CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- MTBE is readily absorbed following inhalation or oral exposure, and to a lesser extent following dermal exposure.
- Absorbed MTBE is initially widely distributed; the liver contains a large percentage of the initial body burden; smaller amounts are found in lungs, kidney, and testes.
- Most absorbed MTBE is rapidly metabolized; hepatic first-pass metabolism of MTBE is likely following oral exposure.
- Biotransformation of MTBE is generally similar between rats and humans and between sexes.
- MTBE metabolites are rapidly excreted, predominantly in the urine.

3.1.1 Absorption

Results from human studies that employed single inhalation exposures to MTBE at concentrations in the range of 0.5–75 ppm for time periods ranging from 30 minutes to 8 hours indicate that inhaled MTBE is rapidly absorbed from the respiratory tract (e.g., Amberg et al. 1999; Cain et al. 1996; EPA 2003a; Johanson et al. 1995; Lee et al. 2001; Nihlén et al. 1998b; Prah et al. 2004; Vainiotalo et al. 2007). For example, in a study of volunteers exposed at rest to airborne MTBE for 4 hours at 4 or 40 ppm, mean blood MTBE concentrations measured 1.9 and 6.7 μM, respectively, immediately following cessation of exposure (Amberg et al. 1999). Collectively, these studies demonstrate that MTBE plasma levels reach steady state as early as 30 minutes to 2 hours following initiation of exposure and that plasma levels rapidly decrease upon cessation of exposure. Pulmonary retention of inhaled MTBE has been estimated to range from 32 to 66% in humans (Lee et al. 2001; Nihlén et al. 1998b; Vainiotalo et al. 2007). Another study showed that following a 30-minute exposure to 0.5 ppm, the mean fraction of absorbed MTBE dose was 0.73, with blood levels of 0.9–2.5 μg/L at the end of exposure (EPA 2003a). The mean uptake residence time was 5.7 minutes.

The toxicokinetics of inhaled MTBE have been studied in rats (e.g., Amberg et al. 1999; Benson et al. 2001; Miller et al. 1997). Amberg et al. (1999) demonstrated similarities between humans and rats regarding absorption of inhaled MTBE. Following 4-hour exposures to MTBE at 4 ppm, blood MTBE levels measured 1.9 and 2.3 μM among humans and rats, respectively. Similar exposure at 40 ppm resulted in blood MTBE levels of 6.7 and 5.9 μM in humans and rats, respectively.

Benson et al. (2003) exposed male rats to ^{14}C-MTBE by 4-hour inhalation (nose-only) at 4, 40, or 400 ppm. MTBE equivalents were rapidly absorbed into the blood; peak blood concentrations were achieved prior to the end of the 4-hour exposure period and increased with increasing MTBE exposure level. In a rat study involving exposure to high levels of MTBE (400 or 8,000 ppm) for 6 hours, plasma concentrations of MTBE increased rapidly to apparent steady state within 2 hours following the end of a single exposure; peak plasma concentrations reached 14 and 493 μg/mL, respectively (Miller et al. 1997).

Blood MTBE levels were generally related to inhaled dose in rats repeatedly exposed to MTBE at 50, 100, or 300 ppm for 2–5 weeks (Savolainen et al. 1985). At the lower exposure concentrations, peak blood MTBE levels of 11 and 24 nmol/g, respectively (about 1.3 and 2.9 μg/mL, respectively) were reached at 6 weeks. At the highest exposure concentration, the peak blood level of 72 nmol/g (about 8.6 μg/mL) was reached at 15 weeks after decreasing from 66 nmol/g at week 6 to 55 nmol/g at week 10. Thus, MTBE blood levels continue to rise for a substantial amount of time during prolonged exposure, indicating a relatively long time for steady state to be reached following repeated exposures.

MTBE is readily absorbed from the gastrointestinal tract of humans. Among three male and three female volunteers administered ^{13}C-MTBE orally (in water) at 5 or 15 mg, MTBE blood concentrations averaged 0.10 and 0.69 μM, respectively, at 1-hour posttreatment and declined rapidly thereafter (Amberg et al. 2001). In another study of 14 volunteers administered 2.8 mg of MTBE in 250 mL of Gatorade®, a mean peak blood MTBE level of 0.17 μmol/L was reached at 15 minutes posttreatment and declined rapidly thereafter (Prah et al. 2004).

MTBE is also readily absorbed from the gastrointestinal tract of rats. Peak MTBE blood concentrations (average of nearly 20 μg/mL) were reached within 15 minutes posttreatment at 40 mg MTBE/kg (Miller et al. 1997). Blood MTBE levels declined rapidly thereafter. A peak blood concentration of 5.9 μg/mL MTBE was reached in 0.9 hours in rats given a single oral dose of MTBE at 0.379 mg/kg (Li et al. 1991).

Limited information regarding absorption of dermally applied MTBE indicates that absorption occurs to a lesser extent than absorption from the respiratory or gastrointestinal tract. Prah et al. (2004) immersed the hand and forearm of 14 male volunteers in a sealed container (mean concentration of 50 μg MTBE/mL) for 1 hour and took periodic blood samples during and following the exposure period. Dermal absorption peaked at 0.05 μmol/L at 65 minutes following the initiation of exposure. An estimated permeability coefficient (K_p) was 0.028 cm/hour. Another study evaluated dermal uptake in volunteers showering or

bathing with water containing 150 μg/L MTBE for 30 minutes using continuous breath analysis (EPA 2003b). Small increases in breath concentrations of MTBE indicated dermal absorption from bath water, with the mean uptake residence time of 21.2 minutes. No measurable increase in exhaled MTBE was observed following a 30-minute shower (EPA 2003b).

