ppm for 2 hours during light exercise (Johanson et al. 1995; Nihlén et al. 1998a). Additionally, neurobehavioral test evaluating symbol-digit substitution, switching attention, and mood scales did not show any differences in performance when measured 1 hour prior to exposure or during the last 15 minutes of a 1-hour exposure to ≤1.7 ppm (Cain et al. 1996; Prah et al. 1994).

A number of clinical studies of patients receiving MTBE therapy for gallstone dissolution have recorded neurological effects that are typical of transient CNS depression and have been described as drowsiness, mild sedation, somnolence, confusion, coma, vertigo, and dizziness (Allen et al. 1985a; Bonardi et al. 1986; Brandon et al. 1988; Di Padova et al. 1986; Eidsvoll et al. 1993; Kaye et al. 1990; McNulty et al. 1991; Murray et al. 1988; Neoptolemos et al. 1990; Ponchon et al. 1988; Saraya et al. 1990; Thistle et al. 1989; Tobio-Calo et al. 1992; van Sonnenberg et al. 1986; Williams et al. 1990).

Laboratory animal studies conducting neurobehavioral assessments have reported a number of alterations following inhalation exposure. An acute-duration rat study examined neurobehavior using a functional observation battery (FOB) and motor activity evaluation after a 6-hour exposure to MTBE (Daughtrey et al. 1997). Findings included abnormal gait (duck walk progressing to ataxia) in females at ≥4,000 ppm and males at 8,000 ppm. Other effects noted at 8,000 ppm in males included labored respiration pattern, decreased muscle tone, decreased performance on a treadmill, and increased hind limb splay. Other effects noted in females included decreased hind limb grip strength at ≥4,000 ppm and labored respiration and increased latency to rotate on the inclined screen at 8,000 ppm. These effects were seen at 1 hour after exposure, but not at 6 or 24 hours after exposure, consistent with transient CNS depression. The time course of changes in motor activity corresponded with the functional observation battery findings and supported the exposure-related CNS depression. No neurological effects were observed at 800 ppm for 6 hours.

A detailed neurobehavioral assessment was also conducted in rats in a preliminary 13-day range-finding study (Dodd and Kintigh 1989). Results of the detailed behavioral observations included ataxia, decreased startle and pain responses, and/or decreased muscle tone immediately after exposure for all males and most females in the 8,000-ppm group. No treatment-related behavioral alterations were found in any rats at 2,000 or 4,000 ppm when tested immediately following exposure or for the 8,000 ppm groups when retested one hour after the initial testing. However, during the exposure period, clinical signs of CNS depression were observed at ≥2,000 ppm (lowest concentration tested), including hypoactivity at ≥2,000 ppm and ataxia at ≥4,000 ppm. In a 13-week study, ataxia was observed after

exposure to 8,000 ppm during the first 4 weeks, but no exposure-related, toxicologically relevant changes were observed in FOB or motor activity testing after 4, 8, or 13 weeks of exposure (Daughtrey et al. 1997).

Another intermediate-duration study evaluated neurobehavioral effects (barbiturate-induced sleeping time, spontaneous motility, motor activity, righting reflex, grasping reflex on horizontal and vertical poles, and inclined screen test) in mice exposed to MTBE at 50,000 ppm for 10 minutes/day or 80,000 ppm for 5 or 10 minutes/day for 30 days (5 days/week). No exposure-related clinical signs or changes in neurobehavior were observed (Snamprogetti 1980).

Numerous other inhalation studies that did not rigorously test neurobehavior also reported clinical signs of CNS depression at high exposure levels; findings apparently did not increase in incidence or severity with duration of study. In acute-duration lethality studies, 4-hour exposures ≥18,892 ppm (lowest concentration tested) resulted in ataxia, loss of righting reflex, hyperpnea, incoordination, and prostration in rats (ARCO 1980). Repeat-exposure studies across all durations consistently reported transient signs of CNS depression in rats, mice, and rabbits, transient signs of CNS depression were consistently reported during 6-hour exposure periods, including hypoactivity at ≥2,000 ppm, blepharospasm and decreased startle response at ≥3,000 ppm, ataxia and drowsiness at ≥4,000 ppm, and prostration and decreased muscle tone at 8,000 ppm (Bevan et al. 1997a, 1997b; Bird et al. 1997; Dodd and Kintigh 1989; Lington et al. 1997; Moser et al. 1996; MTBE Committee 1990a; Vergnes and Chun 1994; Vergnes and Morabit 1989). In a 13-week intermediate-duration study, MTBE induced anesthesia was observed in rats exposed intermittently to 250–1,000 ppm (Greenough et al. 1980), but the lowest concentration resulting in anesthesia was not specified. The incidence and severity of effect did not increase with duration of study. However, one 9-day inhalation study did not report clinical signs of neurotoxicity in rats during or following 6-hour exposure periods to concentrations up to 3,000 ppm (Texaco Inc. 1981), and no clinical signs were observed in mice exposed to concentrations up to 8,000 ppm for 1 or 2 days (Vergnes and Kintigh 1993).

Consistent with inhalation studies, gavage studies reported clinical signs of CNS depression at high exposure levels. In general, onset of neurological signs was rapid, but they disappeared or were markedly reduced within 24 hours. In acute-duration studies in rats, findings included drowsiness at ≥400 mg/kg/day, lethargy and ataxia at ≥600 mg/kg/day, and transient anesthesia/sedation at ≥1,000 mg/kg/day; no clinical signs of neurotoxicity were observed at 40 mg/kg/day (ARCO 1980; de Peyster et al. 2003, 2014; Dong-mei et al. 2009; MTBE Committee 1990b; Robinson et al. 1990). In

mice, acute-duration gavage exposure resulted in ataxia and lethargy at 2,000 mg/kg/day, but not 1,000 mg/kg/day (de Peyster et al. 2008). In intermediate-duration studies, clinical signs of neurotoxicity included transient hypoactivity at ≥440 mg/kg/day and transient ataxia and anesthesia at ≥1,200 mg/kg/day; no clinical signs of neurotoxicity were observed at 90 mg/kg/day (Amoco 1992; Robinson et al. 1990). However, no behavioral changes were reported in rats following exposure to gavage doses up to 1,000 mg/kg/day, 4 days/week, for 104 weeks (Belpoggi et al. 1995, 1997).

In contrast to gavage studies, no clinical signs of neurotoxicity were observed in drinking water studies in male rats at doses up to 972 mg/kg/day for 13 weeks, 384 mg/kg/day for 6 or 12 months, or 330 mg/kg/day for 2 years, or female rats at doses up to 1,153 mg/kg/day for 13 weeks, 1,119 mg/kg/day for 12 months, or 1,042 mg/kg/day for 2 years (Bermudez et al. 2012; Dodd et al. 2013).

