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 of the toxicology of methyl *tert*-butyl ether (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.

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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 first by route of exposure-inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure 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 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 end point 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 end points. 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.

The significance of the exposure levels shown in the LSE tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of MTBE are indicated in Tables 2-1 and 2-2, and Figures 2-l and 2-2.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for MTBE. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding death in humans following inhalation exposure to MTBE.

Information regarding death in animals was located for rats, mice, and rabbits. Acute inhalation 4-hour LC_{50} (lethal concentration, 50% kill) values in rats for 2 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 LC_{50} in mice following inhalation of MTBE for 10 minutes was determined to be 180,000 ppm (Snamprogetti 1980). The LT_{50} (time at which death occurs in 50% of exposed animals) in mice following inhalation exposure to 209,300 ppm MTBE was 5.6 minutes. No deaths occurred in male or female Fischer rats exposed to $\leq$ 8,000 ppm for 6 hours (Bioresearch Labs 1990d; Gill 1989). Intermittent inhalation exposure of Sprague-Dawley rats of both sexes to MTBE for 9 days to concentrations ranging from 100 to 3,000 ppm did not result in any treatment-related deaths (Biodynamics 1981). Intermittent exposure of rats for 5 days (Vergnes and Morabit 1989), rats and mice for 13 days (Dodd and Kintigh 1989), or mice for 1 or 2 days (Vergnes and Chun 1994; Vergnes and Kintigh 1993) to concentrations $\leq$8,000 ppm MTBE was not lethal. Similarly, no deaths occurred in pregnant Sprague-Dawley rats (Conaway et al. 1985), pregnant CD-l mice (Conaway et al. 1985; Tyl and Neeper-Bradley 1989), or pregnant New Zealand rabbits exposed intermittently to $\leq$ 8,000 ppm (Tyl 1989) during gestation.

Intermediate-duration exposure of rats (5-10 minutes per day, 5 days per week for 30 days) to 50,000 and 80,000 ppm MTBE caused no mortality (Snamprogetti 1980). However, in mice similarly exposed, death occurred in 1 of 30 mice exposed to 80,000 ppm for 10 minutes per day, 5 days per week for 30 days, but no mice died after exposure to 50,000 ppm for 10 minutes per day or to 80,000 ppm for 5 minutes per day. No mortality was observed in rats or mice exposed to $\leq$8,000 ppm MTBE 6 hours per day, 5 days per week for 4 or 5 weeks (Chun and Kintigh 1993). No rats died during intermittent exposure to $\leq$8,000 ppm MTBE for I3 weeks (Dodd and Kintigh 1989; Greenough et al. 1980) or to $\leq$2,500 ppm for 16-28 weeks (Biles et al. 1987).

In a 24-month inhalation study, increased mortality and decreased mean survival time occurred in male rats exposed intermittently to 3,000 and 8,000 ppm MTBE (Chun et al. 1992). These groups were sacrificed at weeks 97 and 82, respectively. A slight increase in mortality and a significantly decreased

mean survival time also occurred in the male rats exposed to 400 ppm (lowest concentration tested). This group was maintained until the scheduled sacrifice. Chronic progressive nephropathy was the main cause of death. Although female rats had slightly more deaths from chronic progressive nephropathy, the increased mortality was not statistically significant. As discussed for Renal Effects in Section 2.2.1.2, 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 α_{2u}-globulin or another unknown protein unique to male rats. In mice exposed to the same concentrations of MTBE for 18 months, increased mortality and decreased mean survival time, due to a slightly increased incidence of obstructive uropathy, occurred in males at 8,000 ppm (Burleigh-Flayer et al. 1992). No increased mortality occurred in female mice. The LC_{50} values in rats and mice, the LT_{50} value in mice, and the concentrations associated with increased mortality in male mice in the chronicduration studies are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.2. Systemic Effects

Studies regarding the systemic effects in humans and animals after inhalation exposure to MTBE are discussed below. The highest NOAEL values and the LOAEL values for each systemic effect from all reliable studies for each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. Since MTBE has been used as a gasoline additive to increase octane levels and more recently to reduce the levels of carbon monoxide emissions; some humans have reported respiratory symptoms while adding gasoline to automobile tanks or while driving. Such anecdotal reports have prompted studies by the Centers for Disease Control and Prevention (CDC). Preliminary investigations were conducted by the state of Alaska, with the assistance of CDC (Belier and Middaugh 1992; Chandler and Middaugh 1992). To determine whether symptoms were occurring, whether symptoms occurred in a consistent pattern, and whether symptoms could be related to exposure to oxyfuel in Fairbanks and Anchorage, Alaska, people believed to travel routinely in motor vehicles (taxi cab drivers and health-care workers) were asked if they experienced an increase in health complaints during 1 or 2 months since oxyfuel programs were initiated. In the study in Fairbanks (Beller and Middaugh 1992), university students were used as controls. No appropriate control group was identified in Anchorage; university students did not live in dormitories on a campus as isolated as in Fairbanks, and thus could not serve as a control group (Chandler and Middaugh 1992). In the Fairbanks study (Beller and Middaugh 1992), the percentage of persons who met the case definition (an 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;

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (NS)	4 hr				33370 (4-hr LC_{50})	ARCO 1980
2	Mouse (Swiss Albino)	3-12 min				209300 M (LT_{50}=5.6 min)	Snamprogetti 1980
3	Mouse (Swiss Albino)	10 min				180000 M (10-min LC_{50})	Snamprogetti 1980
Systemic							
4	Human	1 hr	Resp	1.7			Cain et al. 1994
5	Human	1 hr	Resp	1.39			Prah et al. 1994
6	Rat (NS)	4 hr	Resp			18892 (nasal discharge, tachypnea, respiration slowing until death)	ARCO 1980

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
7	Rat (Sprague-Dawley)	9 d 5 d/wk 6 hr/d	Resp		1000	(chronic inflammation of nasal mucosa and trachea)			Biodynamics 1981
			Cardio	3000					
			Gastro	3000					
			Hemato	3000					
			Musc/skel	3000					
			Hepatic	1000	3000	(increased relative liver weight)			
			Renal	3000					
			Endocr	3000					
			Bd Wt	3000					
8	Rat (Fischer 344)	2 wk 6 hr/d 5 d/wk	Bd Wt	3000			8000	(24-49% decreased body weight gain)	Chun and Kintigh 1993
9	Rat (Fischer 344)	5 d 6 hr/d	Renal	400 M 8000 F	3000 M	(increased proliferation of epithelial cells in the proximal convoluted tubules)			Chun and Kintigh 1993
10	Rat (Sprague-Dawley)	10 d Gd 6-15 6 hr/d	Hepatic	2500 F					Conaway et al. 1985
			Bd Wt	2500 F					

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
11	Rat (Fischer 344)	13 d 6 hr/d	Resp	8000			Dodd and Kintigh 1989
			Hepatic	2000	4000 (10-13% increased relative liver weight in both sexes; 14% increased absolute liver weight in females only)		
			Renal	2000 M	4000 M (8% increased relative kidney weight)		
				4000 F	8000 F (8% increased absolute kidney weight)		
			Bd Wt	2000 M		4000 M (65% decreased body weight gain during days 1-3)	
				4000 F		8000 F (36% decreased body weight gain during days 1-7)	
12	Rat (Fischer 344)	1-14 d 5 d/wk 6 hr/d	Bd Wt	800 M	4000 M (12% decrease in body weight gain)	8000 (decrease in body weight gain, 60% in males, 41% in females)	Dodd and Kintigh 1989
				4000 F			
13	Rat (Fischer 344)	6 hr	Bd Wt	8000			Gill 1989
14	Mouse (CD-1)	5 d 6 hr/d ≤ 23 exp	Hepatic	3000 F 8000 M	8000 F (increased proliferation of hepatocytes)		Chun and Kintigh 1993
15	Mouse (CD-1)	10 d Gd 6-15 6 hr/d	Hepatic	2500 F			Conaway et al. 1985
			Bd Wt	2500 F			

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
16	Mouse (CD-1)	13 d 6 hr/d	Hepatic	4000 M	8000 M (13% increased relative liver weight)		Dodd and Kintigh 1989
					2000 F (13% increased relative liver weight and 16% increased absolute liver weight)		
			Bd Wt	8000			
17	Mouse (Swiss-Webster)	1 hr	Resp		4604 M (RD_{50} [50% decrease in respiratory rate] indicative of sensory irritation)		Tepper et al. 1994
18	Mouse (CD-1)	10 d Gd 6-15 6 hr/d	Resp	4000 F		8000 F (labored breathing)	Tyl and Neeper-Bradley 1989
			Hepatic	8000 F			
			Bd Wt	4000 F	8000 F (reduction in body weight and weight gain by 7-16% accompanied by reduction in food consumption)		
19	Mouse (CD-1)	1-2 d 6 hr/d	Bd Wt	8000			Vergnes and Kintigh 1993
20	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d	Resp	8000 F			Tyl 1989
			Hepatic	4000 F	8000 F (increased relative liver weight)		
			Bd Wt	1000 F	4000 F (reduction in maternal weight gain and reduced food consumption)		

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
Immunological/Lymphoreticular									
21	Human	1 hr		1.7					Cain et al. 1994
22	Rat (Sprague-Dawley)	9 d 5 d/wk 6 hr/d		3000					Biodynamics 1981
23	Rat (Fischer 344)	13 d 6 hr/d		8000					Dodd and Kintigh 1989
Neurological									
24	Human	1 hr		1.7					Cain et al. 1994
25	Human	1 hr		1.39					Prah et al. 1994
26	Rat (NS)	4 hr					18892	(incoordination, prostration, and loss of righting reflex)	ARCO 1980
27	Rat (Sprague-Dawley)	9 d 5 d/wk 6 hr/d		3000					Biodynamics 1981
28	Rat (Fischer 344)	6 hr		400			8000	(ataxia and drowsiness)	Bioresearch Labs 1990d
29	Rat (Fischer 344)	≤ 28 d 5 d/wk 6 hr/d		400			3000	(ataxia, hypoactivity, lack of startle response, blepharospasm)	Chun and Kintigh 1993
30	Rat (Fischer 344)	13 d 6 hr/d			2000	(hypoactivity)	4000	(hypoactivity, ataxia)	Dodd and Kintigh 1989
31	Rat (Fischer 344)	1-14 d 5 d/wk 6 hr/d		800	4000	(hypoactivity)	8000	(ataxia)	Dodd and Kintigh 1989

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
32	Rat (Fischer 344)	6 hr		800[b]			4000	(dose-related increased incidence/severity ataxia and duck walk gait)	Gill 1989
33	Mouse (CD-1)	≤ 23 exp 5 d/wk 6 hr/d		400			3000	(ataxia, hypoactivity, lack of startle response)	Chun and Kintigh 1993
34	Mouse (CD-1)	13 d 6 hr/d			2000	(hypoactivity)	4000	(hypoactivity and ataxia)	Dodd and Kintigh 1989
35	Mouse (CD-1)	10 d Gd 6-15 6 hr/d		1000 F			4000 F	(hypoactivity, ataxia)	Tyl and Neeper-Bradley 1989
36	Mouse (CD-1)	2 d 6 hr/d		400	3000	(hypoactivity, lack of startle response)	8000	(hypoactivity, abdominal breathing, ataxia, prostration)	Vergnes and Chun 1994
37	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d		4000 F			8000 F	(ataxia, hypoactivity)	Tyl 1989
	Reproductive								
38	Rat (Sprague-Dawley)	9 d 5 d/wk 6 hr/d		3000					Biodynamics 1981
39	Rat (Sprague-Dawley)	10 d Gd 6-15 6 hr/d		2500 F					Conaway et al. 1985
40	Mouse (CD-1)	10 d Gd 6-15 6 hr/d		2500 F					Conaway et al. 1985
41	Mouse (CD-1)	13 d 6 hr/d		8000 M					Dodd and Kintigh 1989

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL				Reference
					Less serious (ppm)		Serious (ppm)		
42	Mouse (CD-1)	10 d Gd 6-15 6 hr/d		8000 F					Tyl and Neeper-Bradley 1989
43	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d		8000 F					Tyl 1989
	Developmental								
44	Rat (Sprague-Dawley)	10 d Gd 6-15 6 hr/d		2500					Conaway et al. 1985
45	Mouse (CD-1)	10 d Gd 6-15 6 hr/d		2500					Conaway et al. 1985
46	Mouse (CD-1)	10 d Gd 6-15 6 hr/d		1000	4000	(reduced skeletal ossification, reduced fetal body weight)	8000	(increased nonviable implants/litter and late resorption; increased incidence of cleft palate in fetuses)	Tyl and Neeper-Bradley 1989
47	Rabbit (New Zealand)	13 d Gd 6-18 6 hr/d		8000					Tyl 1989

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
INTERMEDIATE EXPOSURE							
Systemic							
48	Rat (Sprague-Dawley)	16-28 wk 5-7 d/wk 6 hr/d	Resp	2500			Biles et al. 1987
			Bd Wt	2500			
49	Rat (Fischer 344)	4-5 wk 5 d/wk 6 hr/d	Hepatic	400	3000 (increased absolute and relative liver weight)		Chun and Kintigh 1993
			Renal	400	3000 (increased protein accumulation & proliferation of epithelial cells in proximal convoluted tubules in males; increased absolute and relative kidney weight in females)		
			Bd Wt	3000 M 8000 F		8000 M (24-35% decreased body weight gain)	

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
50	Rat (Fischer 344)	13 wk 5 d/wk 6 hr/d	Resp	8000			Dodd and Kintigh 1989
			Cardio	8000			
			Gastro	8000			
			Hemato		800 M (mild decrease in RBC and WBC; increase in MCV, and MCH)		
				4000 F	8000 F (increased neutrophils and hematocrit)		
			Musc/skel	8000			
			Hepatic		800 M (increased relative liver		
				800 F	4000 F weight)		
			Renal		800 M (increased relative		
				800 F	4000 F kidney weight)		
			Endocr	4000	8000 (increase in serum corticosterone)		
			Bd Wt	8000			
51	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Resp	1000			Greenough et al. 1980
			Cardio	1000			
			Gastro	1000			
			Hemato	1000			
			Musc/skel	1000			
			Hepatic	1000			
			Renal	500 M		1000 M (significant increase in BUN)	
				1000 F			
			Endocr	1000			
			Bd Wt	1000			

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
52	Rat (Sprague-Dawley)	14-19 wk 5-7 d/wk 6 hr/d	Resp	8000			Neeper-Bradley 1991
			Gastro	8000			
			Hepatic	400 M 3000 F	3000 M 8000 F (increased relative liver weight in F1 parents)		
			Renal	8000			
			Endocr	8000			
			Bd Wt	3000 M	8000 M (10.3% reduction in body weight gain)		
53	Rat (Wistar)	2-15 wk 5 d/wk 6 hr/d	Musc/skel	300 M			Savolainen et al. 1985
			Hepatic	300 M			
			Renal	300 M			
			Bd Wt	300 M			
54	Mouse (CD-1)	28 d 5 d/wk 6 hr/d	Hepatic	400 F	3000 F (increased absolute and relative liver weight)		Chun and Kintigh 1993
				3000 M	8000 M (centrilobular hepatocellular hypertrophy)		
			Renal	8000			
			Endocr	8000			
			Bd Wt	8000			
Immunological/Lymphoreticular							
55	Rat (Fischer 344)	13 wk 5 d/wk 6 hr/d		4000 M	8000 M (hyperplasia of submandibular lymph nodes)		Dodd and Kintigh 1989
				8000 F			
56	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		1000			Greenough et al. 1980

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
57	Rat (Sprague-Dawley)	14-19 wk 5-7 d/wk 6 hr/d		8000			Neeper-Bradley 1991
	Neurological						
58	Rat (Fischer 344)	4-5 wk 5 d/wk 6 hr/d		400		3000 (ataxia, hypoactivity, lack of startle response, blepharospasm)	Chun and Kintigh 1993
59	Rat (Fischer 344)	13 wk 5 d/wk 6 hr/d		800 M	4000 M (hypoactivity; decreased hind limb grip strength) 800 F (increased motor activity)	8000 (ataxia and hypoactivity)	Dodd and Kintigh 1989
60	Rat (Sprague-Dawley)	14-19 wk 5-7 d/wk 6 hr/d		400[c]		3000 (hypoactivity, lack of startle response, blepharospasm)	Neeper-Bradley 1991
61	Rat (Wistar)	2-15 wk 5 d/wk 6 hr/d		300 M			Savolainen et al. 1985
62	Mouse (CD-1)	28 d 5 d/wk 6 hr/d		400		3000 (ataxia, hypoactivity, lack of startle response)	Chun and Kintigh 1993
	Reproductive						
63	Rat (Sprague-Dawley)	16-28 wk 5-7 d/wk 6 hr/d		2500			Biles et al. 1987
64	Rat (Fischer 344)	13 wk 5 d/wk 6 hr/d		8000			Dodd and Kintigh 1989