In rats exposed to MTBE via 6-hour dermal application at 40 or 400 mg/kg in isotonic saline under occlusive conditions, MTBE was detected in plasma within 10 minutes following the initiation of treatment and peak plasma MTBE concentration was achieved within 2–4 hours after dosing (Miller et al. 1997). Based on mass balance studies, MTBE absorption was estimated to have been approximately 16 and 34% of the administered radioactivity at the low and high dose, respectively. Schenk et al. (2018) reported an *in vitro* steady-state flux of 0.00194 g/cm^2/hour (19.4 g/m^2/hour) and a permeability coefficient of 0.000585 cm/hour for MTBE across pig skin.

3.1.2 Distribution

As MTBE is a small molecular weight, volatile, lipophilic compound, it is expected to readily cross biological membranes during its transport via the blood. Although no studies were located regarding distribution of MTBE in humans after inhalation, oral, or dermal exposure, there is no reason to expect that the tissues to which MTBE distributes would differ from those of animals (fatty tissue, brain, liver kidney). Furthermore, MTBE has been detected in fatty tissue and breast milk of patients who received MTBE via intracystic infusion for dissolution of gallstones (Leuschner et al. 1991).

Benson et al. (2003) evaluated the disposition of ^{14}C-MTBE equivalents in rats following 4-hour inhalation exposures at 4, 40, or 400 ppm. Immediately following cessation of exposure, MTBE equivalents were highest in the liver and accounted for approximately 10, 8, and 3% of the initial body burden for the 4, 40, and 400 ppm exposure groups, respectively. At 72 hours postexposure, MTBE equivalents in the liver were only 1, 0.8, and 0.4% of the initial body burden. At all exposure levels, other tissues (lung, kidney, heart, brain, and testes) each accounted for ≤ 2% of the initial body burden immediately following cessation of exposure and <0.2% of the initial body burden at 72 hours postexposure.

Levels of MTBE in samples of blood, cerebral hemispheres, and perirenal fat were monitored in Wistar rats sacrificed at selected times following repeated inhalation exposure to MTBE for up to 15 weeks at 50, 100, or 300 ppm (Savolainen et al. 1985). Blood MTBE levels were generally related to exposure

concentration. At the two lower exposure concentrations, peak blood MTBE levels of 11 and 24 nmol/g, respectively (about 1.3 and 2.9 μg/mL, respectively) were reached at 6 weeks. At the highest exposure concentration, the peak blood level of 72 nmol/g (about 8.6 μg/mL) were reached at 15 weeks. Brain MTBE levels peaked at 2–6 weeks at all exposure levels, with brain levels of 11, 28, and 87 nmol/g at 10, 100, and 300 ppm, respectively (about 1.3, 3.4, and 10 μg/mL, respectively). MTBE levels were considerably higher in perirenal fat than in blood and brain tissues at all exposure concentrations. At 2 weeks, the perirenal fat concentrations of MTBE were 184, 245, and 642 nmol/g at 50, 100, and 300 ppm, respectively. The perirenal concentration of MTBE declined to 81–92 nmol/g at 6–15 weeks in the 50-ppm exposed group but remained relatively unchanged at the higher exposure concentrations.

The disposition of radioactivity was evaluated in rats exposed to ^{14}C-MTBE by nose-only inhalation (400 or 8,000 ppm once for 6 hours or 400 ppm repeatedly for 15 days), dermal application (40 or 400 mg/kg for 6 hours), or intravenous injection (40 mg/kg) (Miller et al. 1997). Radioactivity from expired air, urine, and feces was determined for 48 hours following inhalation exposures and for 7 days following dermal and intravenous injection exposures. Determination of total radioactivity in tissues/carcass was performed at sacrifice.

Radioactivity recovered from tissue/carcass following single inhalation exposure was approximately 13% in the 400-ppm exposure group and 4% in the 8,000-ppm exposure group (Miller et al. 1997). The higher percentage of radioactivity in the tissues after the low dose may be due to shifts in metabolic and elimination pathways as enzyme systems become saturated at high doses (e.g., increased exhalation of unchanged MTBE at 8,000 ppm; see Section 3.1.4 for more details). In the rats repeatedly exposed at 400 ppm for 15 days, the percentage of total radioactivity recovered from tissues/carcass was approximately 11%. Percentages of radioactivity in tissues/carcass following dermal exposure at 40 or 400 mg/kg were 0.12 and 0.07%, respectively. Only 0.42% of the administered radioactivity was recovered in the tissues/carcass of rats administered ^{14}C-MTBE intravenously at 40 mg/kg. In both single and repeated exposure studies, mean radioactivity in various tissues (e.g., liver, kidneys, lungs, heart, brain, gonads, femur, perirenal fat, muscle) was very low (<1% of the total dose), indicating that MTBE or its metabolites do not accumulate in tissues after short-term exposure (MTBE Committee 1990a, 1990b).