There is no evidence of structural damage to the central or peripheral nervous system following inhalation exposure to MTBE. No exposure-related, biologically relevant changes in brain weight were observed at concentrations up to 8,000 ppm for 13 or 28 days in rats or mice (Bird et al. 1997; Dodd and Kintigh 1989), or 13 weeks in rats (Daughtrey et al. 1997; Lington et al. 1997). No gross or histological lesions of the brain were observed in rats intermittently exposed to concentrations up to 3,000 ppm for 9 days (Texaco Inc. 1981). No gross and histological lesions of the brain, spinal cord, and/or sciatic nerve were observed at 8,000 ppm in rats following exposure for 13 weeks or 24 months (Bird et al. 1997; Daughtrey et al. 1997; Greenough et al. 1980; Lington et al. 1997), or in mice following exposure for 18 months (Bird et al. 1997).

There are sporadic findings of alterations in brain weight reported in oral studies. In 2-week gavage studies, inconsistent alterations in brain weight included a significant 7% decrease in absolute (but not relative) brain weight in male rats at 1,428 mg/kg/day, but not in male rats exposed to doses up to 1,071 mg/kg/day or female rats at doses up to 1,428 mg/kg/day (Robinson et al. 1990); a significant 6–12% increase in relative (but not absolute) brain weight was observed in male rats at ≥600 mg/kg/day (de Peyster et al. 2014); and no exposure-related changes were observed in male or female rats at doses up to 1,600 mg/kg/day (Dong-mei et al. 2009). In intermediate-duration studies, no exposure related changes in brain weight were observed in male or female rats at gavage or drinking water doses up to 1,750 or 1,153 mg/kg/day, respectively (Amoco 1992; Dong-mei et al. 2009; Robinson et al. 1990). In a 1-year drinking water study, relative, but not absolute, brain weights were significantly increased by 11–14% in all male exposure groups (≥29 mg/kg/day), but a dose-related trend was not observed. Additionally, no exposure-related changes in brain weight were observed at drinking water doses up to 1,042 mg/kg/day

for 2 years (Dodd et al. 2013). None of these brain weight findings are considered biologically relevant because they are inconsistent, associated with alterations in body weight, and/or are small in magnitude. Additionally, there is no evidence of histopathological changes in studies that evaluated nervous tissue histology. No histopathological lesions were observed in the central or peripheral nervous system of rats exposed to acute-duration gavage doses up to 1,428 mg/kg/day (Robinson et al. 1990), intermediate-duration gavage or drinking water doses up to 1,750 or 1,153 mg/kg/day, respectively (Amoco 1992; Bermudez et al. 2012), or chronic-duration gavage or drinking water doses up to 1,000 or 1,119 mg/kg/day, respectively (Belpoggi et al. 1995, 1997; Bermudez et al. 2012; Dodd et al. 2013).

A number of studies have been conducted in animals by intravenous and intraperitoneal injection, or by infusion into the gallbladder to determine possible side effects of MTBE therapy for gallstone dissolution. Effects noted in animals after administration of MTBE by other routes (Allen et al. 1985b; Dai et al. 1989; McGahan et al. 1988; Snamprogetti 1980; Tritapepe et al. 1989) are similar to those noted for inhalation and oral exposure.

Mechanisms of Neurotoxicity. The presence of MTBE and/or *tert*-butanol in the brain may account for the CNS toxicity of MTBE. A proposed mechanism for CNS depression at high MTBE exposure levels is interaction of MTBE and/or *tert*-butanol with the γ-aminobutyric acid$_A$ ($GABA_A$) receptor, which has been shown to be a target of several classes of well-known volatile anesthetics (e.g., ethers, alcohols) (Martin et al. 2002, 2004). Since potentiation of the $GABA_A$ receptor is correlated with induction of anesthesia for these compounds, the ability of MTBE and its metabolites to bind to the $GABA_A$ receptor was evaluated *in vitro* using a competitive binding assay with *tert*-butylbicycloorthobenzoate (TBOB), a known $GABA_A$ ligand *(*Martin et al. 2002). Both MTBE and *tert*-butanol resulted in concentration-dependent inhibition of TBOB. Further analysis showed that MTBE and *tert*-butanol enhanced $GABA_A$ receptor function in a concentration-related manner, resulting in increased chloride uptake in isolated synaptoneurosomes composed of pre- and postsynaptic membranes from adult rat cerebral cortex (Martin et al. 2004). MTBE was a more potent $GABA_A$ agonist than *tert*-butanol. Together, these studies suggest that MTBE and/or *tert*-butanol can directly interact with the $GABA_A$ receptor, resulting in decreased neuronal excitability via increased chloride conductance. The biological outcome of this interaction would be CNS depression, consistent with observed effects in laboratory animals.

An intracerebral exposure study in rats by Zheng et al. (2009) further supports that MTBE interacts with the $GABA_A$ receptor and associates this interaction with altered neurobehavior. In this study, rats exposed to MTBE via intracerebroventricular injection at doses of 50% MTBE (v/v in saline) or 100%

MTBE showed a dose-related impairment in spatial learning and memory (as assessed by the Morris water maze), compared with controls given saline injections only. Study authors attribute impaired learning and memory to observed increases in the $GABA_A$ receptor subunit α1 density in the hippocampus coupled with reduced phosphorylation of extracellular-signal regulated kinase 1/2 (ERK1/2) in the hippocampus, as the cascade initiated by ERK1/2 phosphorylation is considered essential for hippocampus-dependent learning and memory.

Neither rat brain succinate dehydrogenase nor acetylcholinesterase activity was affected by exposure for 15 weeks to 50–300 ppm, indicating these pathways do not contribute to MTBE neurotoxicity (Savolainen et al. 1985).

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans following exposure to MTBE. Based on animal inhalation studies, the male and female reproductive tract do not appear to be primary targets of MTBE toxicity. Based on animal oral studies, there is some evidence of male reproductive toxicity in rats; however, findings are inconsistent across studies and exposure durations. There is no evidence of impaired female fertility or damage to the female reproductive system following oral exposure.