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL			Reference
					Less serious (ppm)		Serious (ppm)	
65	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		1000				Greenough et al. 1980
66	Rat (Sprague-Dawley)	14-19 wk 5-7 d/wk 6 hr/d		8000				Neeper-Bradley 1991
Developmental								
67	Rat (Sprague-Dawley)	16-28 wk 5-7 d/wk 6 hr/d		2500				Biles et al. 1987
68	Rat (Sprague-Dawley)	14-19 wk 5-7 d/wk 6 hr/d		400	3000	(reduced F1 and F2 pup weight)		Neeper-Bradley 1991

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
CHRONIC EXPOSURE							
Death							
69	Mouse (CD-1)	18 mo 5 d/wk 6 hr/d				8000 M (increased mortality and decreased survival time)	Burleigh-Flayer et al. 1992
Systemic							
70	Rat (Fischer 344)	24 mo 5 d/wk 6 hr/d	Resp	8000			Chun et al. 1992
			Cardio	8000			
			Gastro	8000			
			Hemato	8000			
			Musc/skel	400 F		3000 F (osteodystrophy, secondary to chronic progressive nephropathy)	
			Hepatic	400 F 8000 M	3000 F (increased absolute and relative liver weight)		
			Renal	400[d] F		3000 F (dose-related increased incidence/severity of chronic progressive nephropathy)	
			Endocr	400		3000 F (hyperplasia of parathyroid, secondary to chronic progressive nephropathy)	
			Bd Wt	3000		8000 (13-19% decreased absolute body weight and 22-29% decreased body weight gain)	

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
71	Mouse (CD-1)	18 mo 5 d/wk 6 hr/d	Resp	8000					Burleigh-Flayer et al. 1992
			Cardio	8000					
			Gastro	8000					
			Hemato	8000					
			Musc/skel	8000					
			Hepatic	400	3000	(increased absolute and relative liver weight)			
			Renal	3000			8000	(obstructive uropathy in males, increased relative kidney weight in females)	
			Endocr	8000					
			Bd Wt	3000			8000	(15-24% decreased body weight gain)	
Immunological/Lymphoreticular									
72	Rat (Fischer 344)	24 mo 5 d/wk 6 hr/d		8000					Chun et al. 1992
73	Mouse (CD-1)	18 mo 5 d/wk 6 hr/d		8000					Burleigh-Flayer et al. 1992
Neurological									
74	Rat (Fischer 344)	24 mo 5 d/wk 6 hr/d		400			3000	(blepharospasm, hypoactivity, ataxia, lack of a startle reflex)	Chun et al. 1992
75	Mouse (CD-1)	18 mo 5 d/wk 6 hr/d		400			3000	(blepharospasm, hypoactivity, ataxia, lack of startle reflex, stereotypy)	Burleigh-Flayer et al. 1992

Table 2-1. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL			Reference
					Less serious (ppm)	Serious (ppm)		
Reproductive								
76	Rat (Fischer 344)	24 mo 5 d/wk 6 hr/d		8000				Chun et al. 1992
77	Mouse (CD-1)	18 mo 5 d/wk 6 hr/d		8000				Burleigh-Flayer et al. 1992
Cancer								
78	Rat (Fischer 344)	82-97 wk 5 d/wk 6 hr/d				3000 M	(CEL - renal tubule adenoma and carcinoma)	Chun et al. 1992
79	Mouse (CD-1)	18 mo 5 d/wk 6 hr/d				8000	(CEL - hepatocellular adenoma and carcinoma)	Burleigh-Flayer et al. 1992

[a]The number corresponds to entries in Figure 2-1.

[b]Used to derive an acute inhalation minimal risk level (MRL) of 2 ppm. Concentration adjusted for intermittent exposure, converted to an equivalent concentration in humans, and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive an intermediate inhalation MRL of 0.7 ppm. Concentration adjusted for intermittent exposure, converted to an equivalent concentration in humans, and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[d]Used to derive a chronic inhalation MRL of 0.7 ppm. Concentration adjusted for intermittent exposure, converted to an equivalent concentration in humans, and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Bd Wt = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; exp = exposure(s); F = female; Gastro = gastrointestinal; Gd = gestational day; Hemato = hematological; hr = hour(s); LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observable-adverse-effect level; LT_{50} = time to 50% kill; M = male; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; RBC = red blood cell; Resp = respiratory; WBC = white blood cell; wk = week(s)

Figure 2-1. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) - Inhalation

Acute (≤14 days)

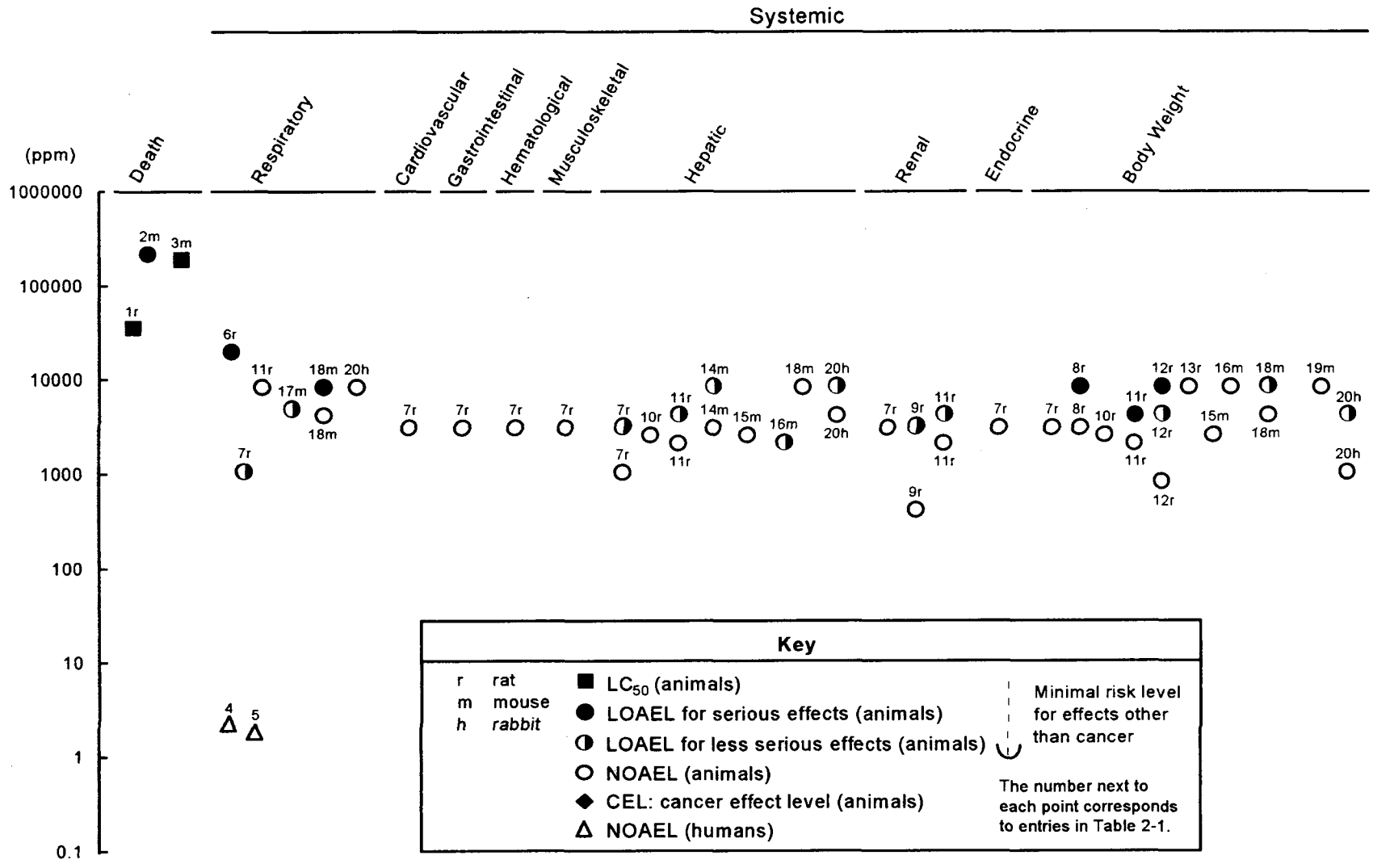


Figure 2-1. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) - Inhalation (cont.)

Acute (≤14 days)

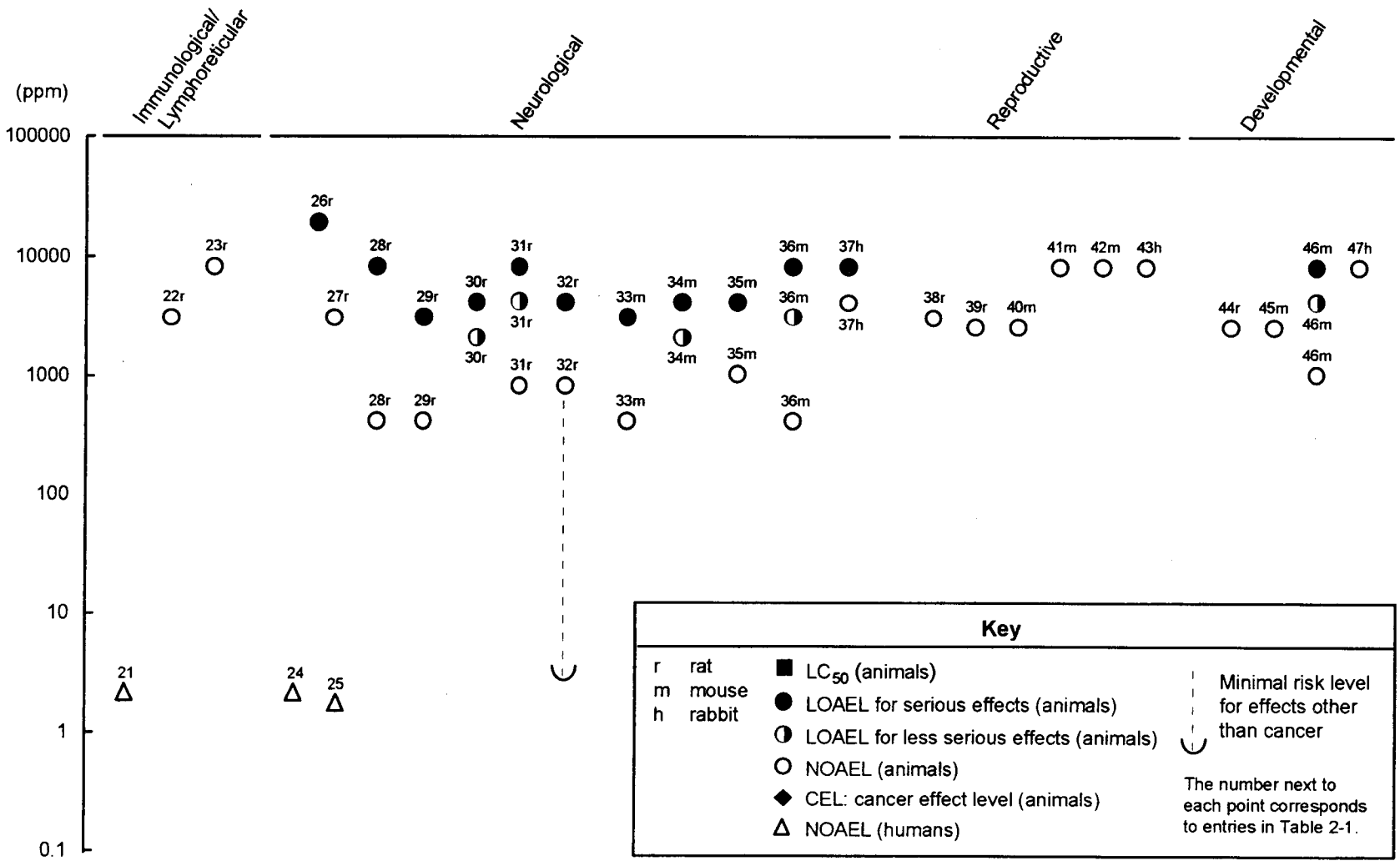


Figure 2-1. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) - Inhalation (cont.)

Intermediate (15-364 days)