In rats that received a single intraperitoneal dose of 232 mg/kg ^{14}C-MTBE, the total accumulation of radioactivity in tissues averaged 3.39, 1.94, and 1.14% of the administered dose at 15 minutes, 6 hours, and 24 hours after dosing, respectively (API 1984). At 15 minutes, radioactivity was found primarily in

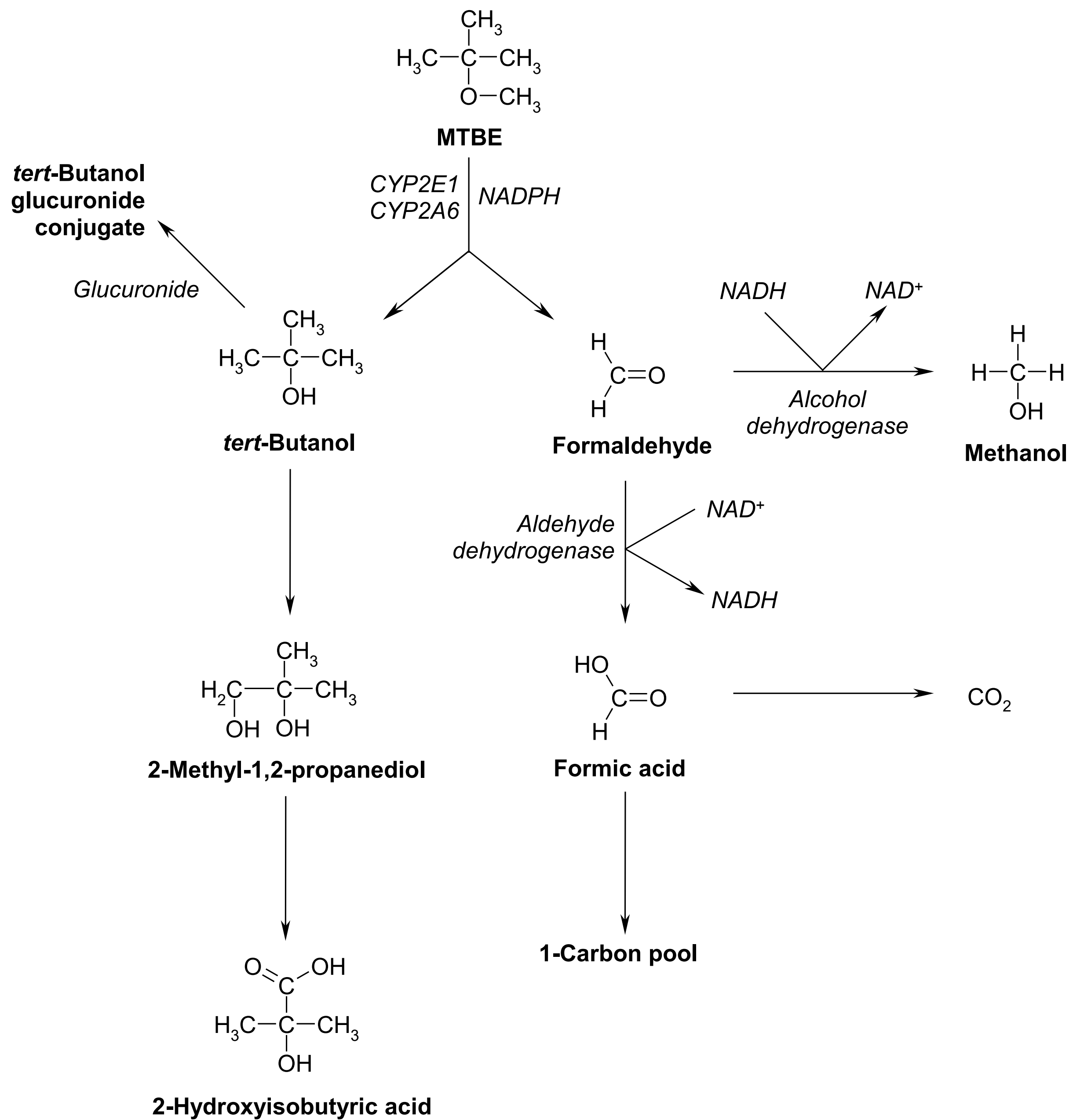
mesenteric fat (325 ppm in males, 138.5 ppm in females), liver (93.7 ppm in males, 68.7 ppm in females), and kidney (49.8 ppm in males, 19.4 ppm in females). At 6 hours, mesenteric fat radioactivity levels were more comparable to liver and kidney levels, and radioactivity levels declined in all three tissues at 24 hours. Radioactivity levels in liver were 65.5 ppm at 6 hours and 37.7 ppm at 24 hours. Levels in the kidney were 40 ppm at 6 hours and 28.5 ppm at 24 hours. Qualitative data indicated that radiolabeled formic acid and methanol were present in the liver and kidney, but quantification of the metabolites was not possible.

3.1.3 Metabolism

The proposed metabolic scheme for MTBE biotransformation presented in Figure 3-1 is based on available information from toxicokinetic studies of rats and humans and *in vitro* assays using selected rodent and human tissues. According to the scheme, MTBE rapidly undergoes CYP-dependent demethylation in the liver to form equimolar amounts of *tert*-butanol and formaldehyde. Additional rapid biotransformation in the liver includes the oxidation of *tert*-butanol to 2-methyl-1,2-propanediol and its subsequent oxidation to α-hydroxyisobutyric acid. Both 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid are urinary metabolites. *tert*-Butanol may undergo glucuronide conjugation and subsequent excretion in the urine. Formaldehyde is reduced to methanol or oxidized to formic acid and CO_2; the carbon atom may enter the physiological 1-carbon pool. The biotransformation of MTBE is generally similar among rats and humans and between sexes (Amberg et al. 1999).

Prah et al. (2004) indicated that the degree of metabolism in humans differs based on exposure route, with increased metabolism to *tert*-butanol following oral ingestion (compared to inhalation or dermal exposure) due to first-pass metabolism. However, Amberg et al. (2001) indicated that MTBE biotransformation observed following oral exposure in humans is similar to what they observed for inhalation exposure (Amberg et al. 1999), with no evidence of significant first-pass metabolism. Higher exposure levels in the studies by Amberg et al. (1999, 2001), compared to the study by Prah et al. (2004), may contribute to this discrepancy (15 versus 2.8 mg in oral studies, 40 versus 3.1 ppm in inhalation studies).