Studies evaluating reproductive function following MTBE exposure include 1- and 2-generation inhalation studies in rats, an oral fertility study in male rats, and an ovarian function and *in vitro* fertilization study following acute-duration oral exposure in female rats. No evidence of reproductive toxicity was observed in 1- and 2-generation inhalation studies in rats (Bevan et al. 1997b; Biles et al. 1987). In the 1-generation study, evaluation of fertility, and reproductive systems, performance of male and female rats, and the reproductive development of offspring revealed that MTBE had no structural effect on the reproductive system or effect on reproductive performance of male and female rats following exposure to ≤2,500 ppm for 16–28 weeks (pre-mating through gestation of two litters) (Biles et al. 1987). In the 2-generation study, F0 and F1 adult exposure to concentrations up to 8,000 ppm for 14–19 weeks (premating through lactation) had no effect on F0 or F1 reproductive parameters including gestational length and mating, fertility, and gestational indices (Bevan et al. 1997b). No histological lesions were seen in the vagina, uterus, ovaries, testes, epididymides, seminal vesicles, or prostate. However, in the only oral exposure study evaluating male fertility, a 40% decrease in fertility was observed in male rats exposed to 1,600 ppm MTBE for 30 days and mated with unexposed females (Khalili et al. 2015). In a specialized acute-duration female reproductive study in rats, exposure to

520 mg/kg/day via drinking water for 2 weeks did not alter the percentage of female ovulations, number of oocytes per female, fragility of oocytes, *in vitro* fertilization rates of harvested oocytes (incubated with sperm from untreated rats), or number of penetrated sperm/oocyte (Berger and Horner 2003).

Additional inhalation studies evaluating reproductive endpoints, but not reproductive function, are limited to general toxicity studies that included measurement of weight and histopathological examination of male and female reproductive tissues. More data are available from oral studies that evaluated the potential of MBTE to induce alterations in reproductive hormones in male rats and mice, organ weight, and histological alterations of reproductive tissues in male and female rats and mice, and sperm alterations in rats and mice.

In inhalation studies, no changes in testicular weight or histology of male reproductive tissues (testes, epididymides, prostate, and/or seminal vesicles) were observed in rats following exposure to ≤8,000 ppm in acute-duration studies (Dodd and Kintigh 1989; Texaco Inc. 1981), intermediate-duration studies (Greenough et al. 1980; Lington et al. 1997), or chronic-duration studies (Bird et al. 1997). Similar to rats, no changes in testicular weight or histology of male reproductive tissues (testes, epididymides, prostate, and/or seminal vesicles) were observed in mice following chronic-duration inhalation exposure to ≤8,000 ppm (Bird et al. 1997).

In general, oral exposure studies in rats have not reported alterations in testes weights following acute-duration exposure to ≤1,428 mg/kg/day (Bermudez et al. 2012; de Peyster et al. 2014; Robinson et al. 1990), intermediate-duration exposure to ≤1,600 mg/kg/day (Bermudez et al. 2012; de Peyster et al. 2003; Gholami et al. 2015; Li et al. 2008; Robinson et al. 1990), or chronic-duration exposure to ≤1,000 mg/kg/day (Belpoggi et al. 1995, 1997; Dodd et al. 2013). Similarly, no alterations in testes weight were observed in mice exposed to 2,000 mg/kg/day every other day for 1 week (Billitti et al. 2005; de Peyster et al. 2008), or 1 mg/kg/day for 4 weeks (de Peyster et al. 2008). One study reported a decrease in testicular weight in rats exposed to ≥400 mg/kg/day for 2 weeks (Dong-mei et al. 2009), and one study reported an increase in relative testicular weight in rats exposed to 1,500 mg/kg/day for 28 days, but not 15 days (Williams et al. 2000). Inconsistent results were also found for histological alterations in the testes. In a 2-week study in rats exposed via gavage, histopathological examination of testicular tissues showed a compact and regular arrangement of cells in the seminiferous tubules of control animals; however, rats exposed to 1,600 mg/kg/day showed fewer compact cells in testicular tissue compared to controls (Li et al. 2008). A 4-week study by this group reported abnormally arranged cells and shedding of the seminiferous epithelium in rats exposed to ≥800 mg/kg/day (Li et al. 2008). A

third study in rats reported damage to seminiferous tubules, including pyknosis, decreased cell layers, increased cell distance, and vacuoles at doses of ≥400 mg/kg/day; observed lesions increased in severity with increasing dose (Gholami et al. 2015). An increase in the incidence of testicular Leydig cell tumors following chronic-duration exposure to 1,000 mg/kg/day has also been reported (Belpoggi et al. 1995, 1997). Other studies in rats reported no non-neoplastic alterations in the testes following acute-duration exposure to ≤1,428 mg/kg/day (Bermudez et al. 2012; Robinson et al. 1990), intermediate-duration exposure to ≤1,750 mg/kg/day (Amoco 1992; Bermudez et al. 2012; Robinson et al. 1990; Williams et al. 2000), or chronic-duration exposure to ≤1,000 mg/kg/day (Belpoggi et al. 1995, 1997; Bermudez et al. 2012; Dodd et al. 2013). A similar inconsistency was observed in mouse studies. Two studies reported no histological alterations in the testes in mice exposed to 2,000 mg/kg/day for 1 week (de Peyster et al. 2008), or 1 mg/kg/day for 4 weeks (de Peyster et al. 2008). Another 1-week study reported a significant increase in the mean number of seminiferous tubules with gross disruption (compared with controls) in the testes of mice exposed to 2,000 mg/kg/day (Billitti et al. 2005). However, there was no evidence of increased seminiferous epithelial vacuolization, multinucleated giant cells, marginated chromatin, or sloughing, and the study authors did not consider gross disruption alone to be a biologically relevant finding. No alterations in prostate, seminal vesicles, or epididymides weight or histology were observed in rats exposed to ≤1,500 mg/kg/day in intermediate-duration studies (Bermudez et al. 2012; Williams et al. 2000) or ≤1,000 mg/kg/day in chronic-duration studies (Belpoggi et al. 1995, 1997; Bermudez et al. 2012; Dodd et al. 2013).

A small number of studies evaluated potential effects on sperm. Dose-related decreases in the number of spermatocytes and spermatids were observed in rats exposed to ≥800 mg/kg/day for 30 days; no exposure-related changes were observed in Sertoli cells or spermatogonia (Gholami et al. 2015). In a 4-week study in rats, a significant dose-related increase in the percentage of abnormal sperm in the seminiferous tubules was observed in rats at ≥400 mg/kg/day (Li et al. 2008). A third study did not find any alterations in sperm count in rats exposed to 1 mg/kg/day for 4 weeks (de Peyster et al. 2008). In mice, gavage exposure ≤1,000 mg/kg/day for 3 weeks resulted in no effects on the frequency of germ cells in the testes (Ward et al. 1994).

Male reproductive toxicity associated with MTBE exposure may be attributable to direct toxic effect of MTBE on testicular cells, including Sertoli cells and germ cells. Decreased viability, increased plasma membrane damage, and increased ratio of necrotic cells were observed in cultured spermatogenic cells following *in vitro* exposure to MTBE for ≥12 hours; findings were associated with altered sperm morphology (Li and Han 2006; Li et al. 2007). A direct cytotoxic effect was also noted in Sertoli cells

following *in vitro* exposure to MTBE for ≥12 hours (Li et al. 2009). In both cell types, reactive oxygen species production and lipid peroxidation were enhanced with MTBE exposure, suggesting that observed cytotoxicity may be mediated via oxidative stress (Li et al. 2007, 2009).