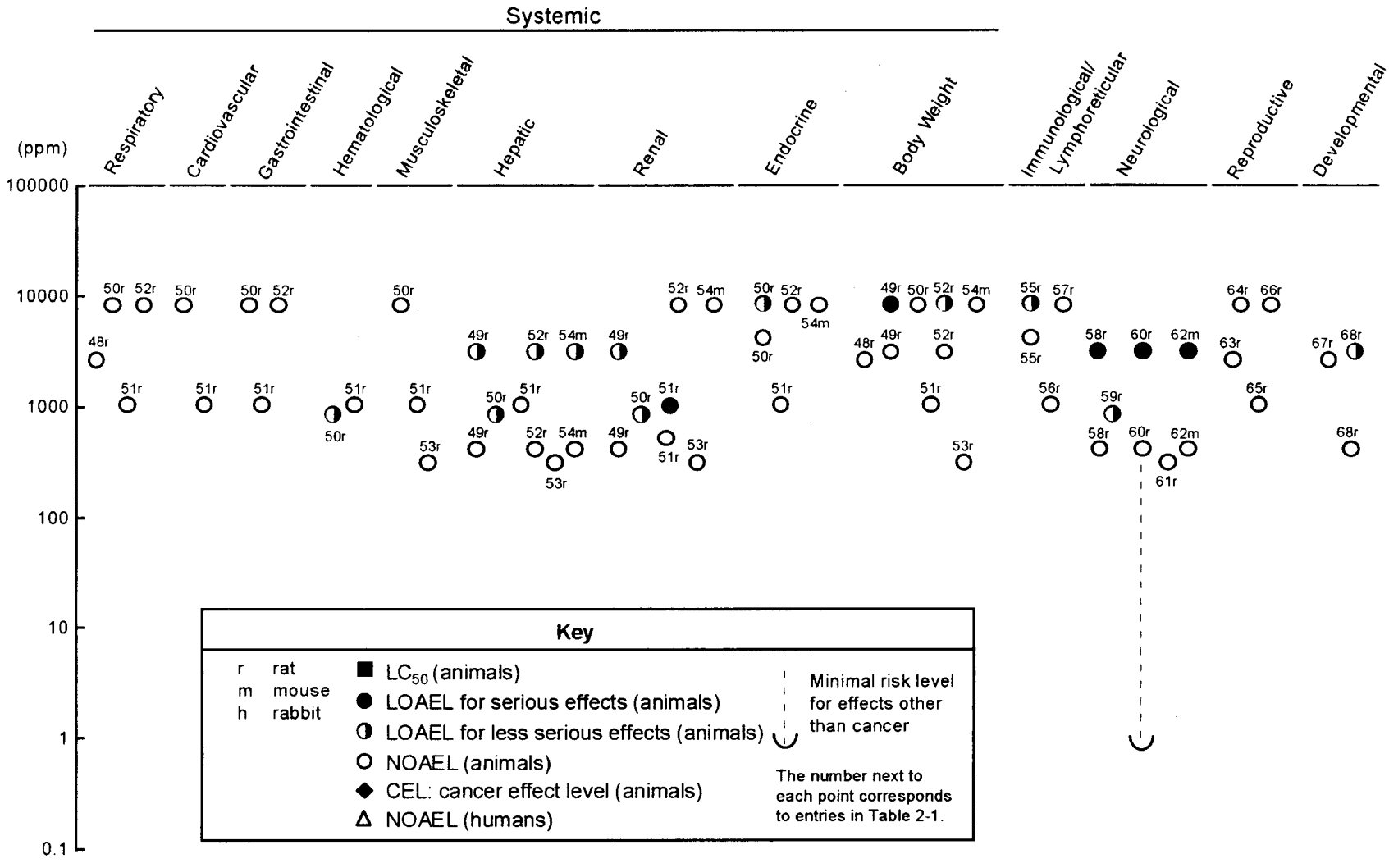
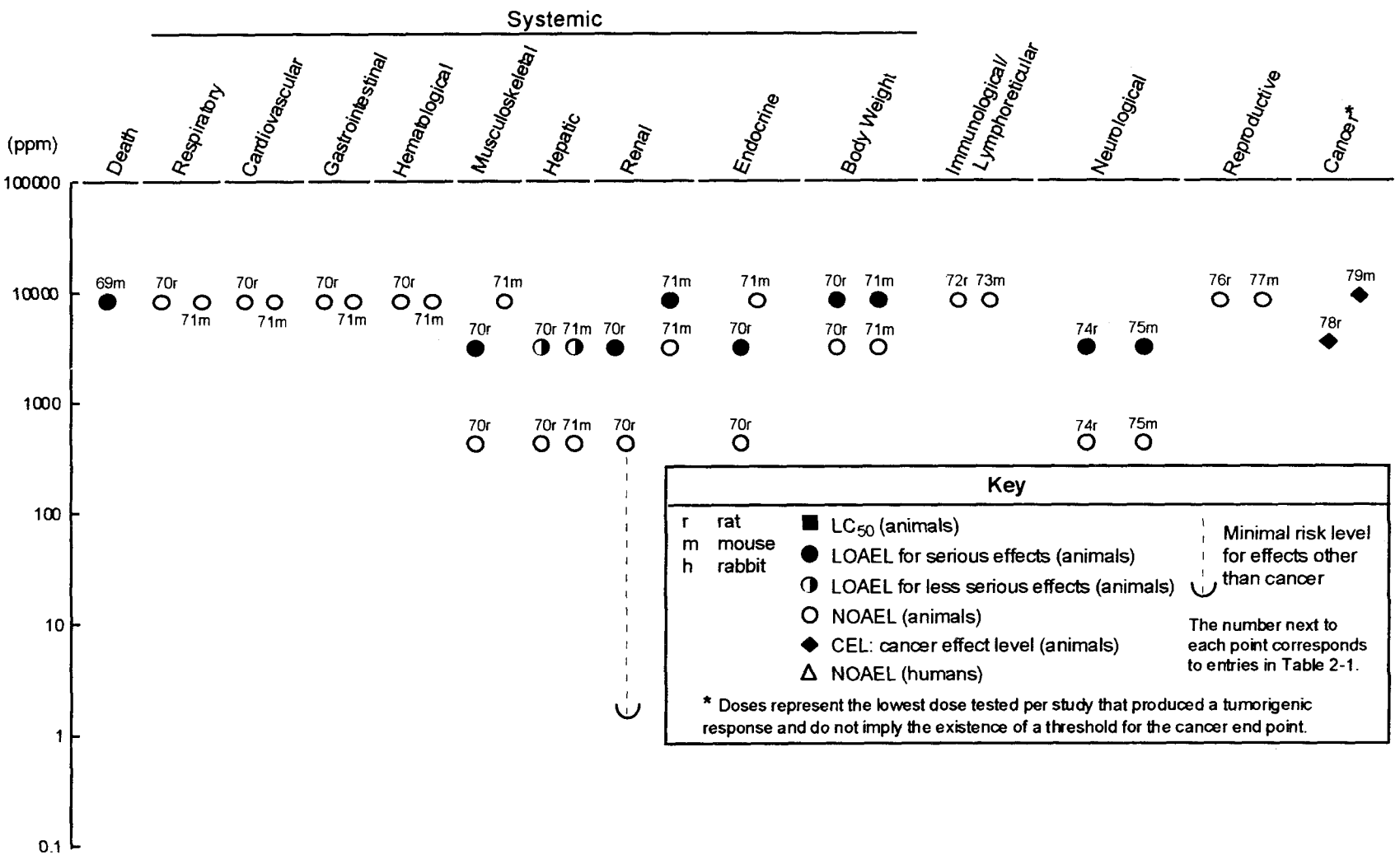


Figure 2-1. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) - Inhalation (cont.)

Chronic (≥365 days)



spaciness or disorientation; eye irritation) was highest in taxi drivers (4 of 12 or 33%), followed by health – care workers (26 of 90 or 29%); the percentage of Fairbanks university students who met the case definition was 15% (15 of 101). The proportion of cases in which symptoms while traveling were reported was 3 of 4 taxi drivers, I1 of 26 health-care workers, and 3 of 15 students; the proportion of cases in which symptoms while fueling were reported was 1 of 4 taxi drivers, 9 of 26 health-care workers, and 3 of 15 students. Of the cases, burning sensation in the nose or throat was reported by 0 of 4 (0%) taxi drivers, 2 of 26 (8%) health workers, and 3 of 15 (20%) students, and cough was reported by 1 of 4 (25%) taxi drivers, 8 of 26 (31%) health-care workers, and 3 of 15 (20%) students. On average, taxi drivers, healthcare workers, and students reported spending 69, 7.7, and 0.8 hours/week, respectively, traveling by motor vehicle. In the Anchorage study (Chandler and Middaugh 1992), the proportion of persons who met the case definition, as defined above, was 12 of 25 (48%) taxi drivers and 36 of 137 (26%) health-care workers. The proportion of cases in which symptoms while traveling were reported was 12 of 12 taxi drivers and 27 of 36 health-care worker, and the proportion of cases in which symptoms while fueling were reported was 9 of 12 taxi drivers and 19 of 36 health-care workers. Of these cases, nose or throat burning was reported by 5 of 12 (42%) taxi drivers and 13 of 36 (36%) health-care workers, and cough was reported by 4 of 12 (33%) taxi drivers and 13 of 36 (36%) health-care workers. The authors of these reports noted that these investigations do not provide definitive evidence that symptoms are due to the oxyfuel programs, but since these investigations were initiated with only modest goals and had many limitations, no definitive results could be expected. Limitations of these studies include the observations that the university students were interviewed shortly before their final examinations took place (when students historically experience more headaches) and that some of the students had access to motor vehicles, factors which could bias results against finding an association between symptoms and exposure to oxyfuel. In addition, the investigations were not conducted when the temperature dropped below 0 ºF, when many people reported that health complaints were worse. Furthermore, the results of these investigations could have been heavily influenced by widespread media coverage and public opposition to the oxyfuel program. Several Anchorage taxi drivers mentioned issues that could bias their symptom reporting: that oxyfuels cost more money, that their cabs were running poorly, that they experienced decreased gas mileage, and that they lost profits because the airport was charging the taxi drivers more.

CDC studies were conducted in Fairbanks, Alaska (CDC 1993a; Moolenaar et al. 1994) and in Stamford, Connecticut (CDC 1993b; White et al. 1995), during oxygenated fuel programs. In the Fairbanks study, people exposed to MTBE in gasoline were monitored during the first 2 weeks of December, 1992 (Phase I), when the oxygenated fuel program was in full effect, and during the beginning of February, 1993 (Phase II), after the oxygenated fuel program had ended on December 22, 1992 (CDC 1993a; Moolenaar et

al. 1994). In Phase I, the subjects consisted of 10 mechanics and workers at service stations and automobile dealerships and 8 subjects who spent most of their workdays in motor vehicles (animal control officers, meter and telephone technicians, and a garbage collector). The median 8-hour time-weighted average (TWA) concentration of MTBE in the workplace air of the service stations and dealership garages was 0.1 ppm, with a range of 0.01-0.81 ppm. The frequencies of key respiratory symptoms were 9 of 18 for burning sensation of nose or throat and 5 of 18 for cough. In Phase II, the subjects consisted of 12 of the original 18 participants in Phase I and an additional 16 workers from service stations and garages (n=28 workers). The median 8-hour TWA concentration of MTBE in the workplace air was 0.04 ppm, with a range of nondetectable to 0.14 ppm. The difference in the median TWAs of MTBE concentrations in Phase I and Phase II was statistically significant. The frequency of key respiratory symptoms in the Phase II subjects was 0 of 28 for both cough and burning sensations of the nose or throat. As discussed in Section 2.3.1.1, the median preshift concentrations of MTBE in blood of occupationally exposed workers were 1.15 μg/L (range, 0.1-27.8 μg/L) in Phase I and 0.20 μg/L (range, 0.05 to 4.35 μg/L) in Phase II, which were statistically significantly different. Likewise, postshift blood concentrations of MTBE in occupationally exposed workers were statistically significantly higher in Phase I (median 1.80 μg/L and range 0.2-37.0 μg/L) than in Phase II (median 0.24 μg/L and range 0.05-I .44 μg/L). The authors reported that when the Phase I postshift blood concentrations were separated into quartiles, all of the 4 workers whose levels were in the top quartile (above 9.6 μg/L) had one or more of the 7 key health complaints (headache, eye irritation, burning of the nose of throat, cough, nausea or vomiting, dizziness, and a sensation of spaciness or disorientation) on the day the blood samples were obtained, compared with 9 of 14 of those whose levels were in the lower 3 quartiles. The Stamford, Connecticut study (CDC 1993b; White et al. 1995) was conducted from April 5 to 16, 1993, about 5 months after gasoline stations began selling gasoline containing 15% MTBE (no Phase II in Stamford study). The study participants were divided into 4 groups: Group 1 consisted of 48 male mechanics or gasoline station attendants; Group 2 consisted of 57 male taxi or limousine drivers; Group 3 consisted of 12 “other” male workers who spent most of their time around traffic or motor vehicles (e.g., meter readers); and Group 4 consisted of 59 men not occupationally exposed, but who commuted to work in their cars. The frequency of key respiratory symptoms was as follows: burning nose or throat in 7 of 48 (14.6%) mechanics (Group 1), in 0 of 57 (0%) professional drivers (Group 2), in 4 of 12 (33.3%) “other” workers (Group 3), and in 4 of 59 (6.8%) commuters (Group 4); cough in 7 of 48 (14.6%) mechanics, in 3 of 57 (5.3%) professional drivers, in 5 of 12 (41.7%) “other” workers, and in 9 of 59 (15.3%) in commuters. Approximate median blood levels of MTBE were 0.1 μg/L in 14 commuters, 2 μg/L in 2 1 car repairers, 0.1 μg/L in 6 “other” workers, and 25 μg/L in 3 gas station attendants. The 11 individuals with the highest blood MTBE levels (>2.4 μg/L) were significantly more likely to report one or more key symptoms. Both personal breathing zone levels

of MTBE for the mechanics and workroom levels of MTBE were measured. Personal breathing zone levels of mechanics ranged from <0.03 to 12.04 ppm, while workroom levels ranged from 0.001 to 0.429 ppm. In both the Stamford and the Fairbanks studies, the accuracy of the measured workroom levels was questionable since the content of the air a worker actually breathed may have been substantially different from the air level measured by the monitoring devices. In addition, in the Stamford study, many of the windows and doors were open due to the mildness of the weather, leading to an underestimation of the exposure levels (CDC 1993b; White et al. 1995). Furthermore, the breathing zone levels, which were measured approximately one week earlier, were questioned because exposure to higher levels of MTBE probably occurred while the workers were close to the gasoline tank. Other limitations of these studies include the participation of only healthy working adults, no control groups, small to modest numbers of participants limiting the statistical power, and the possibilities that an unknown contaminant or combustion product of MTBE, interaction between MTBE and other gasoline components, or the other components alone, may have been responsible for the symptoms. In addition, it is possible that persons willing to participate in the study may have been more likely to report symptoms; or that participants and investigators may have been biased due to awareness of the purpose of the study, publicity, the cost of oxygenated fuel, the odor, or negative personal views.

A telephone interview survey was conducted in metropolitan Milwaukee, Wisconsin, and in metropolitan Chicago, Illinois (the use of reformulated gasoline was required in both of these areas), and in the rest of Wisconsin exclusive of metropolitan Milwaukee (where the use of reformulated gasoline was not required) (Anderson et al. 1995). In the Milwaukee metropolitan area, 23% of the telephone survey respondents reported experiencing unusual symptoms since the reformulated gasoline program started, while in the Chicago metropolitan area and in the rest of Wisconsin, only 6% of the respondents reported experiencing unusual symptoms. Therefore, the proportion in Chicago was not statistically different from that found in Wisconsin. Prevalence of respiratory symptoms associated with MTBE exposure in the questionnaire (throat irritation, difficulty breathing, sinus congestion, funny smells) was statistically higher in Milwaukee than in either Chicago or Wisconsin; the prevalence was not different between Chicago and Wisconsin for any symptom. These results suggest that factors other than reformulated gasoline use contributed significantly to the differences in symptom prevalence between Milwaukee and the other two areas. Knowledge about reformulated gasoline, including the likely awareness of the potential negative effects of reformulated gasoline in Milwaukee and Wisconsin, may have biased the symptom reporting. It was noted that following the nationally televised story about the health effects of MTBE on January 19, 1995, a local Milwaukee television station ran a week-long feature about MTBE, which prompted an increasing number of complaints from Milwaukee residents. 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. The authors concluded that the study was unable to attribute the increased prevalence of symptoms in Milwaukee to reformulated gasoline use, but did not rule out subtle effects or the possibility that a relatively small number of individuals in Milwaukee may have had a greater sensitivity to reformulated gasoline mixtures.

In May 1993, CDC and New York state health officials investigated whether exposure to MTBE in fuels was measurable in occupationally and nonoccupationally exposed people in Albany, where MTBE is used in small concentrations (CDC 1993c). Questionnaires regarding MTBE-associated symptoms (headache, eye irritation, burning of the nose or throat, cough, nausea, dizziness, and spaciness) were administered. Group 1 consisted of 34 automobile repair shop workers and service station attendants exposed to gasoline fumes; Group 2 consisted of 48 policemen, toll booth workers, and parking garage attendants exposed to automobile exhaust; and Group 3 consisted of 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. Ambient air and worksite levels of MTBE were nondetectable (<5 ppb), but MTBE was detectable in the personal air monitoring zone of 2 of 13 auto mechanics and gas station attendants, one at a level of 140 ppb. The proportion of subjects who reported burning nose or throat was 2 of 34 (6%) in Group I, 2 of 48 (4%) in Group 2, and 24 of 182 (13%) in Group 3. The proportion of subjects who reported cough was 5 of 34 (15%) in Group 1, 12 of 48 (25%) in Group 2, and 37 of 182 (20%) in Group 3. All key symptoms were slightly more prevalent and the presence of 2 or more key symptoms was 2-3 times more prevalent in Groups 2 and 3 than in Group 1, but the increases were not statistically significant. Thus, no increase in health complaints among people with higher gasoline exposures was detected. Limitations of the this study included that the study sample may not have accurately represented the population in the various exposure categories; that subjects in Group 3 may have been more likely to report symptoms; and that the study was conducted in early May, when the people may have been driving with their car windows open, when car repair garages typically had their bays open, and when allergies related to pollen may have affected the symptom reporting.

A cross-sectional cohort study investigated self-reported symptoms (headache, nausea, cough, lightheadedness, sleepiness while driving, daytime sleepiness, eye irritation) of garage workers in the state of New Jersey exposed to high (115 workers in northern New Jersey during the wintertime oxyfuel program) and low (122 workers in southern New Jersey 10 weeks after the phase-out date for oxyfuel program) MTBE concentration environments; the results of the questionnaire regarding respiratory symptoms (cough) over the last 30 days showed no significant difference (12% in the northern workers

and 17% in the southern workers, p=0.23) (Mohr et al. 1994). Results of the pre- and postshift questionnaires revealed that all workers felt worse at the end of the workday with regard to symptoms associated with MTBE (not otherwise specified), but no difference was found between the groups or in reporting rates between groups across the work shift. No difference in 30-day symptom reporting was found in subgroups of 13 northern New Jersey workers and 15 southern New Jersey workers who spent an average of ≥5 hours per day pumping gasoline. However, analysis of the pre- and postshift questionnaires showed a higher rate of symptom reporting (p=0.03 for MTBE-associated symptoms) for the northern gas pumpers than for the southern gas pumpers, but not when the same analysis was repeated with northern gas pumpers and age-, sex-, and education-matched southern gas pumpers. Active air sampling done for 1 hour, which was converted to 8-hour TWAs, yielded high MTBE levels (1.66-6.1 ppm) in northern New Jersey garages and nondetectable (<0.28 ppm) to low (0.28-0.83 ppm) MTBE levels in southern New Jersey garages. Passive samplers worn by some of the workers indicated high (1.66-6.1 ppm) or very high (>6.1 ppm) MTBE levels in northern New Jersey. For the southern New Jersey workers, some of the samplers had high levels, but most were low or nondetectable. The authors concluded that no increased rate of symptom reporting was found, even though the northern New Jersey workers received higher exposures. Nevertheless, even 10 weeks after the phase-out date of oxyfuels in southern New Jersey, some workers still received significant exposure, probably due to changing fuel filters and pumps in vehicles that still contained the wintertime oxyfuel.