Figure 3-1. Proposed Metabolic Pathway for Methyl *tert*-Butyl Ether (MTBE) in Rats



Sources: API 1984; Brady et al. 1990; Dekant et al. 2001; MTBE Committee 1990a, 1990b, 1990d, 1991; Phillips et al. 2008; Snyder 1979

3.1.4 Excretion

Excretion of MTBE metabolites occurs primarily via the urine. Urinary metabolites of MTBE common to humans and rats are *tert*-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyric acid. Very little unchanged MTBE is excreted in the urine.

Amberg et al. (1999) designed studies to evaluate the biotransformation and excretory kinetics of inhaled MTBE in humans and rats. Six human subjects (three men and three women) were exposed to MTBE by 4-hour inhalation at 4 or 40 ppm (the two exposure levels were separated by a 4-week rest period). Urine was collected prior to MTBE exposure and for 72 hours postexposure. Urine samples taken prior to MTBE exposure at 4 ppm contained small amounts of *tert*-butanol (0.6 μmol) and 2-methyl-1,2-propanediol (0.2 μmol), and a relatively larger amount of 2-hydroxyisobutyric acid (42.7 μmol); MTBE was not detected. Following MTBE exposure, total urinary excretion was 0.3 μmol for MTBE, 3.4 μmol for *tert*-butanol, 16.5 μmol for 2-methyl-1,2-propanediol, and 78.9 μmol for 2-hydroxyisobutyric acid. Markedly greater increases in the urinary concentrations of MTBE and its metabolites were observed following MTBE exposure at 40 ppm. Pre-exposure urine samples from similarly exposed rats assigned to the 4 ppm MTBE exposure level contained 0.2 μmol *tert*-butanol, 0.1 μmol 2-methyl-1,2-propanediol, and 0.8 μmol 2-hydroxyisobutyric acid; MTBE was not detected. Following MTBE exposure, total urinary excretion was 0.3 μmol for *tert*-butanol, 0.7 μmol for 2-methyl-1,2-propanediol, and 1.7 μmol for 2-hydroxyisobutyric acid; MTBE was not detected. Markedly greater increases in the urinary concentrations of MTBE metabolites were observed following MTBE exposure at 40 ppm; urinary MTBE was not detected in the urine from rats of the 40-ppm exposure level.

The human and rat studies identified 2-hydroxyisobutyric acid as the major urinary metabolite of MTBE. All identified MTBE metabolites excreted in the urine were rapidly eliminated in both species. Approximately 35–69% of the MTBE retained at the end of the inhalation exposure period was eliminated in the urine of both species.

Several other human studies evaluated the toxicokinetics of MTBE following inhalation exposure (Johanson et al. 1995; Lee et al. 2001; Nihlén et al. 1998b; Vainiotalo et al. 2007). Urinary excretion of MTBE and *tert*-butanol represented only a very small portion of absorbed MTBE. These studies did not monitor the urinary metabolites, 2-methyl-1,2-propanediol and 2-hydroxyisobutyric acid. Vainiotalo et al. (2007) estimated that approximately 2.5% of absorbed MTBE was excreted in exhaled breath as *tert*-butanol during 48 hours postexposure.

Two studies evaluated exhalation of isotopically labeled MTBE in volunteers during and after 30-minute inhalation exposure to 0.5 ppm or dermal exposure to 150 μg/L (EPA 2003a, 2003b). In the inhalation study, the fraction of exhaled MTBEd12, compared to air concentrations, was 0.29 (EPA 2003a). The breath decay phase data, which fit a two-compartment model, estimated decay residence times of 3.8 and 61 minutes for the first and second compartments, respectively. In the dermal study, the fraction of exhaled MTBEd12, compared to water concentrations, was 0.00011 (EPA 2003b). The mean residence time for decay (assumed one-compartment model) was 41.5 minutes. However, Prah et al. (2004) indicated that excretion via all routes in humans follows a three-compartment model. Half-lives for the first, second, and third compartment for blood were calculated to be 14.9, 102.0, and 417.3 minutes, respectively, for oral exposure to 2.8 mg; 1.9, 59.0, and 313.7 minutes, respectively, for inhalation exposure to 3.1 ppm for 1 hour; and 5.5, 126.6, and 403.1 minutes, respectively, for dermal exposure to 51.3 μg/mL for 1 hour. For breath, first-, second-, and third-compartment half-lives following oral exposure were 13.0, 63.1, and 254.0 minutes, respectively. Half-lives in breath following inhalation and dermal exposure were only reported for the first and second compartment and were 30.2 and 265.7 minutes and 58.4 and 256.0 minutes, respectively.

Limited information regarding excretion of MTBE and its metabolites was provided in a study of 27 patients who received MTBE via intracystic infusion for dissolution of gallstones (Leuschner et al. 1991). Urine samples were collected before treatment, immediately after treatment, and for up to 18 hours after treatment; levels of MTBE, methanol, *tert*-butanol, formic acid, and formaldehyde were determined. Mean urinary levels of MTBE were about 0.018 mg/mL at 5 hours after treatment and <0.005 mg/mL at 12–18 hours. Mean urinary levels of *tert*-butanol were higher than levels of MTBE and were approximately 0.036 mg/mL at 5 hours and 0.03 mg/mL at 12–18 hours after treatment. Trace levels of methanol were detected in the urine from three patients; no formaldehyde or formic acid were detected in the urine.