Several studies have evaluated the effect of MTBE on reproductive hormone levels in male rats following oral exposure. Decreases in serum testosterone levels were observed at ≥800 mg/kg/day in 2-week studies (de Peyster et al. 2003; Li et al. 2008) or ≥800 mg/kg/day in 28–30-day studies (de Peyster et al. 2003; Khalili et al. 2015; Li et al. 2008; Williams et al. 2000); intermediate-duration studies to lower doses did not result in significant alterations (de Peyster et al. 2003). However, one study did not observe exposure-related changes in serum or testicular testosterone levels in rats at gavage doses as high as 1,200 mg/kg/day for 2 weeks (de Peyster et al. 2014). A decrease in dihydrotestosterone levels was also observed in rats exposed to 1,500 mg/kg/day for 28 days (Williams et al. 2000). Inconsistent results have been found in acute- and intermediate-duration studies evaluating serum luteinizing hormone (LH) levels, with one study reporting transient increases at ≥400 mg/kg/day for 2 weeks (Li et al. 2008), some studies reporting decreases at 1,200 mg/kg/day for 2 weeks (de Peyster et al. 2003) or 1,500 mg/kg/day for 28 days (Williams et al. 2000), and others reporting no alterations at doses of 800 or 1,600 mg/kg/day for 4 weeks (de Peyster et al. 2003; Li et al. 2008). Khalili et al. (2015) reported reduced serum LH levels after exposure to 800 mg/kg/day, but not 1,600 mg/kg/day, for 30 days. Most studies examining serum follicle-stimulating hormone (FSH) levels did not find alterations at doses as high as 1,600 mg/kg/day for 2 weeks, 28 days, or 30 days (de Peyster et al. 2003; Khalili et al. 2015; Williams et al. 2000); Li et al. (2008) reported an increase following exposure to ≥800 mg/kg/day for 2 weeks, but not following exposure to ≤1,600 for 4 weeks. For serum estradiol, one 2-week exposure study reported increased levels in rats exposed to 1,200 mg/kg/day (de Peyster et al. 2003), while two other studies reported no exposure-related changes at similar doses for 2 weeks or 28 days (de Peyster et al. 2014; Williams et al. 2000). Two studies evaluated serum prolactin levels; one study found a decrease in rats exposed to 1,500 mg/kg/day for 15 days, but not for 28 days (Williams et al. 2000), and the second study did not find alterations at 800 mg/kg/day for 4 weeks (de Peyster et al. 2008). A small number of studies evaluated reproductive hormone levels in male mice. No alterations in serum testosterone levels were observed in mice exposed to 2,000 mg/kg/day every other day for 1 week (Billitti et al. 2005; de Peyster et al. 2008), or to 1 mg/kg/day for 4 weeks (de Peyster et al. 2008). Collectively, the results of these studies in male rats suggest that oral exposure to high doses of MTBE can decrease serum testosterone levels but does not appear to affect other reproductive hormone levels.

Additional inhalation studies evaluating female reproductive endpoints, but not reproductive function, are limited to general toxicity studies evaluating female reproductive organs. No histological alterations in female reproductive tissues, including uterus, cervix, vagina, fallopian tubes, and/or mammary glands, were observed in rats following inhalation exposure to ≤8,000 ppm in acute-duration studies (Texaco Inc. 1981), intermediate-duration studies (Greenough et al. 1980; Lington et al. 1997), or chronic-duration studies (Bird et al. 1997). In female mice exposed to 8,000 ppm for 4 or 8 months, a 77–83% decrease in absolute and relative uterus weight; a 46–55% decrease in absolute and relative ovary weight; fewer uterine ducts and glands; reduced convolution of the tubular glands in the uterus, cervix, and vagina; reduced epithelial layers in the cervix and vagina; and deceased cell proliferation in the uterus were observed (Moser et al. 1998). No treatment-related gross or histopathological lesions were observed in the reproductive organs of female mice exposed to concentrations up to 8,000 ppm for 18 months (Bird et al. 1997).

Similar to inhalation, additional oral studies evaluating female reproductive endpoints, but not reproductive function, are limited to general toxicity studies evaluating female reproductive organs. No adverse effects on female reproductive organ weights or histology were reported in oral exposure studies. Gavage administration of ≤1,428 mg/kg/day MTBE in rats for 14 days (Robinson et al. 1990), ≤1,750 mg/kg/day in rats for 4 weeks (Amoco 1992), ≤1,200 mg/kg/day for 90 days (Robinson et al. 1990), or ≤1,000 ppm for 2 years (Belpoggi et al. 1995, 1997) did not result in alterations in ovarian weight or histological alterations in female reproductive tissues. In drinking water studies, no biologically significant changes in the weight and/or histopathology of the ovary or uterus were reported in rats at doses up to 1,153 mg/kg/day for 13 weeks (Bermudez et al. 2012), or 1,119 mg/kg/day for 1 year (Bermudez et al. 2012). Similarly, no histopathological changes were observed in the ovary, uterus, vagina, or mammary glands of rats exposed to drinking water doses up to 1,042 mg/kg/day for 2 years (Dodd et al. 2013). In mice, gavage exposure to ≤1,000 mg/kg/day for 3 weeks resulted in no effects on the frequency of germ cells in the ovaries (Ward et al. 1994).

2.17 DEVELOPMENTAL

Human studies were limited to a single cohort study evaluating the potential association between MTBE exposure during birth year and diagnosis of autism spectrum disorder (ASD). No studies were located regarding birth outcomes in humans following exposure to MTBE. In animals, developmental toxicity was only observed following inhalation exposure to high concentrations associated with frank maternal

toxicity. Available oral studies in animals are inadequate to comprehensively evaluate potential developmental effects following oral MTBE exposure.

In a case-control study, a significant positive correlation was reported between estimated MTBE exposure during the year of birth and risk of ASD diagnosis (see Table 2-1 for odds ratios) (Kalkbrenner et al. 2018). Further analyses indicated that the MTBE association with ASD diagnosis persisted after adjustment for other traffic pollution toxics, including diesel particulate matter and xylenes. However, no associations were observed between estimated MTBE exposure during the year of birth and continuous measures of autism-related traits from the Social Responsiveness Scale (SRS), or measures of autism severity using the Calibrated Severity Score (see Table 2-1 for odds ratios). The study authors acknowledge that the analysis was not adjusted for all measured air toxics, and that the sample size was inadequate for a comprehensive mixture analysis.