To address some of the issues identified in the occupational and field studies, an experimental double - blind study was conducted in 22 healthy men and 21 healthy women, who were examined for both objective and subjective effects (Cain et al. 1994). In this study, half of the subjects were exposed sequentially to 1.7 ppm MTBE for 1 hour on 1 day, to uncontaminated air for 1 hour 2 days later, and to 7.1 ppm of a 17-component mixture of volatile organ compounds (VOCs) for 1 hour 2 days later. The other half of the subjects were similarly exposed in the reverse order. The subjects were able to detect the odor of MTBE, but expressed little objection to it. Analysis of nasal lavage material for differential cell counts of polymorphonuclear neutrophilic leukocytes (PMNs), epithelial cells, monocytes, eosinophils, and lymphocytes revealed no statistically significant differences across the three exposure conditions. In addition, statistical analysis of the results of questionnaires administered every 10 minutes during the various exposure conditions revealed no differences for irritation of the nose or throat, dry or sore throat, stuffy or runny nose, sinus congestion, cough, wheezing, chest tightness, or shortness of breath when the subjects were exposed to MTBE or when they exposed to air. In a similar study, 19 healthy men and 18 healthy women were exposed for 1 hour to clean air and 1.39 ppm MTBE in separate sessions at least 1 week apart (Prah et al. 1994). The order of exposure was randomly selected, but because of the odor of

MTBE, it is likely that subjects were aware of the exposure conditions. Results of questionnaires administered prior to exposure, immediately upon entering the exposure chamber, after 30 minutes of exposure, and during the last 5 minutes of exposure revealed no differences for irritation of the nose, cough, wheezing, chest tightness, shortness of breath, stuffy or runny nose, or irritation of the throat. No differences were found for nasal inflammation by the determination of neutrophils, interleukin-8, albumin and prostaglandin D2 in nasal lavage material. Female subjects reported that the air quality in the MTBE condition was worse than in the clean air condition. The odor threshold was determined to be approximately 0.18 ppm. Thus, other than detection of the odor and the reported poor air quality in these experimental studies, no reactions to exposure to MTBE were observed or reported under the conditions of the studies. Although the exposure concentrations used in these experimental studies were chosen on the basis of airborne concentrations of MTBE to which commuters are exposed, the studies could not resolve whether multiple exposures, exposure to higher concentrations, and exposure for longer durations, which are more relevant for real-life exposure of motorists to MTBE, would have caused cumulative effects.

In a study conducted to determine whether symptoms associated with MTBE were reported at an increased rate among subjects known to be sensitive to chemicals and in situations where exposure to MTBE was likely to be greatest, 14 individuals with multiple chemical sensitivities, 5 individuals with chronic fatigue syndrome, and 6 normal control individuals were interviewed regarding symptoms in response to situations in which gasoline containing MTBE was used (driving a car, gasoline stations) and not used (shopping malls, grocery stores, office buildings, parks) (Fiedler et al. 1994). The symptoms of interest included cough and burning sensation in the nose. Although multiple chemical sensitivity subjects and chronic fatigue syndrome subjects reported more symptoms than normal controls in some situations, no significant differences were found among the groups for driving a car or visiting gas stations. The authors concluded that the study did not provide clear evidence to support that an unusually high rate of symptoms or an increase of symptoms occurred uniquely where MTBE exposure was likely.

Respiratory effects have been observed in animals following inhalation exposure to MTBE. A 4-hour exposure of rats to $\geq$ 19,621 ppm ARCO MTBE (96.2% MTBE) caused hyperpnea, while a 4-hour exposure of rats to $\geq$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 study to determine the RD_{50} (the concentration that results in 50% decrease in respiratory rate) for respiratory irritancy of MTBE, mice were exposed to 83,277,832, 2,774, or 8,321 ppm MTBE for 1 hour (Tepper et al. 1994). A threshold irritant response (13%) in respiratory rate occurred at 83 ppm, and a 52% decrease in breathing frequency occurred at 8,321 ppm. No pulmonary irritation was observed at $\leq$ 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 MTBE resulted in high incidence (27 of 40 in both groups) and increased severity of chronic inflammation of the nasal mucosa and trachea (Biodynamics 1981). In mated female rabbits exposed to ≤ 8,000 ppm MTBE on gestational days 6 through 18, no gross lesions were observed in the respiratory tract of the exposed dams when sacrificed on gestational day 29 (Tyl 1989), but histological examination was not performed. However, labored breathing was observed in mouse dams exposed to 8,000 ppm, but not 1,000 or 4,000 ppm, MTBE on gestational days 6-15 (Tyl and Neeper-Bradley 1989). Necropsy revealed color changes in the lungs of 1 dam in the control group, 3 dams in the 4,000 ppm group, and 5 dams in the 8,000 ppm group, but histological examination was not performed. In rats exposed intermittently for 16-28 weeks to concentrations ≤ 2,500 ppm MTBE, nasal discharge and rales were among the most common observations, but control animals were reported to have similar types and incidences of these clinical signs (Biles et al. 1987). However, male and female rats exposed intermittently for 13 days or 13 weeks to ≤ 8,000 ppm had no gross or microscopic lesions in the lungs (Dodd and Kintigh 1989). Rats exposed intermittently for 13 weeks to 1,000 ppm MTBE had significant reductions in absolute and relative lung weights (Greenough et al. 1980). This appears to be a nonspecific effect; gross necropsy of the respiratory tract of males and females exposed to 250-l,000 ppm revealed froth in the airways, congestion of the lung tissues, and small grey foci over the lungs, but these changes were not sex- or doserelated. Rats of both sexes exposed intermittently for 14-19 weeks to concentrations ≤ 8,000 MTBE showed no treatment-related lesions in the gross examination or in the histological evaluation of the upper and lower respiratory tract (Neeper-Bradley 1991). In chronic-duration inhalation studies in rats (Chun et al. 1992) and mice (Burleigh-Flayer et al. 1992) exposed intermittently to 400, 3,000, or 8,000 ppm MTBE, gross and histological examination of lungs, nasopharynx, trachea, and larynx revealed no treatment-related lesions.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to MTBE.

MTBE does not appear to have adverse effects on the cardiovascular system of rats. No treatment-related gross or histopathological lesions in the heart were found in rats exposed intermittently for 9 days to ≤ 3,000 ppm (Biodynamics 1981) or for 13 weeks to ≤ 1,000 ppm (Greenough et al. 1980) or ≤ 8,000 ppm (Dodd and Kintigh 1989). Similarly, histological examination of the aorta revealed no treatment-related

lesions following 13 weeks exposure to ≤ 1,000 ppm MTBE (Greenough et al. 1980). In chronic-duration inhalation studies in rats (Chun et al. 1992) and mice (Burleigh-Flayer et al. 1992) exposed intermittently to 400, 3,000, or 8,000 ppm MTBE, gross and histological examination of the heart revealed no treatment-related lesions.

Gastrointestinal Effects. Nausea or vomiting were among the symptoms reported by motorists or gas station workers during oxygenated fuel programs in which MTBE had been added to gasoline to reduce carbon monoxide emissions. Since these symptoms may be related to the neurological symptoms, the gastrointestinal symptoms are also discussed in Section 2.2.1.4. In a preliminary investigation to determine whether symptoms were occurring, whether symptoms occurred in a consistent pattern, and whether symptoms could be related to exposure to oxyfuel in Fairbanks, Alaska, nausea or vomiting was reported by 3 of 4 (75%) taxi drivers, 9 of 26 (35%) health-care workers, and 6 of 15 (40%) students who met the case definition (4 of 12 taxi drivers, 26 of 90 health-care workers, and 15 of 101 students) (Beller and Middaugh 1992). In the preliminary investigation in Anchorage, Alaska,, nausea or vomiting was reported by 5 of 12 (42%) taxi drivers and 9 of 36 (25%) health-care workers who met the case definition (12 of 25 taxi drivers and 36 of 137 health-care workers) (Chandler and Middaugh 1992).

In the more definitive CDC study in Fairbanks, Alaska, the frequency of nausea or vomiting in the Phase I subjects, when the oxyfuel program was in full effect, was 6 of 18 in occupationally exposed workers (CDC 1993a; Moolenaar et al. 1994). In the Phase II subjects, after the oxyfuel program was terminated, the frequency of nausea or vomiting was 1 of 28. In the CDC study in Stamford, Connecticut, when the oxyfuel program was in full effect (no Phase II in Stamford), nausea or vomiting was reported by 1 of 48 (2.1%) mechanics and gas station attendants, 0 of 57 (0%) professional drivers, I of 12 (8.3%) in “other” workers, and 0 of 59 (0%) commuters (CDC 1993b; White et al. 1995).

In the telephone interview survey conducted in metropolitan Milwaukee, Wisconsin and in metropolitan Chicago, Illinois (where the use of reformulated gasoline was required in both of these areas) and in the rest of Wisconsin exclusive of metropolitan Milwaukee (where the use of reformulated gasoline was not required), the prevalence of nausea associated with MTBE exposure was statistically higher in Milwaukee than in either Chicago or Wisconsin; prevalence was not different between Chicago and Wisconsin for any symptom (Anderson et al. 1995). As discussed under Respiratory Effects in Section 2.2.1.2, these results suggest that factors other than reformulated gasoline use, such as knowledge about reformulated gasoline and the likely awareness of potential negative effects, significantly contributed to the differences in symptom prevalence between Milwaukee and the other two areas.

In a cross-sectional cohort study of self-reported symptoms of garage workers in the state of New Jersey exposed to high (115 workers in northern New Jersey during the win*ter*time oxyfuel program) and low (122 workers in southern New Jersey 10 weeks after the phase-out date for oxyfuel program) MTBE concentration environments, the results of the questionnaire regarding nausea over the last 30 days indicated that southern New Jersey workers experienced nausea (35%) more often than the northern New Jersey workers (27%) ($p=0.03$) (Mohr et al. 1994).

In the study conducted in Albany, New York, the proportion of subjects who reported nausea was 2 of 34 (6%) in Group 1 (automobile repair shop workers and service station attendants), 3 of 48 (6%) in Group 2 (policemen, toll booth workers, and parking garage attendants), and 14 of 182 (8%) in Group 3 (office workers and college students) (CDC 1993c). Key symptoms were slightly more prevalent and the presence of 2 or more key symptoms was 2-3 times more prevalent in Groups 2 and 3 than in Group 1, but the increases were not statistically significant. Thus, no increase in health complaints among people with higher gasoline exposures was detected.

In the study conducted to determine whether symptoms associated with MTBE were reported at an increased rate among subjects known to be sensitive to chemicals and in situations where exposure to MTBE was likely to be greatest, the symptoms of interest included gastrointestinal upset (Fiedler et al. 1994). As discussed in the Respiratory Effects category in Section 2.2.1.2, although multiple chemical sensitivity subjects and chronic fatigue syndrome subjects reported more symptoms than normal controls in some situations, no significant differences were found among the groups for driving a car or visiting gas stations. The authors concluded that the study did not provide clear evidence that an unusually high rate of symptoms or an increase of symptoms occurred uniquely where MTBE exposure was likely. (See Respiratory Effects in Section 2.2.1.2 for a more complete discussion of the human studies regarding protocols, limitations, and conclusions.)

The gastrointestinal tract was not affected in rats exposed to MTBE by inhalation. Intermittent exposure of rats for 9 days to 3,000 ppm MTBE caused no gross or histological changes of the stomach, duodenum, jejunum, ileum, colon, or rectum (Biodynamics 1981). Likewise, gastrointestinal tract tissue of rats exposed intermittently for 13 weeks to 8,000 ppm did not exhibit microscopic lesions (Dodd and Kintigh 1989). Histological examination of the salivary glands, tongue, esophagus, and gastrointestinal tract revealed no treatment-related lesions in rats exposed intermittently for 13 weeks to 1,000 ppm (Greenough et al. 1980) or for 14-19 weeks to 8,000 ppm MTBE (Neeper-Bradley 1991). In chronic-duration inhalation studies in rats (Chun et al. 1992) and mice (Burleigh-Flayer et al. 1992) exposed intermittently

to 400, 3,000, or 8,000 ppm MTBE, gross and histological examination of the gastrointestinal tract revealed no treatment-related lesions.

Hematological Effects. No studies were located regarding hematological effects in humans after inhalation exposure to MTBE.

Rats exposed intermittently for 9 days to ≤ 3,000 ppm MTBE had mean hematological values that were not significantly different from controls (Biodynamics 1981). Male and female rats exposed intermittently for 13 weeks to 1,000 ppm MTBE exhibited a slight increase in white blood cell counts, while males also showed a slight but statistically significant increase in hemoglobin levels (Greenough et al. 1980). Both of these increases were considered of no biological or toxicological significance. All other hematological parameters were normal. Intermittent exposure of male rats for 13 weeks to 800, 4,000, or 8,000 ppm MTBE caused mild decreases (2-4%) in erythrocyte count and mean corpuscular hemoglobin concentration (MCHC) and mild increases (2-5%) in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and reticulocytes (Dodd and Kintigh 1989). This exposure also induced a 15% decrease in the leukocyte count, primarily in the lymphocyte population, in male rats. At the end of the treatment period, female rats of the 8,000 ppm group had increased hematocrit and segmented neutrophil count. In chronic-duration inhalation studies in rats (Chun et al. 1992) and mice (Burleigh-Flayer et al. 1992) exposed intermittently to 400, 3,000, or 8,000 ppm MTBE, no treatment-related changes in hematological parameters were found. In addition, histological examination of the bone marrow revealed no treatment-related lesions.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to MTBE.

Gross and histological examination of bone and skeletal muscle of rats revealed no treatment-related lesions following intermittent exposure to 3,000 ppm MTBE for 9 days (Biodynamics 1981) or to 1,000 ppm for 13 weeks (Greenough et al. 1980). Similarly, an intermittent exposure for 13 weeks to concentrations ≤ 8,000 ppm MTBE produced no microscopic lesions in bones of treated rats (Dodd and Kintigh 1989). Intermittent exposure for 2-l 5 weeks to 300 ppm MTBE 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 I5 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. In chronic-duration

inhalation studies in rats (Chun et al.1992) and mice (Burleigh-Flayer et al. 1992) exposed intermittently to 400, 3,000, or 8,000 ppm MTBE, gross and histological examination of the gastrocnemius muscle revealed no treatment-related lesions. No treatment-related histopathological lesions were found in the bone tissue of mice (Burleigh-Flayer et al. 1992); however, fibrous osteodystrophy, which was secondary to chronic progressive nephropathy, was observed in male rats at all concentrations and in female rats at 3,000 and 8,000 ppm (Chun et al. 1992). As discussed for Renal Effects in Section 2.2.1.2, 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 α_{2u},-globulin or another unknown protein unique to male rats.

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation exposure to MTBE.

Acute-, intermediate-, or chronic-duration inhalation exposure to MTBE did not produce substantial hepatic toxicity in rats. Intermittent exposure for 9 days to $\leq$ 3,000 ppm MTBE had no effect in serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), serum alkaline phosphatase, serum bilirubin, and serum cholesterol (Biodynamics 1981). Relative liver weights were significantly increased in both sexes of rats exposed to 3,000 ppm MTBE. In addition, exposure of male and female rats to MTBE intermittently for 13 days resulted in increased relative liver weights in both sexes at 8,000 ppm (17-24%) and 4,000 ppm (10-l 3%), and increased absolute liver weights in female rats at 8,000 ppm (21%) and 4,000 ppm (14%), but not in males (Dodd and Kintigh 1989). No changes in liver weight or gross liver lesions were observed in mated rats exposed to 2,500 ppm intermittently from day 6-l 5 of gestation (Conaway et al. 1985). At 8,000 ppm MTBE, liver weight of female rabbits exposed on gestational days 6-l8 was significantly increased (Tyl 1989). This increase probably resulted from induction of metabolizing enzymes.