Miller et al. (1997) evaluated the disposition of radioactivity in rats exposed to ^{14}C-MTBE by nose-only inhalation (40 or 400 ppm once for 6 hours or repeatedly for 15 days), dermal application (40 or 400 mg/kg for 6 hours), or intravenous injection (40 mg/kg). Radioactivity from expired air, urine, and feces was determined for 48 hours following inhalation exposures and for 7 days following dermal and intravenous injection exposures. For inhalation studies, recoveries of radioactivity were based on the total amount measured in the excreta, expired air, and tissues (48 hours postexposure) because the total radioactivity inhaled by the rats could not be determined. Following single inhalation exposure at

40 ppm, radioactivity in expired air, urine, and feces accounted for 21.2, 64.7, and 0.76%, respectively, of the total radioactivity recovered (Miller et al. 1997). The disposition of excreted radioactivity was similar for both single and repeated inhalation exposures at 40 ppm. A larger fraction of radioactivity was eliminated in exhaled breath of the 400-ppm exposure group. For all inhalation exposure scenarios, >90% of the exhaled radioactivity occurred during the first 6 hours postexposure. Following exposure at 40 ppm, MTBE accounted for 66–69% and *tert*-butanol accounted for 31–34% of the exhaled radioactivity. A higher proportion of MTBE was exhaled following exposure at 400 ppm. Initial urinary excretion was more rapid in the rats exposed at 40 ppm (27–38% at 6 hours versus 10% in the 400-ppm group).

For single dermal exposure at 40 mg/kg, radioactivity in expired air, urine, and feces accounted for 7.58, 6.33, and 0.25%, respectively, of the total dose during the 7-day post-exposure evaluation period; MTBE and *tert*-butanol were not detected (lower limit of quantitation: 0.1 μg/mL), indicating that the radioactivity in expired air was largely in the form of $^{14}CO_2$ (Miller et al. 1997). At the 400 mg/kg dose level, radioactivity in expired air, urine, and feces accounted for 18.9, 16.2, and 0.39%, respectively, of the total dose. MTBE and *tert*-butanol accounted for 96.7 and 3.3% of the radioactivity in expired air monitored for 9 hours posttreatment at 400 mg/kg.

Following intravenous injection at 40 mg/kg, radioactivity in expired air, urine, and feces accounted for 59.9, 34.9, and 2.2%, respectively, of the total dose over a 7-day post-exposure period (Miller et al. 1997). Approximately 91% of the radioactivity in exhaled air was recovered during the first 3 hours posttreatment and was identified as MTBE (97.4%), *tert*-butanol (1.0%), and $^{14}CO_2$ (1.6%).

Urinary metabolic profiles were assessed in the rats exposed to MTBE by inhalation (Miller et al. 1997). The urinary metabolites, 2-hydroxyisobutyric acid and 2-methyl-1,2-propanediol, accounted for 70 and 14% of the total urinary radioactivity. Two additional unidentified metabolites accounted for 15% of the radioactivity in the urine. MTBE and *tert*-butanol were not detected the urine of rats treated by inhalation, dermal, or intravenous exposure.

The biotransformation of absorbed MTBE to *tert*-butanol occurs rapidly, as demonstrated by rapid elimination of MTBE from blood and increasing blood levels of *tert*-butanol. Elimination half-lives of 36–156 minutes for MTBE have been reported in studies of volunteers exposed to MTBE by inhalation (Amberg et al. 1999; Cain et al. 1996; Nihlén et al. 1998b; Prah et al. 2004). Amberg et al. (2001) reported that elimination of MTBE from blood of volunteers administered MTBE orally occurred in three

phases. The average half-lives were 0.8, 1.8, and 8.1 hours following dosing with 5 mg MTBE and 0.7, 1.2, and 3.7 hours following dosing at 15 mg MTBE.

MTBE was rapidly eliminated from the blood of male rats following inhalation, oral, or dermal exposure (Miller et al. 1997). Calculated half-lives of elimination were 0.51–0.57 hours following single inhalation exposure at 400 or 8,000 ppm or repeated exposures at 400 ppm, 0.52 and 0.79 hours following oral dosing at 40 or 400 mg/kg, respectively, 2.3 and 1.8 hours following dermal exposure at 40 or 400 mg/kg, respectively, and 0.45 hours following intravenous injection at 40 mg/kg.

Benson et al. (2003) determined blood MTBE equivalent elimination half-times among male rats exposed to ^{14}C-MTBE for 4 hours at 4, 40, and 400 ppm, respectively. The half-time for the exposure at 400 ppm (30.1 hours) was significantly longer than half-times at 4 and 40 ppm (14.7 and 16.5 hours, respectively).

Only small amounts of unchanged MTBE are excreted in the urine. Most of the *tert*-butanol formed from MTBE metabolism is further oxidized to 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid; only small amounts of *tert*-butanol are excreted. *In vitro* assays using human microsomes have identified CYP2A6 as the major liver enzyme responsible for MTBE metabolism (Hong et al. 1997, 1999; Le Gal et al. 2001; Shamsipur et al. 2012). Results from rats exposed to MTBE by inhalation at 400 or 8,000 ppm or oral administration at 40 or 400 mg/kg indicate that MTBE metabolic pathways are saturated at the high-dose level, as evidenced by increased proportions of excreted MTBE and/or *tert*-butanol via the pulmonary route compared to the renal excretion route (Miller et al. 1997).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