MTBE did not cause adverse developmental effects in rats and rabbits following inhalation exposure during gestation. Exposure of female rats to concentrations up to 2,500 ppm from GD 6 to 15 had no effect on percentage of resorption, percentage of live fetuses, mean fetal weights, crown-rump distances, incidence of external malformations, or incidence of fetal soft-tissue and fetal skeletal malformations (Conaway et al. 1985). Similarly, exposure of rabbits to concentrations up to 8,000 ppm from GDs 6 to 18 did not change the number of total nonviable fetuses (such as early or late resorptions or dead fetuses), viable implantations, percentage of pre- or post-implantation loss, fetal body weight, sex ratio, or incidence of fetal malformations (Bevan et al. 1997a).

Long-term exposure of male and female rats to concentrations up to 2,500 ppm during premating, mating, and gestational periods for a total of 16–28 weeks had no effect on pup viability, mean pup body weight, external malformations, gross pathology on GD 1, or gonad histology on GD 1 (Biles et al. 1987). A slight, but not statistically significant, increase in the incidence of dilated renal pelvis was observed at 250 and 1,000 ppm; however, incidence at 2,500 ppm was comparable to controls. Exposure of rats for 10 weeks prior to mating, 3 weeks during gestation, and 3 weeks during the postnatal period resulted in an approximate 10% reduction in F1 female and F2 male and female body weights at ≥3,000 ppm during lactation (Bevan et al. 1997b). The body weight effects occurred only at exposure levels associated with parental toxicity, including signs of clinical neurotoxicity at ≥3,000 ppm and decreased body weights at 8,000 ppm in both F0 and F1 adults. Parental exposure to MTBE did not affect F1 or F2 pup live birth and survival indices, litter size, or sex ratio. In a study designed to evaluate male fertility in rats, no exposure-related changes were observed in offspring number, sex ratio, or gross anomalies following

paternal exposure to gavage doses up to 1,600 mg/kg/day for 30 days prior to mating with unexposed females (Khalili et al. 2015).

MTBE has been found to produce developmental effects in mice at concentrations associated with maternal toxicity. Maternal inhalation exposure to concentrations of 250–2,500 ppm from GDs 6–15 had no effects on percentage of resorption, percentage of live fetuses, mean fetal weights, crown-rump distances, incidence of external malformations, or incidence of fetal soft-tissue malformations (Conaway et al. 1985). A slight, but not statistically significant, increase in fused sternebrae was observed in the offspring at ≥250 ppm, while no fused sternebrae were present in controls. In a similar study that exposed mice to higher concentrations (400–8,000 ppm), adverse developmental effects included a 7–21% decrease in fetal weights at ≥4,000 ppm, with increased post-implantation loss/litter, a 29% reduction in live fetuses, increased incidence of cleft palate, and four completely resorbed litters observed at 8,000 ppm (Bevan et al. 1997a). Decreased skeletal ossification was observed in several areas at ≥4,000 ppm (cervical centra, thoracic centra, caudal centra, skull plates/bones, forepaws, hindpaws, and sternebrae); however, overall litter incidence of skeletal malformations (including reduced ossification) was similar across exposure groups. Maternal toxicity consisted of reduced maternal body weight, reduced maternal weight gain, and reduced food consumption at 8,000 ppm, and increased incidence of treatment-related clinical signs of CNS depression at 4,000 and 8,000 ppm. The study authors speculated that the cleft palate could have resulted from maternal stress, which may be related to elevated endogenous maternal blood levels of corticosterone (see Section 2.13, Endocrine), which may produce cleft palate in susceptible strains of mice.

Alterations were observed in the male reproductive system of rats following prepubertal exposure via gavage for 21 days from postnatal day (PND) 35 to 56 (Zhu et al. 2022). Significantly changed endpoints, compared to controls, included a ≥50% decrease in serum testosterone at ≥300 mg/kg/day, increased apoptosis of Leydig cells at ≥600 mg/kg/day, and decreased number and size of Leydig cells at 1,200 mg/kg/day. No exposure-related changes were observed in body weight or testicular or epididymal weights; serum LH or FSH levels; or number of Sertoli cells. The study authors attributed decreased serum testosterone to Leydig cell toxicity. Companion studies in cultured Leydig cells showed inhibition of testosterone synthesis due to reactive oxygen species generation, mitophagy, and apoptosis (Zhu et al. 2022). In another study in mice, there was no evidence of altered male reproductive development following exposure to low doses (0.02– 2 mg/kg/day) via drinking water for 51 days from PND 25/26 to 76/77 (de Peyster et al. 2008). Reproductive endpoints examined included analysis of serum testosterone and estradiol, reproductive organ weights (testes, epididymides, seminal vesicles), and histopathological

examination of testes and associated epididymides. There were no clinical signs of toxicity and no significant changes to body weight or liver, kidney, brain, spleen, heart, or lung weight.

2.18 OTHER NONCANCER

No studies were located regarding other noncancer effects in humans following exposure to MTBE. A few oral studies in animals reported altered glucose homeostasis following exposure to MTBE; no alterations in glucose parameters were noted in available inhalation studies.

No exposure-related changes in serum glucose were observed in rats following intermittent inhalation exposure to concentrations up to 8,000 ppm for 13 weeks (Greenough et al. 1980; Lington et al. 1997). In a gavage study, serum glucose was significantly increased by 17% in female rats exposed to 1,428 mg/kg/day (Robinson et al. 1990). In contrast, serum glucose was significantly decreased by 13–24% in female rats exposed to gavage doses ≥300 mg/kg/day for 90 days (Robinson et al. 1990). No changes in serum glucose were observed in male rats following exposure up to 1,428 mg/kg/day for 14 days, or 1,200 mg/kg/day for 90 days (Robinson et al. 1990). Additionally, no exposure-related changes in fasting serum glucose or insulin levels or results of glucose or insulin challenge tests were observed in mice following exposure up to 100 mg/kg/day for 14 weeks (Tang et al. 2019).

One study in rats was specifically designed to evaluate zinc and glucose homeostasis in rats following a 3-month exposure to very low doses of MTBE (0.006, 0.03, or 0.15 mg/kg/day) (Saeedi et al. 2017). Endpoints examined included serum analysis of fasting blood glucose, zinc, calcium, and copper and ribonucleic acid (RNA) analysis of genes related to zinc and glucose homeostasis in pancreatic tissues. Fasting blood glucose was significantly increased by 3.3-fold at 0.15 mg/kg/day. A small, but statistically significant, reduction in serum calcium of 5% at 0.15 mg/kg/day was not considered biologically relevant due to its small magnitude. However, a statistically and biologically relevant increase in the copper/zinc ratio was observed at 0.15 mg/kg/day (12-fold increase compared to controls). C-reactive protein was also significantly higher in all MTBE exposed groups by ≥2-fold. Several genes involved in zinc and glucose homeostasis were significantly decreased in exposed groups, including insulin1, insulin2, MT1A, and SLC30A8. Due to the unknown adversity of these findings, limited endpoints evaluated, and lack of consistent findings regarding serum glucose effects following MTBE exposure, a NOAEL/LOAEL determination was not made for this study based on alterations in zinc and glucose homeostasis.