In rats exposed intermittently to 3,000 or 8,000 ppm, but not 400 ppm, MTBE for 4-5 weeks, increased absolute and/or relative liver weights occurred in both sexes (Chun and Kintigh 1993). No clinical or histological evidence of hepatic toxicity was found. Intermittent exposure of rats for 13 weeks to 800-8,000 ppm MTBE caused an increase in liver weight that was greater in male rats than females (Dodd and Kintigh 1989). No treatment-related histopathological hepatic lesions were found. In some rats, there was a decrease in SGOT and SGPT, the toxicological significance of which is unclear. Intermittent exposure to 1,000 ppm MTBE for 13 weeks caused no histological lesions in rat liver, but caused a slight increase in serum lactic dehydrogenase levels in male rats and a decrease in females (Greenough et al.

1980). In a reproductive study, inhalation exposure of rats for 14-19 weeks to MTBE intermittently resulted in increased liver weight in F_1 males at ≥3,000 ppm and in F_1 females at 8,000 ppm, but no liver lesions were found (Neeper-Bradley 1991). Liver microsomal uridine diphosphate glucuronosyl transferase (UDPGT) activity was increased in a dose-related manner in rats exposed to 50, 100, and 300 ppm MTBE for 2 weeks, but not at other times (Savolainen et al. 1985). The biological significance of this finding is not clear. Exposure to these levels for up to 15 weeks did not affect rat liver microsomal cytochrome P-450 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 (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980) indicate that mild microscopic hepatic lesions and increased liver weight due to enzyme induction occur in animals only at much higher exposure levels.

In male and female rats exposed intermittently to 400, 3,000, or 8,000 ppm MTBE for 24 months, increased absolute and relative liver weights (to both body weight and brain weight) in females were concentration-related at 3,000 and 8,000 ppm (Chun et al. 1992). However, gross and histological examination of the liver revealed no treatment-related lesions in females or males at any concentration. The increase in liver weight was probably due to microsomal enzyme induction, which may be considered potentially adverse.

MTBE was slightly more toxic to the liver in mice than in rats. While a 10-day exposure of mated female mice on gestational days 6-15 to 2,500 ppm (Conaway et al. 1985) or 8,000 ppm (Tyl and Neeper-Bradley 1989) MTBE produced no effect on liver weight, increased relative liver weights occurred in both sexes of mice exposed to 8,000 ppm (13-l 8%) and in females exposed to 4,000 ppm (12%) and 2,000 ppm (13%) MTBE intermittently for 13 days (Dodd and Kintigh 1989). Absolute liver weights were increased by 16-20% in female mice at all exposure levels. Intermittent exposure of mice to 400, 3,000, or 8,000 ppm for 28 days also resulted in increased absolute and/or relative liver weight at 3,000 and 8,000 ppm in female mice and 8,000 ppm in male mice, and increased incidence of centrilobular hepatocellular hypertrophy in both sexes at 8,000 ppm (Chun and Kintigh 1993). In addition, in male and female mice exposed to 400, 3,000, or 8,000 ppm for 18 months, increased absolute and relative liver weights (to both body weight and brain weight) were concentration-related in both sexes at 3,000 and 8,000 ppm (Burleigh-Flayer et al. 1992). Increased liver weight in mice at 400 ppm was described as minimal. Gross examination of the livers revealed an increased incidence of liver masses in male and female mice exposed to 8,000 ppm. Histological examination revealed an increased incidence of hepatocellular hypertrophy in both sexes at 8,000 ppm and in males at 3,000 ppm.

In a special experiment for cell proliferation evaluations of hepatocytes, male and female mice were exposed to these concentrations for 5 or 23 exposures (Chun and Kintigh 1993). 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. These results suggest that MTBE initially places an increased metabolic demand on liver cells resulting in a compensatory increase in hepatocellular proliferation that eventually leads to hepatocellular hypertrophy with longer exposure.

Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to MTBE.

Acute intermittent exposure for 9 days to concentrations ranging from 100 to 3,000 ppm MTBE produced no renal toxicity in rats-as indicated by the lack of effect on blood urea nitrogen (BUN) level and urinalysis and by the absence of gross and histological changes in the kidney and urinary bladder (Biodynamics 1981). Exposure of male and female rats to MTBE intermittently for 13 days resulted in increased relative kidney weights in males at 8,000 ppm (14%) and 4,000 ppm (8%) and absolute kidney weights in females at 8,000 ppm (8%) (Dodd and Kintigh 1989). Inhalation exposure of rats for 13 weeks to 1,000 ppm MTBE produced no histological changes of the kidneys and urinary bladder, but male rats exposed to 1,000 ppm exhibited significant increases in BUN (Greenough et al. 1980). No lesions were observed in the gross or histological examination in kidneys of rats exposed to ≤ 8,000 ppm MTBE for 14-19 weeks (Neeper-Bradley 1991). In another intermediate-duration study, exposure of rats to concentrations ≤ 8,000 ppm MTBE had no effect on BUN, creatinine, or albumin levels, but concentrations of ≥800 ppm produced statistically significant increases in kidney weights that were greater in male than female rats (Dodd and Kintigh 1989). No histopathological changes in the kidneys were observed, but male rats exposed to 8,000 ppm had large hyaline droplets in the renal proximal tubules, an effect that may be specific to male rats. The finding of large hyaline droplets in the renal tubules of male rats suggests, but not definitively, the involvement of α_{2u}-globulin accumulation. The accumulation of α_{2u}-globulin complex leads to a nephropathy that is specific for male rats. Male, but not female, rats exposed to ≥ 800 ppm MTBE for 13 weeks showed a treatment-related increase in area and intensity of α_{2u} -globulin positive staining (Swenberg and Dietrich 1991). However, the α_{2u} -globulin positive staining was not dose-related, and α_{2u}-globulin positive proteinaceous casts at the junction of the proximal tubules and thin limb of Henle were not observed, unlike the classical lesions of other α_{2u}-globulin inducing agents.

The possible involvement of α_{2u}-globulin accumulation in MTBE-induced male rat nephropathy has been specifically examined using immunostaining with an antibody to α_{2u}-globulin (Chun and Kintigh 1993). In this study, exposure of male and female rats to 400, 3,000, or 8,000 ppm MTBE for 4-5 weeks resulted in increased protein accumulation and proliferation of epithelial cells in the proximal convoluted tubules of male rats, but not female rats, at 3,000 and 8,000 ppm. However, no evidence of α_{2u}-globulin accumulation was found, suggesting that a mechanism other than a α_{2u}-globulin accumulation (perhaps the accumulation of another protein unique to male rats) may be responsible for the increased cell proliferation in the epithehal cells of the proximal convoluted tubules. The increased proliferation was also observed in males rats, but not female rats, after 5 exposures. Increased absolute and relative kidney weights were observed in male rats at 8,000 ppm and in female rats at $\geq$3,000 ppm after exposure for 4-5 weeks. Urinalysis and urine chemistry evaluations revealed increased urine volume and decreased urinary pH in both sexes at 8,000 ppm, but there was no other indication of renal damage. No renal effects were observed in mice similarly exposed in this study.

The effect of intermittent inhalation exposure to 50-300 ppm MTBE for 2-15 weeks on rat kidney microsomal enzymes has been studied (Savolainen et al. 1985). Cytochrome P-450 content was reported to be significantly increased only after 15 weeks of exposure to 100-300 ppm MTBE, 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 (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980) indicate that microscopic renal lesions and increased kidney weight due to enzyme induction occur in animals only at much higher exposure levels.

In rats intermittently exposed to 400, 3,000, or 8,000 ppm MTBE for up to 24 months, increased absolute kidney weight and kidney weights relative to both body weight and brain weight were concentration- related in females at 3,000 and 8,000 ppm (Chun et al. 1992). Gross examination of kidneys revealed evidence of chronic progressive nephropathy in male rats at all concentrations and in female rats at 3,000 and 8,000 ppm. Histological examination revealed increased incidence and severity of chronic progressive nephropathy, accompanied by fibrous osteodystrophy, hyperplasia in the parathyroid glands, and mineralization in numerous tissues. Chronic progressive nephropathy is an age-related spontaneous disorder of rats that is more severe in males than in females. The exacerbation of chronic progressive nephropathy by MTBE was concentration-related in males at all exposure concentrations and in females at 3,000 and 8,000 ppm. No evidence of renal effects was found in the female rats at 400 ppm. The higher

incidence and greater severity of chronic progressive nephropathy at lower exposure concentrations in male rats as compared with female rats is consistent with the known greater susceptibility of male rats compared with female rats and may be due to the exacerbation of this syndrome by the accumulation of a unique protein in male rats (α_{2u}-globulin or other unknown protein) induced by MTBE. Thus, the enhancement of chronic progressive nephropathy in male rats is generally not considered for MRL derivation. However, since female rats also had enhanced chronic progressive nephropathy, another mechanism not associated with α_{2u}-globulin or other protein accumulation also appears to be involved. Therefore, the chronic inhalation MRL of 0.7 ppm was calculated based on the NOAEL of 400 ppm for kidney effects in female rats as described in the footnote in Table 2-I. The chronic-duration inhalation NOAEL for renal effects is also a NOAEL for clinical signs of neurotoxicity (see Section 2.2.1.4).

In mice similarly exposed to 400, 3,000, or 8,000 ppm for 18 months, increased absolute and relative kidney weights (to both body weight and brain weight) were observed in male mice at all exposure concentrations and in female mice at 8,000 ppm (Burleigh-Flayer et al. 1992). The increase in kidney weight in males, however, was not concentration-related. Furthermore, gross and histological examination revealed no treatment-related lesions in the kidneys of mice. A slight increase in the pH of the urine in male and female mice at 8,000 ppm and a slight increase in the gamma globulin fraction in male mice at 8,000 ppm were found upon urinalysis. Male mice exposed to 8,000 ppm were reported to have increased mortality and decreased mean survival time due to a slightly increased incidence of obstructive uropathy, which may have been due to the increases in pH and gamma globulin fraction.

Endocrine Effects. No studies were located regarding endocrine effects in humans after inhalation exposure to MTBE.

Acute intermittent exposure of rats to 100-3,000 ppm MTBE for 9 days produced no treatment-related lesions in the adrenals, pancreas, and thyroid/parathyroid glands (Biodynamics 1981). No treatment-related changes in adrenal weights were found. However, exposure of male and female rats to MTBE intermittently for 13 days resulted in increased relative and absolute adrenal weights in both sexes at 8,000 ppm, but histological examination of the adrenal glands was not performed (Dodd and Kintigh 1989). In mice exposed to $\leq$8,000 ppm MTBE for 28 days, no effects on thyroid weight and no histopathological lesions of the thyroid were found (Chun and Kintigh 1993). Special blood chemistry evaluation of total T3, total T4, thyroid stimulating hormone (TSH), total bile acid, and estradiol revealed that an increase in total T4 and TSH occurred in male mice at 8,000 ppm. However, these increases were not considered to be biologically significant due to the absence of histological evidence of thyroid lesions.

Decreases in total T4 were found in female mice in the special hepatic cell proliferation studies (see Hepatic Effects above), but net in the main study; therefore, the decrease in T4 was not considered exposure-related. Adrenal weights were significantly increased in male rats exposed to 8,000 ppm and in female rats exposed to 3,000 and 8,000 ppm for 4-5 weeks, but the adrenal glands were not examined histologically (Chun and Kintigh 1993). Although longer exposure (13 weeks) to high concentrations ranging from 800 to 8,000 ppm MTBE had no effect on serum aldosterone or adrenocorticotropic hormone levels in rats, there was an increase in corticosterone level at 8,000 ppm MTBE (Dodd and Kintigh 1989). Exposure to concentrations of 4,000 or 8,000 ppm MTBE produced increases in adrenal weight that were greater in male rats than females. No histopathological changes resulted from this treatment in the adrenals, pancreas, or pituitary glands. These results are in agreement with other intermediate-duration intermittent exposures of rats. In rats exposed to $\leq$ 1,000 ppm MTBE, no treatment-related lesions were found in the pancreas, thyroid, parathyroid, adrenal, or pituitary (Greenough et al. 1980). Similarly, no exposure-related gross or microscopic lesions in the pancreas or pituitary were found in parental F_0 and F_1 rats following exposure for 10 weeks prior to breeding to $\leq$ 8,000 ppm for 5 days per week and through day 19 of gestation (Neeper-Bradley 1991).

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 (Chun et al. 1992). This group was terminated at week 82 because of high mortality from chronic progressive nephropathy. Hyperplasia of the parathyroid gland, which was secondary to chronic progressive nephropathy, was found in male rats at all exposure concentrations and in female rats at 3,000 and 8,000 ppm. No treatment-related histopathological lesions were found in the pituitary, adrenal gland, or pancreas. In mice similarly exposed to the same concentrations for 18 months, no treatment-related histopathological lesions were found in the pituitary, thyroid/parathyroid, or pancreas (Burleigh-Flayer et al. 1992). Adrenal gland weights were increased in high dose males, but were not accompanied by histopathological lesions. However, corticosterone levels were increased at week 79 in both sexes exposed to 8,000 ppm. The toxicological significance of these transient and inconsistent changes in corticosterone levels in rats and mice is questionable.

Dermal Effects. In two experimental studies in which human subjects were exposed to MTBE vapors for 1 hour at 1.7 ppm (Cain et al. 1994) or 1.39 ppm (Prah et al. 1994), no evidence was found that exposure to MTBE vapors resulted in skin rash or dry skin. Rats and mice have been examined for dermal effects after exposure to MTBE vapors in air (Biles et al. 1987; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980). Since any dermal effects in these studies in

humans and animals would probably be due to direct contact of the skin with the vapor, these studies are discussed more fully in Section 2.2.3.2.

Ocular Effects. Eye irritation was among the symptoms reported by motorists or gas station workers during oxygenated fuel programs in which MTBE had been added to gasoline to reduce carbon monoxide emissions. Several survey studies in humans (Anderson et al. 1995; Belier and Middaugh 1992; CDC 1993a, 1993b, 1993c; Chandler and Middaugh 1992; Mohr et al. 1994; Moolenaar et al. 1994; White et al. 1995) and experimental studies in humans (Cain et al. 1994; Prah et al. 1994) have investigated whether the eye irritation was specifically associated with exposure to MTBE vapor in air. In addition, rats and mice have been examined for ocular effects following exposure to MTBE vapors in air (ARCO 1980; Biles et al. 1987; Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun et al. 1992; Conaway et al. 1985; Dodd and Kintigh 1989; Greenough et al. 1980; Neeper-Bradley 1991; Tyl and Neeper-Bradley 1989). Since any ocular effects in these studies in humans and animals would probably be due to direct contact of the eyes with the vapor, these studies are discussed in Section 2.2.3.2.

Body Weight Effects. No studies were located regarding body weight effects in humans after inhalation exposure to MTBE.

A single exposure of rats to $\leq$ 8,000 ppm for 6 hours had no effect on body weight (Gill 1989). Acute exposure of rats intermittently for 9 days to $\leq$ 3,000 ppm MTBE (Biodynamics 1981) or to $\leq$ 2,500 ppm for 10 days during gestational days 6-15 did not affect body weight (Conaway et al. 1985). In contrast, in rats exposed intermittently to 800, 4,000, or 8,000 ppm for 5 days, an unspecified weight loss in males and an unspecified decrease in body weight gain in females occurred at 8,000 ppm (Vergnes and Morabit 1989). Male rats of the 800 and 4,000 ppm groups had marginally depressed body weight gains during the exposure regimen. Similarly, a decrease in body weight gain of 65-66% occurred in male rats during the first l-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 the 13-week study, transient decreases in body weight or body weight gain occurred during the first few weeks, accompanied by reduced food consumption, in the rats exposed to 4,000 or 8,000 ppm, but both body weight and food consumption recovered thereafter. Thus, compared with controls, final body weights were only 6% lower in male rats and 3% lower in female rats exposed to 8,000 ppm. The initial depressions in body weight were, therefore, probably due to the reduced food consumption, which in turn may have been secondary to the reduced motor activity of the 4,000 ppm and 8,000 ppm groups (Dodd and Kintigh 1989). In a

4-5-week study in rats, a 2% loss of body weight was observed in male rats exposed to 8,000 ppm during the first week, and decreased body weight gain occurred throughout the study in male rats exposed to 8,000 ppm, with a decreased body weight gain as high as 49% from exposure days 1-12 (Chun and Kintigh 1993). Similarly, decreased body weight gain (24%) occurred in female rats exposed to 8,000 ppm, but only during the first 2 weeks. Exposure of mated rabbits to ≥ 4,000 ppm (Tyl 1989) and mated mice to 8,000 ppm (Tyl and Neeper-Bradley 1989) during gestation decreased body weight of the dams, and there was a reduction in food consumption. However, exposure of mice for 10 days during gestation to ≤ 2,500 ppm MTBE did not affect the dams' body weight (Conaway et al. 1985). Similarly, intermittent exposure of mice to ≤ 8,000 ppm for 1 or 2 days (Vergnes and Kintigh 1993) for 13 days (Dodd and Kintigh 1989), or for 28 days (Chun and Kintigh 1993) had no effect on body weight. In intermediate-duration studies, no effects on body weight were found in rats exposed to 300-2,500 ppm (Biles et al. 1987; Greenough et al. 1980; Savolainen et al. 1985). However, male rats exposed to 8,000 ppm for 14-19 weeks had a 10.3% reduction in body weight gain (Neeper-Bradley 1991).