A PBPK model for MTBE and *tert*-butanol in male Fischer 344 rats was developed (Borghoff et al. 1996). The model is based on chemical-specific parameters of solubility of MTBE and *tert*-butanol in blood and selected tissues (lung, liver, rapidly perfused tissue, slowly perfused tissue, fat, and kidney) and metabolic rate constants using vial equilibration and gas uptake techniques performed by Borghoff et al. (1996) and the pharmacokinetic data for male Fischer 344 rats obtained in the studies by the MTBE Committee (1990c, I990b, 1990d, 1990a) and for humans in the study by Cain et al. (1996). The model describes MTBE metabolism as occurring via two saturable pathways and predicts gas uptake data up to 2,000 ppm initial concentrations. The model predicted faster blood clearance of MTBE post exposure, thereby underpredicting blood MTBE levels after exposure in humans administered 1.7 ppm of MTBE for 1 hour (Cain et al. 1996). The model accurately predicts blood MTBE levels in rats when exposed via inhalation administration at 400 ppm MTBE for 6 hours, but underpredicts blood MTBE levels after 8,000 ppm exposure (MTBE Committee 1990a, 1990d). The model accurately predicted MTBE blood levels after oral (40 or 400 mg/kg) or intravenous exposure (40 mg/kg) (MTBE Committee 1990c, 1990b). The stomach was included as a compartment for oral exposure scenarios. Since the pharmacokinetics of *tert*-butanol appeared to be more complex than those of MTBE, Borghoff et al. (1996) indicated that additional experimental data on the distribution and elimination of *tert*-butanol were needed to refine the model.

Rao and Ginsberg (1997) expanded the model of Borghoff et al. (1996) to include compartments for the brain, since it is a known target of MTBE toxicity, and skin (to address dermal exposure). The model was validated using published rat and human data. Analysis of the model using animal data indicated that MTBE-induced CNS toxicity was principally attributable to the parent compound rather than the metabolite, *tert*-butanol. Rao and Ginsberg (1997) combined this model with an exposure model for inhalation and dermal exposure to evaluate pharmacokinetics associated with bathing and showering. The combined model results indicated that exposure to MTBE at 1 mg/L during showering or bathing would result in brain concentrations approximately 1,000-fold below levels resulting in CNS effects in animals.

Kim et al. (2007) developed a human PBPK model to predict the metabolism of MTBE and its metabolite, *tert*-butanol, following aggregate inhalation, oral, or dermal exposures. The model is an expansion of the six-compartment model of Borghoff et al. (1996) to include compartments for skin, stomach, and intestine. The model is based on measured blood MTBE and *tert*-butanol levels following controlled human exposures. Inhalation data were derived from 1-hour exposures of human subjects to MTBE at 3.1 ppm. Oral data were derived from the ingestion of 2.8 mg of MTBE (in Gatorade®).

Dermal data were collected following dermal application of 51.3 mg/L MTBE in tap water. The model underpredicted blood MTBE levels in females and underpredicted MTBE blood levels in males. The study authors noted a need for additional information to evaluate human variability such as age, sex, body mass index, and ethnicity.

Blancato et al. (2007) updated the models of Borghoff et al. (1996) and Rao and Ginsberg (1997). The updated model includes inputs for bolus dosing and rate ingestions, intraperitoneal injection, intramuscular injection, dermal exposure, and intravenous bolus and infusion dosing. The model includes compartments for gastrointestinal tract, spleen, liver, carcass, kidney, fat, slowly perfused tissue, rapidly perfused tissue, dermis, brain, and lung. The updated model was configured for humans and assigned values for MTBE metabolism using scaled metabolic parameters from rodents and extrapolated human microsomal values. Model predictions were compared to experimentally derived values. The model underpredicted MTBE blood levels in rats at a modeled lower dose (4.5 ppm) and overpredicted MTBE blood lead levels in rats at a higher dose (38.7 ppm). In humans, the predicted MTBE blood levels were within 3-fold of the reported mean MTBE blood levels. Blancato et al. (2007) performed an impact analysis of variability in metabolism for dose metrics considered of potential usefulness in evaluation of noncancer health risks in humans. For the impact analysis, dose metrics from inhalation exposures included peak MTBE in venous blood, area-under-the-curve (AUC) in venous blood at 24 hours, amount of MTBE metabolized in the liver at 24 hours, and peak *tert*-butanol concentration in venous blood. Modeling of selected scenarios allows for different dose-metric estimates at environmentally relevant exposure levels; results indicated that *tert*-butanol concentration in the blood varied to a much greater extent than MTBE when PBPK metabolic parameters were varied.

Licata et al. (2001) used a flow-limited human model similar in structure to that of Borghoff et al. (1996) in that it included compartments for lung, liver, rapidly perfused tissue, slowly perfused tissue, fat, and kidney. In the model, metabolic rate constants were measured *in vitro* using human liver microsomes and extrapolated to *in vivo* whole-body metabolism. Maximum metabolic rate was assumed to be proportional to body weight raised to the 0.75 power; the affinity constant was assumed to be equal to the *in* vitro value. Data on MTBE blood levels during and after 1-hour inhalation exposure to MTBE at 1.7 ppm and 4-hour exposures at 4 or 40 ppm were used to compare model predictions with measured results. The study authors stated that the model accurately predicted MTBE pharmacokinetics at the 40 and 1.7 ppm exposure levels, but underpredicted early time points at the 4-ppm exposure level. Variability analysis indicated that measured blood MTBE levels varied more than PBPK model-predicted blood levels based on metabolic parameters from *in vitro* human liver samples.

Leavens and Borghoff (2009) designed a PBPK model of MTBE and *tert*-butanol dosimetry in male rats based on binding to α2u-globulin, a mechanism postulated to be responsible for renal effects in rats exposed to MTBE. Borghoff et al. (2010) applied this model to evaluate MTBE and *tert*-butanol dosimetry in rats following a variety of exposure scenarios. The model applies only to the male rat and is not applicable to human risk assessment.