2.19 CANCER

No studies were located regarding cancer in humans following exposure to MTBE. Cancer bioassays in animals are available for rats and mice via inhalation exposure and for rats via oral exposure. Increased renal tubular cell tumors were reported in male rats and hepatocellular adenomas were reported in female mice following chronic-duration inhalation exposure to MTBE. Increased testicular Leydig cell tumors were reported in male rats and lymphomas and leukemia were reported in female rats following chronic-duration gavage exposure to MTBE.

In a 24-month rat inhalation study, neoplastic lesions were observed in both the kidneys and testes of males (Bird et al. 1997). The incidence of renal tubular cell tumors was increased in male rats at ≥3,000 ppm, with a significant increase in combined incidence of adenomas and carcinomas for the 3,000-ppm group compared to controls. Early mortality may have contributed to the lack of a significant increase at 8,000 ppm; this group was terminated at 82 weeks. No renal tubular cell tumors were found in males at 400 ppm. The incidence of testicular interstitial cell adenomas was also increased significantly at 3,000 and 8,000 ppm, compared to controls. However, observed incidences in the exposure groups were within historical controls and the control incidence was low compared to historical data. Since testicular tumors are the most common tumor in this strain of rat, and findings were within historical controls, these tumors were not considered exposure related. No exposure-related tumors were observed in female rats.

In an 18-month mouse inhalation study, a significantly increased incidence of hepatocellular adenoma was observed in female mice at 8,000 ppm (Bird et al. 1997). This finding was accompanied by hepatocellular hyperplasia. For males, there was a nonsignificant increase in hepatocellular carcinomas in the 8,000-ppm group compared to controls. However, re-evaluation and statistical analysis of the incidence of hepatocellular carcinomas, with exclusion of the animals dying before the first tumor occurred, showed a statistically significant increase in male mice at 8,000 ppm compared to controls (Bogen and Heilman 2015; CalEPA 1998). No hepatocellular hyperplasia was observed in males.

In tumor-promotion studies, there was no evidence of hepatic tumor promotion in mice initiated with the known mutagen, N-nitrosodiethylamine, 6 weeks prior to intermittent inhalation exposure to MTBE at concentrations of 8,000 ppm for 16 or 32 weeks (Moser et al. 1996).

In a 104-week gavage study in rats, a dose-related increase in the incidence of lymphomas and leukemia was observed in female rats at 250 or 1,000 mg/kg/day (Belpoggi et al. 1995, 1997). As noted in Section 2.13, there was also an increased incidence in dysplastic proliferation of lymphoreticular tissues in female rats, which was greater at 250 mg/kg/day than at 1,000 mg/kg/day, suggesting that the dysplastic proliferation was preneoplastic. An increase in uterine sarcomas was found in the females only at 250 mg/kg/day. In male rats, there was a statistically significant increased incidence of testicular Leydig cell tumors at 1,000 mg/kg/day. In a 2-year drinking water study in rats, no exposure-related increases in tumor incidences were observed in males exposed to doses up to 330 mg/kg/day or females exposed to doses up to 1,042 mg/kg/day (Dodd et al. 2013).

IARC has determined that MTBE was not classifiable as to its carcinogenicity in humans (IARC 1999). EPA (IRIS 1993) and HHS (NTP 2016) have not classified the potential for MTBE to cause cancer in humans.

Mechanisms of Carcinogenicity. As discussed below in Section 2.20, MTBE is not a strong mutagenic or clastogenic agent, and while there is evidence that MTBE (and/or its metabolites) can bind directly to DNA, evidence of DNA damage following exposure is inconsistent. Therefore, several comprehensive reviews have evaluated potential nongenotoxic mechanisms for carcinogenic action of MTBE.

Several reviews have evaluated potential mechanisms for renal tumors in male rats following inhalation exposure (Ahmed 2001; Bogen and Heilman 2015; Bus et al. 2022; McGregor 2006; Phillips et al. 2008). Collectively, these reviews propose that development of renal tubular cell tumors in male rats is predominantly, if not exclusively, mediated via the well-established α2u-globulin-mediated carcinogenic mode-of-action (MOA), which is not expected to occur in humans. This MOA is characterized by: (1) chemical binding to α2u-globulin; (2) accumulation of the bound protein in lysosomes, forming hyaline droplets; (3) renal tubule cell death; (4) compensatory cell proliferation; (5) population expansion; and (6) renal tubule cell adenoma and carcinoma formation. As discussed in Section 2.10 (Renal), some repeat-exposure studies indicate accumulation of α2u-globulin in hyaline droplets associated with nephropathy in male rats following MTBE exposure. Metabolism of MTBE to *tert*-butanol (Section 3.1.1) likely underlies or at least contributes to this MOA, as α2u-globulin accumulation, protein droplet accumulation, renal cell proliferation, and kidney tumors have been reported in male rats exposed to *tert*-butanol at roughly the same potency as MTBE. An alternate hypothesis for renal tumors is a genotoxic MOA due to metabolic formation of formaldehyde, which is formed in a 1:1 ratio to *tert*-butanol for each molecule of MTBE metabolized (Bogen and Heilman 2015). However, there are

two lines of evidence that do not support this hypothesis: (1) a genotoxic MOA based on formaldehyde production does not explain why renal tumors only occur in male rats, and (2) if formaldehyde production underlies renal tumors following MTBE exposure, carcinogenic potency of MTBE would be expected to be greater than *tert*-butanol instead of similar in potency.

Proposed mechanisms for Leydig testicular cell tumors in male rats following oral exposure to MTBE have also been reviewed (Ahmed 2001; Bogen and Heilman 2015; Bus et al. 2022; McGregor 2006; Phillips et al. 2008). Recognized MOAs for Leydig cell tumor formation include: (1) testosterone biosynthesis inhibition; (2) androgen receptor antagonism; (3) aromatase inhibition; (4) 5α-reductase inhibition; (5) dopamine agonism; and (6) peroxisome proliferation. As discussed in Section 2.16 (Reproductive), alterations in male hormonal signaling have been reported in some studies following oral MTBE exposure, namely decreases in testosterone. Decreases in testosterone may result from increased clearance of circulating testosterone due to induction of enzymes involved in testosterone metabolism, such as CYP enzymes, uridine diphosphate, and UDP-glucuronosyltransferase. In support, *in vitro* testosterone production was decreased by up to 50% in Leydig cells exposed to MTBE or its metabolite *tert*-butanol and decreased circulating testosterone levels in rats exposed to MTBE via gavage were associated with increased hepatic CYP enzymes and decreased testicular aromatase activity (de Peyster et al. 2003). However, while Leydig cell tumorigens are generally associated with elevated serum LH levels (Ahmed 2001; Clegg et al. 1997; McGregor 2006), available oral MTBE studies report inconsistent alterations in LH levels following exposure, with results showing transient increases (Li et al. 2008), decreases (de Peyster et al. 2003; Williams et al. 2000), or no alterations following exposure (de Peyster et al. 2003; Li et al. 2008). Additionally, i*n vitro* screening assays did not show competitive binding to androgen receptors, aromatase inhibition, or inhibition of steroidogenesis (de Peyster et al. 2014). There is no evidence supporting the other proposed MOAs (5α-reductase inhibition, dopamine agonism, peroxisome proliferation) following MTBE exposure. An alternate proposed mechanism is a nonspecific effect associated with a hormonally mediated cytotoxic stress response due to increased mortality from high incidence of chronic progressive nephropathy observed at dose levels associated with Leydig cell tumors (Bogen and Heilman 2015).