In a 24-month inhalation study in rats intermittently exposed to 400, 3,000, or 8,000 ppm MTBE, both male and female rats had decreased absolute body weight and decreased body weight gain at 8,000 ppm (Chun et al. 1992). In mice similarly exposed to the same concentrations for 18 months, slightly decreased absolute body weight and decreased body weight gain were observed in both sexes at 8,000 ppm (Burleigh-Flayer et al. 1992).

2.2.1.3 Immunological and Lymphoreticular Effects

Effects on the immune system were measured by monitoring plasma interleukin-6 levels in 22 volunteers exposed to auto emissions derived from oxyfuels during a 4-week period in late November and early December 1992 at several different locations around Fairbanks, Alaska (Duffy 1994). Blood samples were collected at the beginning of work shifts and the end of the workday and analyzed for interleukin-6 by an immunochemical assay. No differences in interleukin-6 levels were found between the morning and evening blood samples.

In the experimental double-blind study in humans exposed sequentially to 1.7 ppm MTBE for 1 hour on 1 day, to uncontaminated air for 1 hour 2 days later, and to 7.1 ppm of a 17-component mixture of VOCs for 1 hour 2 days later (or the reverse sequence) (see Respiratory Effects in Section 2.2.1.2), nasal lavage material and tear fluid from the eyes were stained for the total numbers of cells and differential counts for polymorphonuclear neutrophilic leukocytes, epithelial cells, monocytes, eosinophils, and lymphocytes

(Cain et al. 1994). No notable changes between pre- and postexposure values were observed for exposure to MTBE or air.

Acute intermittent exposure of rats for 9 days to ≤ 3,000 ppm MTBE did not produce gross or histological lesions in bone marrow, lymph nodes, or spleen (Biodynamics 1981). Similar results were obtained for lymph nodes of rats after exposure for 13 days to ≤ 8,000 ppm MTBE (Dodd and Kintigh 1989). In rats and mice exposed to 400, 3,000 or 8,000 ppm for ≤ 28 days, decreased absolute and relative spleen weights were found in both sexes of rats and in female mice, but not in male mice, at 8,000 ppm, but spleens were not examined histologically (Chun and Kintigh 1993). In some studies in which rats were exposed for 13 weeks to 1,000 ppm (Greenough et al. 1980) or for 14-19 weeks to 8,000 ppm (Neeper-Bradley 1991), MTBE did not cause treatment-related gross or histopathological lesions in the spleen, thymus, or lymph nodes (Greenough et al. 1980; Neeper-Bradley 1991); in another study, however, exposure for 13 weeks to 8,000 ppm of MTBE resulted in a higher incidence of lymphoid hyperplasia in submandibular lymph nodes in male rats (Dodd and Kintigh 1989).

In rats exposed to 400, 3,000, or 8,000 ppm for 24 months, gross and histological examination revealed no treatment-related lesions in the spleen, lymph nodes, thymus, or bone marrow (Chun et al. 1992). In mice exposed to 400, 3,000, or 8,000 ppm 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 (Burleigh-Flayer et al. 1992). Furthermore, no treatment-related lesions were found in the lymph nodes, thymus, or bone marrow.

The highest NOAEL values for acute-, intermediate-, and chronic-duration inhalation exposure to MTBE for immunological and lymphoreticular effects in rats and mice and the LOAEL for lymphoid hyperplasia in rats after intermediate-duration inhalation exposure to MTBE are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.4 Neurological Effects

Headache, nausea or vomiting, dizziness, and a feeling of spaciness or disorientation were among the symptoms reported by motorists or gas station workers during oxygenated fuel programs in which MTBE had been added to gasoline to reduce carbon monoxide emissions. In the preliminary investigation to determine whether symptoms were occurring, whether symptoms occurred in a consistent pattern, and whether symptoms could be related to exposure to oxyfuel in Fairbanks, Alaska, headache was reported by

3 of 4 (75%) taxi drivers, 21 of 26 (81%) health-care workers, and 10 of 15 (67%) students who met the case definition (4 of 12 taxi drivers, 26 of 90 health-care workers, and 15 of 101 students) (Beller and Middaugh 1992). Nausea or vomiting was reported by 3 of 4 (75%) taxi drivers, 9 of 26 (35%) health-care workers, and 6 of 15 (40%) students, and spaciness was reported by 1 of 4 (25%) taxi drivers, 1 of 26 (4%) health-care worker, and 2 of 15 (13%) students who met the case definition. In the preliminary investigation in Anchorage, Alaska, headache was reported by 11 of 12 (92%) taxi drivers and 31 of 36 (86%) health-care workers who met the case definition (12 of 25 taxi drivers and 36 of 137 health-care workers) (Chandler and Middaugh 1992). Nausea or vomiting was reported by 5 of 12 (42%) taxi drivers and 9 of 36 (25%) health-care workers, dizziness was reported by 4 of 12 (33%) taxi drivers and 7 of 36 (19%) health-care workers, and spaciness was reported by 4 of 12 (75%) taxi drivers and 3 of 36 (8%) of health-care workers. As discussed in the Respiratory Effects in Section 2.2.1.2, these investigations do not provide definitive evidence that these symptoms were due to the oxyfuel programs.

In the more definitive CDC study in Fairbanks, Alaska, the frequency of headache in the Phase I subjects, when the oxyfuel program was in full effect, was 13 of 18 in occupationally exposed workers (CDC 1993a; Moolenaar et al. 1994). Nausea or vomiting was reported by 6 of 18, dizziness by 8 of 18, and spaciness by 6 of 18 workers. In the Phase II subjects, after the oxyfuel program was terminated, the frequency of headache was 1 of 28, of nausea or vomiting was 1 of 28, of dizziness was 0 of 18, and of spaciness was 0 of 18. In the CDC study in Stamford, Connecticut, when the oxyfuel program was in full effect (no Phase II in Stamford), headache was reported by 13 of 48 (27.1%) at least once, 12 of 48 (25%) at least twice, and 4 of 48 (8.3%) at least 5 times in mechanics and gas station attendants; by 15 of 57 (26.3%) at least once, 12 of 57 (21.1%) at least twice, and 5 of 57 (8.8%) at least 5 times in professional drivers; by 5 of 12 (41.7%) at least once, 5 of 12 (41.7%) at least twice, and 1 of 12 (8.3%) at least 5 times in “other” workers; and by 15 of 59 (25.4%) at least once, 14 of 59 (23.7%) at least twice, and 3 of 59 (5.1%) at least 5 times in commuters (CDC 1993b; White et al. 1995). Nausea or vomiting was reported by 1 of 48 (2.1%) mechanics and gas station attendants, 0 of 57 (0%) professional drivers, 1 of 12 (8.3%) “other” workers, and 0 of 59 (0%) commuters. Dizziness was reported by 3 of 48 (6.3%) mechanics and gas station attendants, 3 of 57 (5.3%) professional drivers, 2 of 12 (16.7%) “other” workers, and 1 of 59 (1.7%) commuters. Spaciness was reported by 5 of 48 (10.4%) mechanics and gas station attendants, 1 of 57 (1.8%) professional drivers, 1 of 12 (8.3%) “other” workers, and 2 of 59 (3.4%) commuters.

In the cross-sectional cohort study of self-reported symptoms of garage workers in the state of New Jersey exposed to high (115 workers in northern New Jersey during the wintertime oxyfuel program) and low (122 workers in southern New Jersey 10 weeks after the phase-out date for oxyfuel program) MTBE

concentration environments, the results of the questionnaire regarding headache (36% of northern workers, 39% of southern workers, p=0.82), lightheadedness (10% of northern workers, 13% of southern workers, p=0.4 I), sleepiness while driving (20% of northern workers, 24% of southern workers, p=0.37), daytime sleepiness (13% of northern workers, 24% of southern workers, p=0.00l), and nausea (27% of northern workers, 35% of southern workers, p=0.03) over the last 30 days showed either no significant differences or a greater prevalence in southern workers (Mohr et al. 1994).

In the study conducted in Albany, New York, the proportion of subjects who reported headache was 7 of 34 (21%) in Group 1 (automobile repair shop workers and service station attendants), 23 of 48 (47%) in Group 2 (police officers, toll booth workers, and parking garage attendants), and 44 of 182 (24%) in Group 3 (office workers and college students) (CDC 1993c). The proportion of subjects who reported dizziness was 3 of 34 (9%) in Group 1, 6 of 48 (13%) in Group 2, and 5 of 182 (3%) in Group 3. The proportion of subjects who reported spaciness was 0 of 34 (0%) in Group 1, 2 of 48 (4%) in Group 2, and 13 of 182 (7%) in Group 3. Nausea was reported by 2 of 34 (6%) Group 1 subjects, 3 of 48 (6%) Group 2 subjects, and 14 of 182 (8%) Group 3 subjects. Key symptoms were slightly more prevalent and the presence of two or more key symptoms was 2-3 times more prevalent in Groups 2 and 3 than in Group 1, but the increases were not statistically significant. Thus, no increase in health complaints among people with higher gasoline exposures was detected.

A telephone interview survey was conducted in metropolitan Milwaukee, Wisconsin, metropolitan Chicago, Illinois (use of reformulated gasoline was required in both of these areas), and in the rest of Wisconsin exclusive of metropolitan Milwaukee (where the use of reformulated gasoline was not required). Prevalence of headache, dizziness, and spaciness associated with MTBE exposure was statistically higher in Milwaukee than in either Chicago or Wisconsin; prevalence was not different between Chicago and Wisconsin for any symptom (Anderson et al. 1995). As discussed in the Respiratory Effects in Section 2.2.1.2, these results suggest that factors other than reformulated gasoline use, such as knowledge about reformulated gasoline and the likely awareness of potential negative effects, significantly contributed to the differences in symptom prevalence between Milwaukee and the other two areas.

In the study conducted to determine whether symptoms associated with MTBE were reported at an increased rate among subjects known to be sensitive to chemicals and in situations where exposure to MTBE was likely to be greatest, the symptoms of interest included headache, dizziness, nausea, sleepiness, spaciness, and disorientation (Fiedler et al. 1994). Although multiple chemical sensitivity

subjects and chronic fatigue syndrome subjects reported more symptoms than normal controls in some situations, no significant differences were found among the groups for driving a car or visiting gas stations. The authors concluded that the study did not provide clear evidence to support that an unusually high rate of symptoms or an increase of symptoms occurred uniquely where MTBE exposure was likely.

In the experimental double-blind study in humans exposed sequentially to 1.7 ppm MTBE for 1 hour on 1 day, to uncontaminated air for 1 hour 2 days later, and to 7.1 ppm of a 17-component mixture of VOCs for 1 hour 2 days later (or the reverse sequence), neurobehavioral tests evaluating symbol-digit substitution, switching attention, and a profile of mood state (POMS) were administered at 1 hour before exposure and during the last 15 minutes of exposure (Cain et al. 1994). In addition, the subjects were administered questionnaires regarding subjective symptoms of headache; difficulty remembering things or concentrating; feelings of depression; unusual tiredness, fatigue or drowsiness; tension, irritability, or nervousness; dizziness or lightheadedness; mental fatigue or "fuzziness;" and pain or numbness in the hands or wrists. Results of statistical analysis of variance in the neurobehavioral tests revealed no differential effects across the three conditions of exposure to MTBE, air, or VOCs. No differences in reporting of symptoms were noted for exposure to MTBE versus exposure to air. In a similar study, 19 healthy men and 18 healthy women were exposed for 1 hour to clean air and 1.39 ppm MTBE in separate sessions separated by at least 1 week (Prah et al. 1994). The order of exposure was randomly selected, but because of the odor of MTBE, it is likely that the subjects were aware of the exposure conditions. Analysis of the results of the questionnaires administered prior to exposure, immediately upon entering the exposure chamber, after 30 minutes of exposure, and during the last 5 minutes of exposure revealed no differences for 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. Specific neurobehavioral evaluation system tests, which consisted of symbol-digit substitution, switching attention, and mood scales, were completed as baseline before entering the chamber and after 45 minutes of exposure. No measures approached significance. (See Respiratory Effects in Section 2.2.1.2 for a more complete discussion of the human studies regarding protocols, limitations, and conclusions.)

Acute inhalation exposure of laboratory animals to MTBE results in neurological deficits. Rats exposed to 19,62 1 ppm ARCO MTBE (96.2% MTBE) exhibited lacrimation within 3 minutes of exposure (ARCO 1980). After 4 hours of exposure, these animals developed ataxia, loss of righting reflex, hyperpnea, incoordination, and prostration. Similar effects were observed when the rats were exposed to 18,892 ppm of commercial MTBE (99.1 % MTBE). Similarly, in rats exposed to 400 or 8,000 ppm MTBE for 6 hours,

ataxia occurred within 0.5 hours and drowsiness occurred within 1.5 hours (Bioresearch Labs 1990d). In rats exposed intermittently to 4,000 or 8,000 ppm for 5 days, ataxia was noted at 8,000 ppm (Vergnes and Morabit 1989). In addition, clinical signs of neurotoxicity were observed in mice exposed intermittently for 2 days at 3,000 ppm (hypoactivity and lack of startle response) and at 8,000 ppm (hypoactivity, abdominal breathing, ataxia, and prostration) (Vergnes and Chun 1994).

In rats studied for motor activity and given a functional observation battery of tests after a 6-hour exposure to 800, 4,000, or 8,000 ppm, concentration-related increases in incidence and severity of ataxia and duck- walk gait occurred in both males and females at 4,000 and 8,000 ppm (Gill 1989). Other effects noted in high-dose 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 $\geq$ 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, indicating the transient nature. The time course of changes in motor activity corresponded with the functional observation battery findings, and supported the exposure-related central nervous system depression. No neurological effects were observed at 800 ppm for 6 hours. Based on this NOAEL, an acute-duration inhalation MRL of 2 ppm was calculated as described in the footnote in Table 2-l.

In a preliminary 13-day range-finding study for a 13-week study, rats exposed to 4,000 and 8,000 ppm MTBE exhibited hypoactivity and ataxia as well as decreased startle and pain reflexes, and decreased muscle tone (Dodd and Kintigh 1989). The 2,000 ppm level also resulted in hypoactivity. Concentrations of 2,000 or 4,000 ppm did not produce neurobehavioral changes. In the 13-week study, hypoactivity was observed at 4,000 ppm and hypoactivity and ataxia were observed at 8,000 ppm daily after the 6 hour/day exposure, thus representing effects of acute exposure. Similarly, when rats and mice were exposed to MTBE at concentrations of 400, 3,000, and 8,000 ppm 6 hours per day, 5 days per week for $\leq$ 28 days, ataxia, hypoactivity, and lack of startle response were observed at $\geq$ 3,000 ppm during daily exposures (Chun and Kintigh 1993). The rats also showed blepharospasm. Exposure for 9 days to $\leq$ 3,000 ppm showed no treatment-related lesions in rat brains (Biodynamics 1981). While no clinical signs were observed in mice exposed to $\leq$ 8,000 ppm for 1 or 2 days (Vergnes and Kintigh 1993), in a 13-day study in mice, hypoactivity and ataxia occurred at 4,000 and 8,000 ppm during exposure, but only ataxia occurred following exposure in the 8,000 ppm group (Dodd and Kintigh 1989). Hypoactivity was also observed in the 2,000 ppm group during exposures on days 2 and 3, and prostration occurred in 2 of 5 females during exposure to 8,000 ppm on day 9. Also, mice exposed to 4,000 and 8,000 ppm MTBE on gestational days 6-15 exhibited hypoactivity and ataxia (Tyl and Neeper-Bradley 1989). Mice also became prostrate at

8,000 ppm. Similar signs of toxicity were observed in rabbits exposed to ≤ 8,000 ppm MTBE for I3 days during gestation (Tyl 1989).