3.1.6 Animal-to-Human Extrapolations

The limited data in humans suggest some similarities in metabolism between rats and humans, that is, *tert*-butanol as a common metabolite. However, finding the rat urinary metabolites, 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid, in the urine of patients who receive MTBE therapy would provide a better basis for considering the rat a good model to predict the behavior of MTBE in the human body. The data on distribution and excretion are too limited to be compared. Data are not available in other laboratory animal species, which would allow for a determination of whether the disposition of MTBE is similar across species.

The available epidemiological data and laboratory animal data suggest that the toxicity of MTBE may be similar across species, with the possible exception of renal effects observed in male rats. Studies in rats suggest that α2u-globulin may play a role in the observed renal effects. α2u-Globulin-induced renal effects are not relevant to humans (Ahmed 2001; Bogen and Heilman 2015; McGregor 2006; Phillips et al. 2008).

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to MTBE are discussed in Section 5.7, Populations with Potentially High Exposures.

A susceptible population will exhibit a different or enhanced response to MTBE than will most persons exposed to the same level of MTBE in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons, it is expected that the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults.

No specific human populations that are unusually susceptible to the toxic effects of MTBE have been identified. EPA (1995a) and Fiedler et al. (2000) conducted studies to determine whether symptoms associated with MTBE were reported at an increased rate among subjects with known multiple chemical sensitivities (MCS) or chronic fatigue syndrome (CFS) compared to normal control individuals. No significant differences in self-reported respiratory or neurological symptoms were reported in MCS or CFS subjects, compared to healthy referents, while in situations in which gasoline containing MTBE was used (driving a car, gasoline stations) and not used (shopping malls, grocery stores, office buildings, parks) (EPA 1995a). Similarly, no significant differences were found between subjects with self-reported sensitivity (SRS) to MTBE and "non-sensitive" controls for self-reported respiratory or neurological symptoms, psychophysiological measures, or neurobehavioral tests of cognitive performance during 15-minute controlled exposures to clean air, gasoline, gasoline with 11% MTBE, or gasoline with 15% MTBE (Fiedler et al. 2000). Subjects with MCS reported a greater number of symptoms compared to controls when in circumstances more likely to be exposed to MTBE (riding in a car or at a gas station) (EPA 1995a) and subjects with SRS also reported a greater number of symptoms compared to controls when exposed to 15% MTBE (Fiedler et al. 2000). The authors concluded that these studies did not provide clear evidence to support an increase of symptoms occurred uniquely with MTBE exposure in sensitive subjects. It is also possible that some persons are or can become more chronically sensitive to MTBE as a result of prolonged low-level exposure, but no studies were located that specifically addressed this possibility.

Studies in rats (Brady et al. 1990; Snyder 1979) indicate that exposure to microsomal inducers of CYP2B1 and CYP2E1 enhances metabolism of MTBE, suggesting that people who are exposed to inducers of CYP2B1 (e.g., phenobarbital) or CYP2E1 (e.g., acetone, alcohol) may be more susceptible to toxic effects mediated via MTBE metabolites. However, because the toxicity of MTBE relative to the toxicities of its metabolites is unknown, the relative susceptibility cannot be determined.

Pharmacokinetic studies in rats indicated some differences between males and females in absorption and elimination kinetics (MTBE Committee 1990c, 1990b, 1990d, 1990a, 1991). In general, these studies indicated that female rats absorbed more MTBE than males after inhalation, oral, or dermal exposure, and eliminated it more quickly. Whether or not these relations would operate in humans is not known.

Inhalation studies in rats and mice indicate that developmental toxicity was only observed following exposure to high concentrations associated with frank maternal toxicity (Bevan et al. 1997a, 1997b; Conaway et al. 1985), suggesting that the developing animal is not uniquely susceptible to MTBE. No developmental effects were noted in rabbits, even at maternally toxic concentrations (Bevan et al. 1997a). Available oral studies in animals are inadequate to comprehensively evaluate potential developmental effects following oral MTBE exposure. However, one study suggests that the prepubertal male rat may be more susceptible to reproductive toxicity than the mature rat. Zhu et al. (2022) observed a significant decrease in serum testosterone following intermediate-duration exposure to ≥300 mg/kg/day from PND 35 to 56. However, in adult male rats, decreased serum testosterone levels were not observed following intermediate-duration exposures until doses ≥800 mg/kg/day (de Peyster et al. 2003; Khalili et al. 2015; Li et al. 2008; Williams et al. 2000).

Both male and female rats show increased incidence and severity of age-related chronic progressive nephropathy following chronic-duration exposure to MTBE. Since humans often develop age-related nephropathy, elderly people or people with pre-existing nephropathy may be more susceptible to the nephrotoxicity of MTBE.

Mice fed high-fat diets showed alterations in visceral white adipose tissue (increased weight and/or hypertrophy) and alterations in insulin sensitivity following oral exposure to low doses of MTBE (Tang et al. 2019). These effects were not observed in similarly exposed mice fed normal fat diets. Therefore, individuals who eat high-fat diets may be more susceptible to potential metabolic effects following exposure to MTBE.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to MTBE are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for MTBE from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by MTBE are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Much of absorbed MTBE is excreted unchanged in the expired air. Lower levels of its metabolite, *tert*-butanol, are found in expired air. MTBE and *tert*-butanol can be measured in the blood. A strong correlation was found between workroom air levels of MTBE in service stations and dealership garages and the difference in blood concentrations of MTBE between measurements preshift and postshift during an oxygenated fuel program in which MTBE (about 15% by volume) was added to gasoline to reduce emission levels of carbon monoxide (Moolenaar et al. 1994). In a similar study conducted in Stamford, Connecticut, personal breathing zone levels of MTBE were strongly correlated with blood levels of both MTBE and *tert*-butanol, although the breathing zone levels varied widely among the different garage locations, as well as within garages (White et al. 1995). For mechanics, TWA concentrations of personal breathing zone levels ranged from <0.03 to 12.04 ppm. Vainiotalo et al. (1998) reported a strong correlation between MTBE in the breathing zone of fuel tanker drivers during loading procedures and blood MTBE levels approximately 20 minutes later; there was no significant correlation between MTBE in the breathing zone and urinary *tert*-butanol. MTBE and *tert*-butanol blood levels were generally related to inhalation exposure concentrations in rats (Savolainen et al. 1985).