Several reviews have also evaluated potential mechanisms for hepatocellular adenomas in female mice (Ahmed 2001; Bogen and Heilman 2015; Bus et al. 2022; McGregor 2006; Phillips et al. 2008). One proposed mechanism for hepatic tumor formation is a nongenotoxic MOA mediated via hormonal disruption, such as antiestrogen-like effects proposed for gasoline-induced tumors. However, neither MTBE nor its metabolites, *tert*-butanol or formaldehyde, were found to competitively bind to the estrogen

receptor *in vitro* (Moser et al. 1998). Additionally, exposure to 8,000 ppm MTBE for 4 or 8 months via inhalation did not alter serum estrogen levels or alter the pattern or intensity of estrogen receptor immunoreactivity in female reproductive organs (Moser et al. 1998). Furthermore, a weight-of-evidence assessment reported that MTBE does not have direct endocrine activity based on studies of mammalian and fish models and *in vitro* screening assays (de Peyster and Mihaich 2014). Another proposed hormone-based mechanism suggests that increased estrogen catabolism may occur secondary to induction of cytochrome P450 metabolism by MTBE, resulting in decreased estrogen-mediated suppression of spontaneous liver tumors. It was noted that this mechanism would not be relevant for humans, as human liver tumors are not influenced by estrogen levels. Alternatively, a cytotoxic MOA has been proposed, in which high exposure levels at or exceeding the maximum tolerated dose (MTD) overwhelm cellular defenses resulting in cell death followed by increased cell proliferation in target organs. Lastly, as with renal tumors, a genotoxic MOA based on formaldehyde production has also been considered for MTBE hepatic tumors; however, detoxification of formaldehyde occurs more rapidly in isolated hepatocytes than metabolism of MTBE to formaldehyde (CalEPA 1998). Therefore, a genotoxic MOA based on metabolism to formaldehyde is not considered likely following MTBE exposure.

The mechanism by which MTBE produced leukemia in female rats (Belpoggi et al. 1995, 1997) is not known; however, the authors discussed the possibility that formaldehyde, a known metabolite of MTBE (see Section 3.1.3), is involved, since formaldehyde increased the incidence of lymphomas and leukemias in male and female rats in other studies from their laboratories. Evidence of decreased viability associated with oxidative stress, lipid peroxidation, damage to mitochondria and lysosomes, and glutathione depletion was reported in human blood lymphocytes following *in vitro* exposure to MTBE (Salimi et al. 2016).

2.20 GENOTOXICITY

The majority of available evidence indicates that MTBE is not mutagenic or clastogenic. There is evidence that MTBE (and/or its metabolites) can bind directly to DNA both *in vitro* and *in vivo*, but findings from studies evaluating evidence of DNA damage are inconsistent. In addition, MTBE was predicted to be nongenotoxic in an analysis by a structure activity relational expert system using results generated by the National Toxicology Program (NTP) for rodent carcinogenicity, *Salmonella* mutagenicity, induction of sister chromatid exchanges and chromosomal aberrations, and structural alerts for genotoxicity (Rosenkranz and Klopman 1991). Results of *in vitro* and *in vivo* genetic testing of MTBE are presented in Tables 2-5 and 2-6, respectively, and summarized below.

Table 2-5. Genotoxicity of Methyl *tert*-Butyl Ether (MTBE) *In Vitro*

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference
Prokaryotic organisms				
Salmonella typhimurium TA1535, *TA1537*, TA1538, TA98, TA100	Gene mutation	–	–	Cinelli et al. 1992
S. typhimurium TA98, TA100, TA104, TA1535	Gene mutation	–	–	Kado et al. 1998
S. typhimurium TA102	Gene mutation	–	–	McGregor et al. 2005
S. typhimurium TA98, TA100, YG1041, YG1042	Gene mutation	–	–	Vosahlikova et al. 2006
S. typhimurium TA102	Gene mutation	±	–	Williams-Hill et al. 1999
S. typhimurium TA98, TA100	Gene mutation	–	–	Zhou et al. 2000
Nonmammalian eukaryotic organisms				
Saccharomyces cerevisiae D4	Gene mutation	–	–	ARCO 1980
Mammalian cells				
Mouse lymphoma cells L51785	Gene mutation	+	–	ARCO 1980
Chinese hamster V79 fibroblasts	Gene mutation	–	–	Cinelli et al. 1992
Chinese hamster ovary cells	Sister chromatid exchange	±	–	ARCO 1980
Chinese hamster ovary cells	Chromosomal aberrations	–	–	ARCO 1980
NIH/3T3 murine fibroblast cells	Micronucleus induction	–	NT	Zhou et al. 2000
Human lymphocytes	DNA damage	NT	+	Chen et al. 2008
Human bronchial epithelial cells	DNA damage	NT	+	He et al. 2021
Rat primary hepatocytes	Unscheduled DNA synthesis	NA	–	Cinelli et al. 1992
Rat primary hepatocytes	Unscheduled DNA synthesis	NA	+	Zhou et al. 2000
Isolated DNA				
Calf thymus DNA (ctDNA)	DNA binding	NT	+	Ghasemi and Ahmadi 2014
Anti-parallel human telomeric G-quadruplex DNA (wtTel22)	DNA binding	NT	+	Ghasemi and Ahmadi 2014

– = negative result; + = positive result; ± = equivocal result; DNA = deoxyribonucleic acid; NA = not applicable; NT = not tested