In a 13-week intermediate-duration study, MTBE induced anesthesia in rats exposed intermittently to 250-l,000 ppm (Greenough et al. 1980), but the lowest concentration resulting in anesthesia was not specified. In these animals, MTBE did not result in histopathologic lesions in the brain or the sciatic nerve. In another 13-week study in rats, exposure to ≤ 8,000 ppm did not affect relative brain weights and did not produce microscopic changes, but decreased hindlimb grip strength occurred in males exposed to 4,000 ppm at 1, 2,4, 8, and 13 weeks; decreased motor activity occurred in males exposed to 8,000 ppm at week 8; and increased motor activity occurred in females exposed to 800 and 4,000 ppm at week 8 and at 4,000 ppm at week 13 (Dodd and Kintigh 1989). Exposure of male and female rats for 10 weeks prior to mating and through day 19 of gestation to the concentration of 8,000 ppm MTBE resulted in salivation and hypoactivity in F_0 and F_1 parental rats (Neeper-Bradley 1991). F_0 and F_1 parental groups also showed hypoactivity and lack of startle response, as well as blepharospasm, at 3,000 ppm. A concentration of 400 ppm did not produce any effects. Based on the NOAEL of 400 ppm, an intermediate-duration inhalation MRL of 0.7 ppm was calculated as described in the footnote in Table 2- 1. Neither rat brain succinate dehydrogenase nor acetylcholinesterase activity was affected by exposure for 15 weeks to 50-300 ppm MTBE (Savolainen et al. 1985).

In chronic-duration studies in which rats (Chun et al. 1992) and mice (Burleigh-Flayer et al. 1992) were intermittently exposed to 400, 3,000, or 8,000 ppm MTBE, clinical signs of neurotoxicity were observed at 3,000 and/or 8,000 ppm in males and/or females. These signs consisted of blepharospasm, hypoactivity, ataxia, lack of startle reflex, salivation (rats), stereotypy (mice), and prostration (mice). Gross and histological examination of the brains, spinal cords, and sciatic nerves, however, revealed no treatment- related lesions in either species at any concentration.

The highest NOAEL values and all LOAEL values for each reliable study for neurological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to MTBE.

Acute exposure to MTBE did not produce reproductive toxicity in experimental animals. Exposure of rats for 9 days to ≤ 3,000 ppm MTBE did not result in gross or histological changes in the reproductive system of males or females (Biodynamics 1981). Also, studies on the potential developmental effects of MTBE showed no effects on the dams or the fetuses. Exposure of rats or mice for 10 days during gestation to ≤ 2,500 ppm MTBE did not adversely affect the mean number of corpora lutea, uterine implantations, resorptions, or live fetuses (Conaway et al. 1985). Also, exposure of rabbits for 13 days (Tyl 1989) or mice for 10 days (Tyl and Neeper-Bradley 1989) to ≤ 8,000 ppm MTBE during gestation did not cause gross lesions in the reproductive tract of the dams. This treatment did not affect the number of ovarian corpora lutea, number of total, nonviable, or viable implantations, proportion of pre- or postimplantation loss, fetal body weight, or sex ratio. Only gravid uterine weights were decreased in both species of dams exposed to 8,000 ppm. Exposure of male mice for 13 days at ≤ 8,000 ppm MTBE neither produced microscopic lesions in testes, nor did it cause changes in testicular weight (Dodd and Kintigh 1989).

A study on the effect of long-term exposure (16-28 weeks) to 250-2,500 ppm MTBE on fertility, reproductive systems, performance of male and female rats, and the development of offspring revealed that MTBE had no structural effect on the reproductive system or effect on reproductive performance of male and female rats (Biles et al. 1987). MTBE did not produce microscopic lesions in the prostate, testes, uterus, ovaries, or mammary tissues of rats exposed to ≤ 8,000 ppm for 13 weeks (Dodd and Kintigh 1989; Greenough et al. 1980). A reproductive study was carried out in rats exposed to ≤ 8,000 ppm MTBE for 14-19 weeks including 10 weeks prebreeding period, l-3 weeks breeding period, 3 weeks gestational period, and for females, 3 weeks postnatal period (Neeper-Bradley 1991). This treatment had no effect on F_0 and F_1 reproductive parameters including gestational length and mating, fertility, and gestational indices. No histological lesions were seen in the vagina, uterus, ovaries, testes, epididymis, seminal vesicles, or prostate.

In chronic-duration studies, in which male and female rats (Chun et al. 1992) and male and female mice (Burleigh-Flayer et al. 1992) were intermittently exposed to 400, 3,000, or 8,000 ppm MTBE, gross and histological examination of reproductive organs (testes, epididymides, prostate, seminal vesicles, ovaries, vagina, uterus, fallopian tubes, mammary glands) revealed no treatment-related lesions.

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2-I.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to MTBE. MTBE did not cause adverse developmental effects in rats and rabbits. Exposure of female rats during days 6-15 of gestation to ≤ 2,500 ppm MTBE had no effect on percentage of resorption, percentage of live fetuses, mean fetal weights, crown-rump distances, incidence of external malformations, or the incidence of fetal soft-tissue and fetal skeletal malformations (Conaway et al. 1985). Similarly, exposure of rabbits during gestational days 6-18 to ≤ 8,000 ppm did not change the number of total nonviable fetuses (such as early or late resorptions or dead fetuses), viable implantations, percentage of pre- or postimplantation loss, fetal body weight, or sex ratio (Tyl 1989). This treatment did not increase the incidence of fetal malformations.

MTBE has been found to produce dose-dependent developmental effects in mice. Exposure for 10 gestational days to 250-2,500 ppm MTBE had no effects on percentage of resorption, percentage of live fetuses, mean fetal weights, crown-rump distances, incidence of external malformations, or the incidence of fetal soft-tissue malformations (Conaway et al. 1985). In this study, a slight but not statistically significant increase in fused sternebrae was observed in the offspring of all exposed groups, while no fused sternebrae were present in controls. Thus, while no treatment-related developmental effects were found in mice at ≤ 2,500 ppm (Conaway et al. 1985), exposure of mice to a higher concentration (8,000 ppm) of MTBE resulted in the following developmental effects: increased number of nonviable implantations per litter; increased late resorption; and reduced number of viable implantations, sex ratio (% male fetuses), and fetal body weight (Tyl and Neeper-Bradley 1989). Mice exposed to 8,000 ppm MTBE also exhibited a significant increase in the incidence of cleft palate and a significant reduction in the incidence of partial fetal atelectasis. Skeletal malformations included the following regions: cervical centra, thoracic centra, caudal centra, skull plates/bones, forepaws, hindpaws, and sternebrae. At 4,000 ppm, there was an increased incidence of reduced skeletal ossification, and reduced fetal body weight. 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 central nervous system depression at 4,000 and 8,000 ppm. The 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 Endocrine Effects in Section 2.2.1.2), which may produce cleft palate in susceptible strains of mice.

Long-term exposure of male and female rats to 250-2,500 ppm MTBE during premating and gestational periods for a total of 16-28 weeks (without exposing the litters) had no effect on the offspring when the following parameters were determined: pup viability, mean pup body weight, and renal pelvises (Biles et al. 1987). Exposure of rats for 10 weeks prior to mating, 3 weeks during gestation, and 3 weeks during postnatal period to 400, 3,000, or 8,000 ppm MTBE had no effects on pups except for reduced body weights at 3,000 and 8,000 ppm (Neeper-Bradley 1991). Maternal exposure to MTBE did not affect the total number of live and stillborn F_1 or F_2 litter size or sex ratio. MTBE did not affect F_1 or F_2 pup live birth and survival indices. At 8,000 ppm, pup weights were significantly reduced in F_2 pups from lactational day 7 to lactational day 28. Significant weight reductions were also observed in F_2 pups from lactational day 14 to lactational day 28 at 3,000 ppm. Significant weight gain reductions were observed during most of the lactational period at 3,000 and 8,000 ppm.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and for each duration category are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to MTBE.

Whole body inhalation exposure of male and female Fischer 344 rats to 800, 4,000, and 8,000 ppm MTBE vapor for 6 hours per day for 5 days resulted in no concentration-related increase or significant increase in the number of chromosomal aberrations at any concentration compared with unexposed controls (Vergnes and Morabit 1989). Similarly, no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes (Vergnes and Kintigh 1993) or increases in DNA repair in cultured primary hepatocytes (Vergnes and Chun 1994) were found in male or female CD-l mice exposed to 400, 3,000, or 8,000 ppm MTBE for 6 hours per day for 1 or 2 days compared with controls. 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. Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8. Cancer

No studies were located regarding cancer in humans after inhalation exposure to MTBE.

In a 24-month inhalation study, in which rats were intermittently exposed to 400, 3,000, or 8,000 ppm MTBE, increased mortality occurred in males exposed to 3,000 and 8,000 ppm due to chronic progressive nephropathy (Chun et al. 1992). These groups were terminated at 97 and 82 weeks, respectively. Increased incidences of renal tubular cell adenoma and carcinoma were observed in male rats at 3,000 and 8,000 ppm. The incidences of renal tubular adenoma and carcinoma (combined) were 1 of 50 in controls, 0 of 50 at 400 ppm, 8 of 50 at 3,000 ppm, and 3 of 50 at 8,000 ppm. Renal tubular cell carcinomas were observed only in the 3,000 ppm group at an incidence of 3 of 50. The increase was statistically significant at 3,000 ppm ($p=0.015$), although not at 8,000 ppm. Since the rats in these groups had increased mortality and decreased survival time, it is possible that the number of tumor-bearing rats might have increased if they had survived longer. These renal tumors may have resulted from accumulation of α_{2u}-globulin or other protein unique to male rats (see Renal Effects in Section 2.2.1.2) in the renal tubular cells. In addition, a dose-related increased incidence of interstitial cell adenoma in the testes was observed at 3,000 and 8,000 ppm. The incidence was 32 of 50 in controls, 33 of 50 at 400 ppm, 41 of 50 at 3,000 ppm, and 47 of 50 at 8,000 ppm. However, Fischer 344 rats commonly develop testicular tumors, as seen from the high incidence in the concurrent control group. Furthermore, the incidences in the mid- and high-dose groups were in the range of historic controls, while the incidence in the concurrent control group was lower than the incidence in historic controls.

In an 18-month inhalation study, in which mice were intermittently exposed to 400, 3,000, or 8,000 ppm MTBE, an increased incidence of hepatocellular adenoma was observed in female mice at 8,000 ppm (10 of 50 compared with 2 of 50 in controls, 1 of 50 at 400 ppm, and 2 of 50 at 3,000 ppm) (Burleigh-Flayer et al. 1992). Male mice exposed to 8,000 ppm also had increased incidences of hepatocellular adenoma and carcinoma. The incidences of the hepatic tumors (adenoma and carcinoma combined) were 12 of 49 in controls, 12 of 50 at 400 ppm, 12 of 50 at 3,000 ppm and 16 of 49 at 8,000 ppm. The incidences of hepatocellular carcinoma alone in male mice were 2 of 49 in controls and 8 of 49 at 8,000 ppm ($p=0.045$). The incidence of combined hepatocellular adenoma and carcinoma in high-dose males was reported to be within the range seen in historic controls; therefore, the authors suggested that the increased incidence of hepatic neoplasms in male mice may not have been due to MTBE exposure. However, the increased incidence of hepatocellular carcinoma in the high-dose males was statistically significant compared with the concurrent control, suggesting that the increase was due to MTBE exposure.

Furthermore, the increased incidence of hepatocellular adenoma in high-dose females was attributed to MTBE exposure.

The cancer effect levels (CELs) are recorded in Table 2- 1 and plotted in Figure 2-1.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to MTBE.

MTBE has a low oral acute toxicity in experimental animals. Its oral LD_{50} (lethal dose, 50% kill) was determined by administering the undiluted MTBE by gavage to rats of both sexes and was found to be 3,866 mg/kg (ARCO 1980). The low acute toxicity of MTBE has also been documented in the mouse with a reported oral LD_{50} of 4,000 mg/kg (Little et al. 1979). No treatment-related deaths were observed in rats given MTBE by gavage in water at 400 mg/kg (Bioresearch Labs 1990a) or in rats given MTBE by gavage in oil at $\leq$ 1,428 mg/kg/day for 14 days (Robinson et al. 1990). No deaths in male or female rats resulted from oral gavage doses of 90-l,750 mg/kg/day MTBE for 4 weeks (ITT Research Institute 1992) or 100-l,200 mg/kg/day for 90 days (Robinson et al. 1990). Similarly, no deaths in male or female mice resulted from oral gavage doses of $\leq$ 1,000 mg/kg/day for 3 weeks (Ward et al. 1994). However, in a 104-week carcinogenicity study, in which male and female rats were given gavage doses of 250 or 1,000 mg/kg/day, 4 days per week, a dose-related increased mortality was observed in female rats, beginning at 16 weeks from the start of the study (Belpoggi et al. 1995). The LD_{50} values in rats and mice and the intermediate LOAEL value for increased mortality in female rats are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding systemic effects in humans after oral exposure to MTBE. The systemic effects observed in animals after oral exposure to MTBE are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Table 2-2. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Rat (NS)	once (G)				3866 (LD_{50})	ARCO 1980
2	Mouse (NS)	once (G)				4000 (LD_{50})	Little et al. 1979
	Systemic						
3	Rat (NS)	once (G)	Resp			4080 (labored respiration)	ARCO 1980
4	Rat (Sprague-Dawley)	14 d 7 d/wk 1 x/d (GO)	Resp	1428			Robinson et al. 1990
			Cardio	1428			
			Gastro		357 (diarrhea)		
			Hemato		357 M (decreased monocytes)		
			Hepatic	1428 F 714 M	1071 M (increased SGOT and lactic dehydrogenase) 1428 F (decreased BUN)		
			Renal	1071 M 1428 F	1428 M (increased hyaline droplets)		
			Endocr	1428			
			Bd Wt	714 F 357 M	1071 F (unspecified reduced 714 M weight gain)		
			Other	1071 M 357 F	1428 M (elevated 714 F cholesterol)		

Table 2-2. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference
5	Rat (Sprague-Dawley)	once (GO)	Gastro		100	(diarrhea)			Robinson et al. 1990
	Immunological/Lymphoreticular								
6	Rat (Sprague-Dawley)	14 d 7 d/wk 1 x/d (GO)		1428					Robinson et al. 1990
	Neurological								
7	Rat (NS)	once (G)			1900	(slight to marked CNS depression)	2450	(ataxia)	ARCO 1980
8	Rat (Fischer 344)	once (GW)		40[b]	400	(drowsiness)			Bioresearch Labs 1990b
9	Rat (Sprague-Dawley)	once (G)			90	(salivation)	440 M 1750 F	(hypoactivity and/or ataxia)	ITT Research Institute 1992
10	Rat (Sprague-Dawley)	1-14 d 7 d/wk 1 x/d (GO)		1071			1428	(profound but transient anesthesia)	Robinson et al. 1990
11	Rat (Sprague-Dawley)	once (GO)		900			1200	(profound but transient anesthesia)	Robinson et al. 1990
	Reproductive								
12	Rat (Sprague-Dawley)	14 d 7 d/wk 1 x/d (GO)		1428					Robinson et al. 1990

Table 2-2. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Death						
13	Rat (Sprague-Dawley)	16 wk 4 d/wk 1 x/d (GO)				250 F (increased mortality)	Belpoggi et al. 1995
	Systemic						
14	Rat (Sprague-Dawley)	4 wk 5 d/wk 1 x/d (G)	Resp	1750			ITT Research Institute 1992
			Cardio	1750			
			Gastro	440		1750 (inflammation submucosal edema, epithelial hyperplasia, stomach ulcers)	
			Hemato	1750			
			Musc/skel	1750			
			Hepatic	440	1750 (increased relative liver weight)		
			Renal	90 M	440 M (hyaline droplets in proximal convoluted tubules and increased relative kidney weight)		
				1750 F			
			Endocr	1750			
			Dermal	1750			
			Ocular	1750			
			Bd Wt	1750			
			Other	440	1750 (increased serum cholesterol)		