Pleil et al. (2007) performed a series of controlled human MTBE exposure tests and applied first-order kinetic calculations to estimate the ability of spot measurements of MTBE and *tert*-butanol to predict various exposure scenarios of previous exposures to MTBE. They determined that these biomarkers in both spot blood and breath samples could reliably reconstruct recent inhalation exposure. They also determined that the urinary metabolite, 2-hydroxyisobutyric acid, could serve as a biomarker of very recent exposure to MTBE.

Urinary metabolites found in rats exposed by the inhalation, oral, or dermal routes were identified as *tert*-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyric acid (Amberg et al. 1999; MTBE Committee 1990a, 1990b, 1991). Urinary metabolites found in humans following 4-hour inhalation exposures to MTBE included *tert*-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyric acid (Amberg et al. 1999. The major urinary metabolite in both rats and humans was 2-hydroxyisobutyric acid, although this compound was detected in high amounts in rats and humans prior to MTBE exposure (Amberg et al. 1999).

In rats exposed to ^{14}C-MTBE by inhalation for 6 hours (MTBE Committee 1990a), the rate of excretion of radioactivity via the lungs was rapid, with a total of 82% of the recovered radioactivity in expired air

excreted by 3 hours and 91–92% excreted by 6 hours (MTBE Committee 1990a). Urinary excretion of radioactivity was 96–98% complete by 36 hours after exposure. Similar rates of excretion of radioactivity were found in rats exposed by the oral route (MTBE Committee 1990b). Excretion was considerably slower in rats exposed dermally to MTBE. Half-lives for MTBE and *tert*-butanol for plasma clearance in rats exposed by the inhalation and oral routes were generally <1 or 2 hours (MTBE Committee 1990c, 1990d).

Based on available rat and human data, monitoring of expired air, blood, or urine for MTBE, *tert*-butanol, and/or 2-hydroxyisobutyric acid in humans could be used for determining very recent exposure to MTBE, but after exposure ceases, MTBE is rapidly eliminated from the body (Buckley et al. 1997). Additionally, MTBE metabolites are not unique to MTBE.

3.3.2 Biomarkers of Effect

MTBE exposure can lead to CNS depression characterized by ataxia, hypoactivity, drowsiness, anesthesia, duck-walk gait, decreased muscle tone, prostration, lack of startle response, and lack of righting reflex (see Section 2.15). MTBE exposure can induce hepatic microsomal enzymes (Brady et al. 1990; de Peyster et al. 2003; Moser et al. 1996) or lead to elevated levels of ALT, AST, or LDH (de Peyster et al. 2003; Robinson et al. 1990), and may increase (Greenough et al. 1980) or decrease BUN levels (Robinson et al. 1990). However, many ethers, alcohols, and other chemicals can lead to these effects or combination of effects; therefore, no known effect or combination of effects can be used as a biomarker to identify or quantify effects from exposure to MTBE specifically.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (ATSDR 1990) and for information on biomarkers for neurological effects see OTA (1990).

3.4 INTERACTIONS WITH OTHER CHEMICALS

Benson et al. (2003) exposed rats (nose-only) for 4 hours to MTBE vapor or unleaded gas containing MTBE vapor. Co-exposure to unleaded gas and MTBE resulted in lower tissue burdens of MTBE equivalents and enhanced the elimination of MTBE and its metabolites compared to MTBE exposure alone. The study authors suggested that the toxicity of MTBE alone could potentially be reduced by co-exposure to unleaded gas containing MTBE. However, in a subsequent 2-year rat study designed to

evaluate the carcinogenicity of unleaded gas alone or in combination with MTBE, both exposure scenarios resulted in similar production of renal adenomas and carcinomas (Benson et al. 2011).

Pretreatment of rats with phenobarbital or acetone enhanced the metabolism of MTBE to *tert*-butanol and formaldehyde in liver microsomes, by inducing CYP2B1 and CYP2E1, respectively (Brady et al. 1990; Snyder 1979). *In vitro* assays using human microsomes have identified CYP2A6 as the major liver enzyme responsible for MTBE metabolism (Hong et al. 1997, 1999; Le Gal et al. 2001; Shamsipur et al. 2012). Thus, acetone and phenobarbital, as well as other inducers of these enzymes, would be expected to enhance the metabolism of MTBE. Conversely, competitive metabolic inhibitors may slow the metabolism of MTBE. Whether alterations in metabolism of MTBE would lead to greater or lesser toxicity is not clear, because the toxicity of MTBE relative to the toxicities of its metabolites is not known. Pretreatment of rats with MTBE resulted in a 47-fold induction of liver microsomal pentoxyresorufin O-dealkylase, an activity associated with CYP2B1 (Brady et al. 1990). Thus, MTBE itself is an inducer of CYP2B1, which can lead to the enhanced metabolism and toxicity of other chemicals. Elovaara et al. (2007) designed a rat study to evaluate the effects of MTBE on liver toxicity and induction of CYP2E1 and CYP2B1 by known liver toxicants (ethanol, 13-*cis*-retinoic acid, acetaminophen, phenobarbital, pyrazole). MTBE did not appear to enhance the toxicity of these liver toxicants.