Table 2-6. Genotoxicity of Methyl *tert*-Butyl Ether (MTBE) *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mammals			
Mouse (oral)	*Hrpt* mutant frequency in lymphocytes	–	Ward et al. 1994
Rat (oral)	Chromosomal aberrations	–	ARCO 1980
Rat (oral)	Chromosomal aberrations in bone marrow	+	Darwish and Mosallam 2019
Rats (inhalation)	Chromosomal aberration	–	McKee et al. 1997
Rat (inhalation)	Chromosomal aberrations	–	Vergnes and Morabit 1989
Mouse (oral)	Chromosomal aberrations	–	Ward et al. 1994
Mouse (oral)	Micronuclei in bone marrow	–	Vergnes and Kintigh 1993
Mouse (inhalation)	Micronuclei in bone marrow	–	McKee et al. 1997
Mouse (intraperitoneal)	Micronuclei in bone marrow	–	Kado et al. 1998
Mouse (oral)	DNA adducts (lung, liver, kidney)	+	Du et al. 2005
Mouse (oral)	DNA adducts (lung, liver, kidney)	+	Yuan et al. 2007
Rat (oral)	DNA damage (lymphocytes)	+	Alishahi et al. 2020
Mouse (inhalation)	Unscheduled DNA synthesis (hepatocytes)	–	McKee et al. 1997
Mouse (inhalation)	DNA repair (hepatocytes)	–	Vergnes and Chun 1994
Eukaryotic organisms			
Drosophila melanogaster	Sex-linked recessive lethal	–	McKee et al. 1997
D. melanogaster	Sex-linked recessive lethal	–	Sernau 1989

– = negative result, + = positive result; DNA = deoxyribonucleic acid

In vitro assays generally indicate that MTBE is not mutagenic. In bacterial cells, MTBE did not induce reverse mutation in *Salmonella typhimurium* in the majority of tested strains both with and without metabolic activation, including TA1535, TA1537, TA1538, TA98, TA100, TA104, YG1041, and YG1042 (Cinelli et al. 1992; Kado et al. 1998; Vosahlikova et al. 2006; Zhou et al. 2000). Reported findings for *S. typhimurium* strain TA102 were inconsistent, with McGregor et al. (2005) reporting a lack of mutagenic and Williams-Hill et al. (1999) reporting weakly mutagenic effects in the presence of metabolic activation only. However, the number of revertants/place induced by MTBE in the study by Williams-Hill et al. (1999) remained within 2-fold of control values (~1.7-fold induction at the highest tested concentration; estimated from graphically presented data); therefore, findings are considered inconclusive. In nonmammalian eukaryotic cells, MTBE was not mutagenic in *Saccharomyces cerevisiae* D4 with or without metabolic activation (ARCO 1980). In mouse cells, MTBE induced forward mutations in mouse lymphoma L5178Y tk^+/tk^- cells in the presence, but not in the absence, of metabolic activation (ARCO 1980). The observed mutation is not attributable to the metabolite *tert*-butanol, which

did not induce forward mutations in the L5178Y tk^+/tk^- mouse lymphoma cell assay with or without metabolic activation (McGregor et al. 1988). In hamster cells, MTBE did not induce gene mutation in Chinese hamster V79 fibroblasts (Cinelli et al. 1992).

Available assays indicate that MTBE is not mutagenic *in vivo*. MTBE did not induce sex-linked recessive mutations in *Drosophila melanogaster* (McKee et al. 1997; Sernau 1989). There was no evidence of a dose-related increase in hypoxanthine-guanine phosphoribosyl transferase (hprt) mutant frequency in spleen lymphocytes in mice treated with gavage doses up to 1,000 mg/kg/day for 3 weeks (Ward et al. 1994).

MTBE produced equivocal results for sister chromatid exchange in Chinese hamster ovary cells in the presence of metabolic activation; sister chromatid exchange was not induced without metabolic activation (ARCO 1980). There was no increase in chromosomal aberrations in Chinese hamster ovary cells with or without metabolic activation (ARCO 1980) or micronuclei in NIH/3T3 murine fibroblast cells without metabolic activation (Zhou et al. 2000). Most available data indicate that MTBE is not clastogenic *in vivo.* No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes were found in CD-1 mice bone marrow after oral, inhalation, or intraperitoneal exposure (Kado et al. 1998; McKee et al. 1997; Vergnes and Kintigh 1993). Chromosomal aberrations were not induced in male or female rats following inhalation exposure to MTBE at concentrations up to 8,000 ppm for 6 hours/day for 5 days, compared with unexposed controls (McKee et al. 1997; Vergnes and Morabit 1989). MTBE did not cause chromosomal aberrations in the bone marrow of rats following gavage exposure to 0.04, 0.13, or 0.4 mL/kg/day (30, 96, or 296 mg/kg/day) for 5 days (ARCO 1980). Similarly, no significant induction of chromosome aberrations was observed in spleen lymphocytes in mice following gavage exposure to MTBE doses up to 1,000 mg/kg/day for 3 weeks (Ward et al. 1994). However, a significant dose-related increase in chromosomal aberrations was observed in rat bone marrow cells following oral exposure to 800 or 1,600 mg/kg/day for 14 or 28 days (Darwish and Mosallam 2019).

In isolated human lymphocytes, MTBE induced single and double strand breaks and oxidative-based modifications (Chen et al. 2008). Similarly, MTBE induced DNA damage in isolated human bronchial epithelial cells (He et al. 2021). Unscheduled DNA synthesis was induced in primary rat hepatocytes following *in vitro* exposure to MTBE in one study (Zhou et al. 2000), but not in another (Cinelli et al. 1992).

In vivo, dose-related increases in DNA damage were observed in the lymphocytes of rats following oral exposure to MTBE doses of 5–20 mg/kg for 30 days (Alishahi et al. 2020). In mice, DNA repair and unscheduled DNA synthesis were not induced in hepatocytes following exposure to MTBE vapor at concentrations up to 8,000 ppm 6 hours/day for 1 or 2 days (McKee et al. 1997; Vergnes and Chun 1994). However, in the Vergnes and Chun (1994) study, the MTBE exposed mice were sacrificed 18 hours after the second exposure, which may have been too late to detect DNA repair. The positive control mice, which were treated intraperitoneally with N-nitrosodemethylamine at 10 mg/kg, were sacrificed 2 hours after the dose and showed increased DNA repair (Vergnes and Chun 1994).

MTBE was shown to directly interact (bind) with isolated human telomeric G-quadruplex DNA and calf thymus DNA (Ghasemi and Ahmadi 2014). Similarly, MTBE forms DNA adducts *in vivo.* Du et al. (2005) administered 0.95, 5.71, and 75.59 µg/kg via gavage to male Kunming mice and found DNA adducts in the lungs, livers, and kidneys, with levels peaking at 12 hours post-exposure. A follow-up study by Yuan et al. (2007), exposed male Kunming mice to 1.86, 13.9, 133, 990, and 11,190 µg/kg via gavage and found similar results. Yuan et al. (2007) also demonstrated a positive dose-response relationship between levels of DNA adducts and MTBE oral exposure.