Table 2-2. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
15	Rat (Sprague-Dawley)	90 d 7 d/wk 1 x/d (GO)	Resp	1200			Robinson et al. 1990
			Cardio	1200			
			Gastro		100	(diarrhea)	
			Hemato	900	1200	(increased monocytes, and decreased MCV in males, increased RBC, Hb, Hct, and decreased WBC in females)	
			Hepatic		100 c	(decreased BUN values)	
			Renal	900 M	1200 M	(hyaline droplets, granular casts)	
				1200 F			
			Endocr	1200			
			Bd Wt	1200			
			Other	300 M	900 M 100 F	(elevated cholesterol levels)	
16	Mouse (CD-1)	3 wk 5 d/wk (GO)	Bd Wt	1000			Ward et al. 1994
	Immunological/Lymphoreticular						
17	Rat (Sprague-Dawley)	4 wk 5 d/wk 1 x/d (G)		1750			ITT Research Institute 1992

Table 2-2. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
18	Rat (Sprague-Dawley)	90 d 7 d/wk 1 x/d (GO)		1200			Robinson et al. 1990
	Reproductive						
19	Rat (Sprague-Dawley)	4 wk 5 d/wk 1 x/d (G)		1750			ITT Research Institute 1992
20	Rat (Sprague-Dawley)	90 d 7 d/wk 1 x/d (GO)		1200			Robinson et al. 1990
21	Mouse (CD-1)	3 wk 5 d/wk (GO)		1000			Ward et al. 1994

Table 2-2. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	CHRONIC EXPOSURE						
	Systemic						
22	Rat (Sprague-Dawley)	104 wk 4 d/wk 1 x/d (GO)	Resp	1000			Belpoggi et al. 1995
			Cardio	1000			
			Gastro	1000			
			Musc/skel	1000			
			Hepatic	1000			
			Renal	1000			
			Endocr	1000			
			Dermal	1000			
			Bd Wt	1000			
	Immunological/Lymphoreticular						
23	Rat (Sprague-Dawley)	104 wk 4 d/wk 1 x/d (GO)		1000 M		250 F (dysplastic proliferation of lymphoreticular tissues, possibly preneoplastic)	Belpoggi et al. 1995
	Reproductive						
24	Rat (Sprague-Dawley)	104 wk 4 d/wk 1 x/d (GO)		1000			Belpoggi et al. 1995

Table 2-2. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
	Cancer							
25	Rat (Sprague-Dawley)	104 wk 4 d/wk 1 x/d				250 F	(CEL - lymphoma and leukemia)	Belpoggi et al. 1995
		(GO)				1000 M	(CEL - testicular Leydig cell tumor)	

[a]The number corresponds to entries in Figure 2-2.

[b]Used to derive an acute oral minimal risk level (MRL) of 0.4 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive an intermediate oral MRL of 0.1 mg/kg/day; dose divided by an uncertainty factor of 300 (3 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CNS = central nervous system; d = day(s); Endocr = endocrine; F = female; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; (GW) = gavage in water; Hb = hemoglobin; Hct = hematocrit; Hemato = hematological; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; RBC = red blood cell; Resp = respiratory; SGOT = serum glutamic oxaloacetic transaminase; WBC = white blood cell; wk = week(s); x = times

Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) - Oral

Acute (≤14 days)

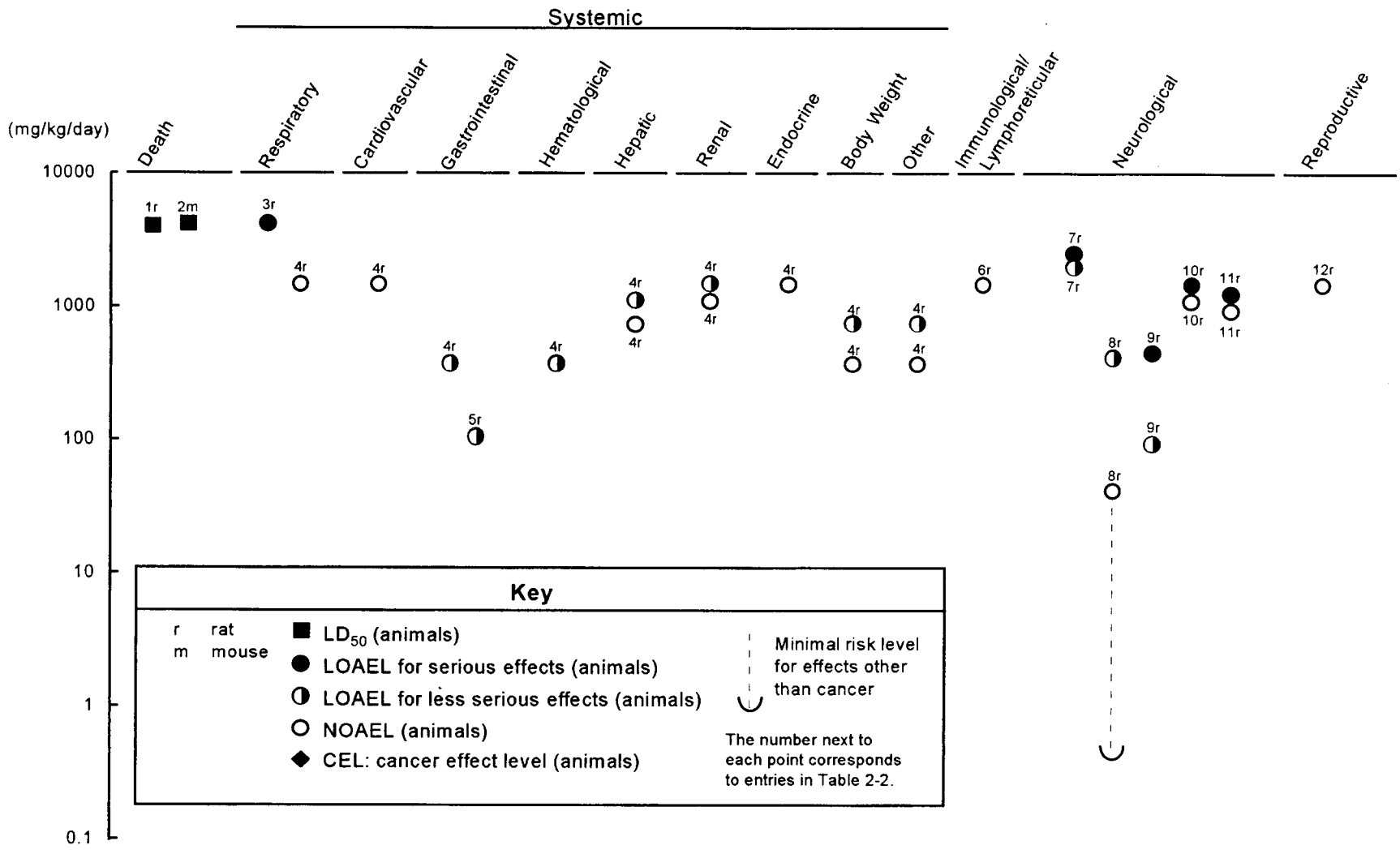


Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) - Oral (cont.)

Intermediate (15-365 days)

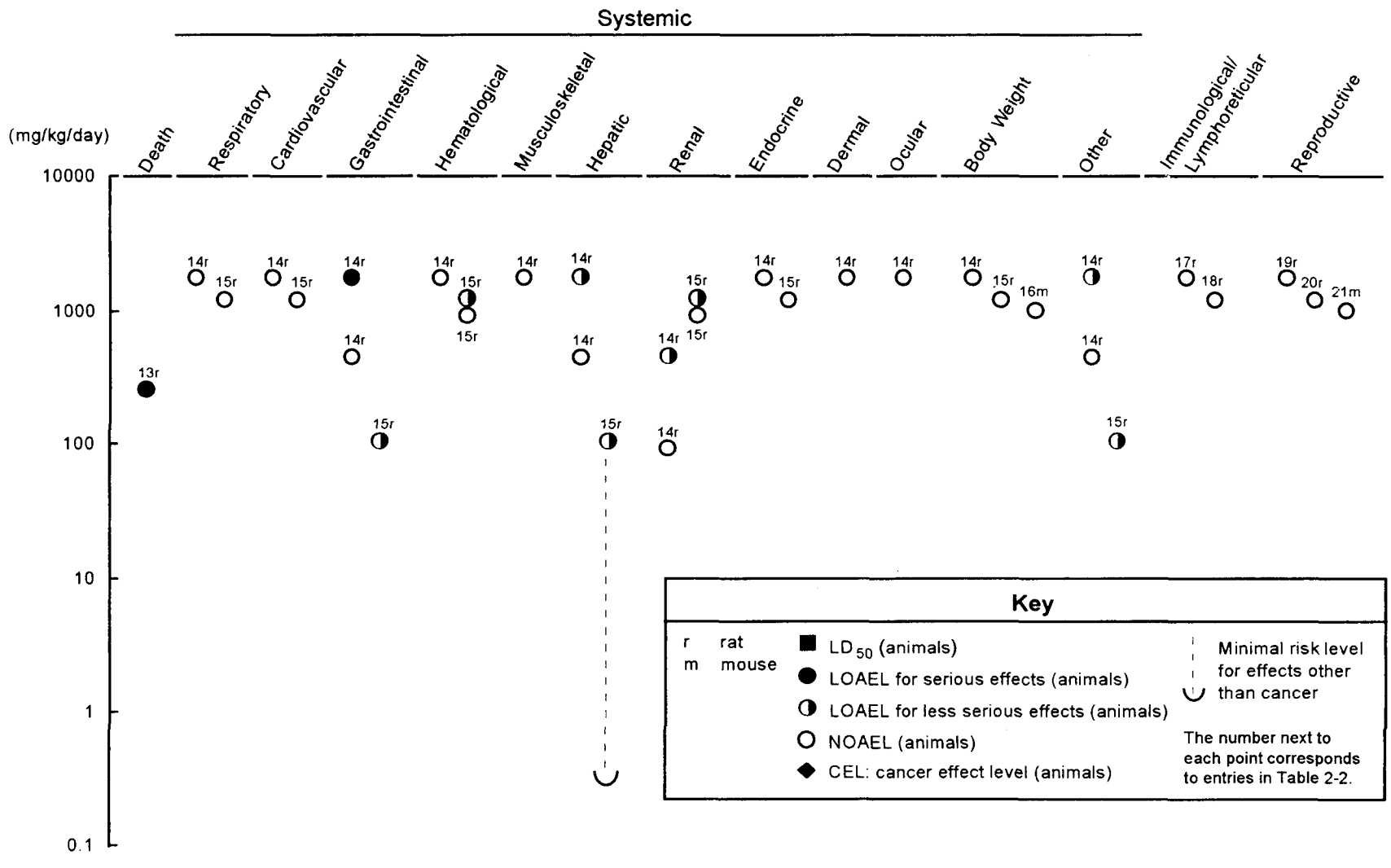
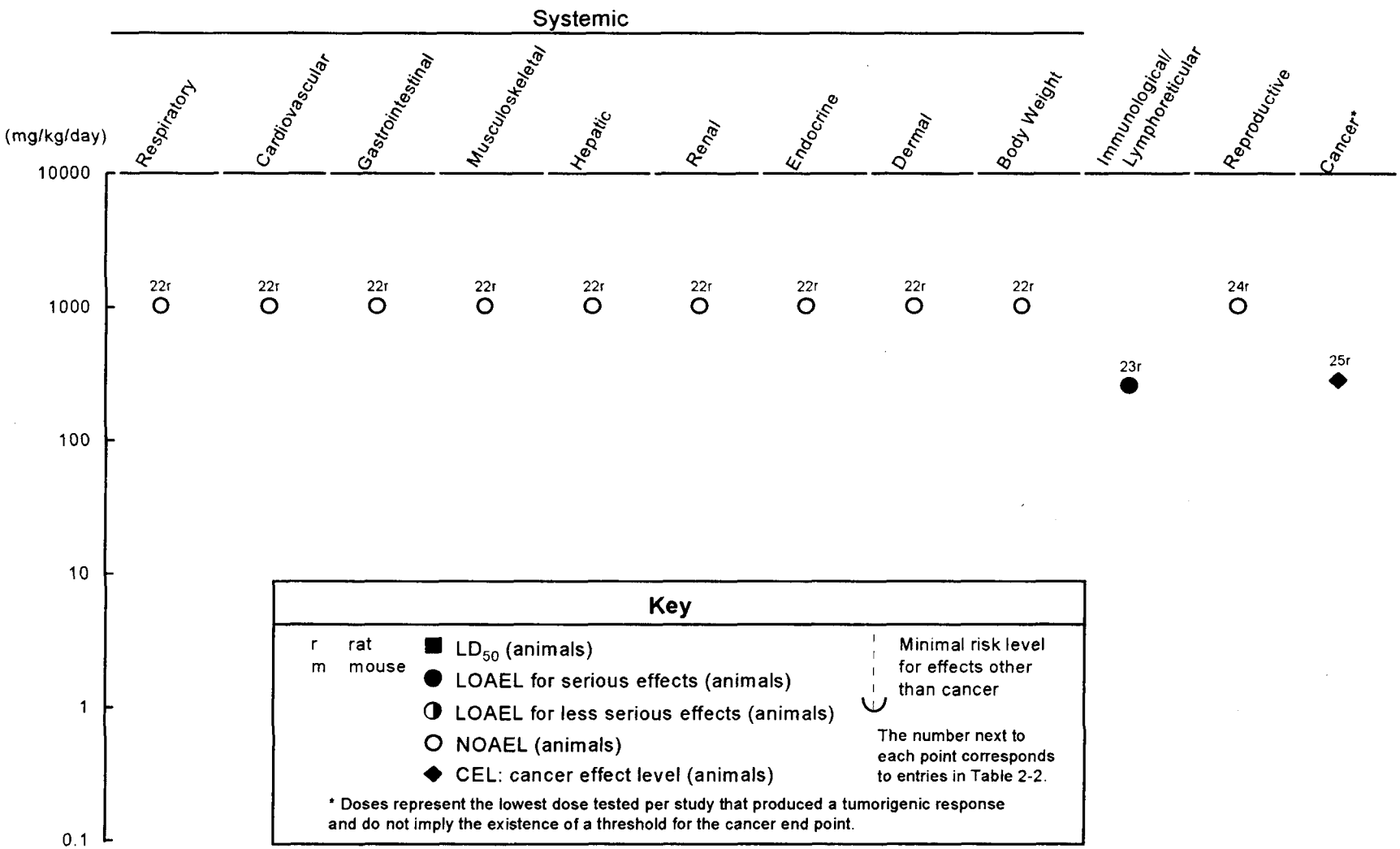


Figure 2-2. Levels of Significant Exposure to Methyl *tert*-Butyl Ether (MTBE) - Oral (cont.)

Chronic (≥365 days)



Respiratory Effects. High oral doses (≥ 4,080 mg/kg) of MTBE caused labored respiration in rats (ARCO 1980). This treatment, however, did not produce grossly observable changes in the major organ systems at 1,900 mg/kg, and at higher doses (≥ 2,450 mg/kg) the few gross signs were attributed to the irritating nature of MTBE. In rats given daily oral doses of 357, 714, 1,071, or 1,428 mg/kg/day for 14 days, absolute and relative lung weight was decreased at all doses in female rats but only at 714 mg/kg/day in male rats (Robinson et al. 1990). Histological examination of the lungs, however, revealed no treatment-related lesions at any dose: No gross lesions were found in the lungs, larynx, nasal turbinates, or trachea, and no histopathological lesions were found in the lungs or trachea (other respiratory tissues were not examined microscopically) of rats given ≤ 1,750 mg/kg/day for 4 weeks (ITT Research Institute 1992). Similarly, while absolute and relative lung weights were significantly increased in males gavaged with 1,200 mg/kg/day MTBE for 90 days, no significant changes in lung weights were found in female rats, and no treatment-related histopathological lesions were found in the lungs of either sex at any dose (100-l,200 mg/kg/day) (Robinson et al. 1990). 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 per week, histological examination of the nasal cavity, pharynx, larynx, diaphragm, trachea, lung, and mainstream bronchi revealed no treatment-related lesions (Belpoggi et al. 1995).

Cardiovascular Effects. Based on histological examination of the heart and aorta of rats, oral exposure to MTBE appears to be without effects on the cardiovascular system. Daily oral administration of ≤ 1,428 mg/kg/day MTBE for 14 days had no significant effects on heart weights and did not produce histopathological lesions in the heart (Robinson et al. 1990). Histological examination of the heart and aorta of rats given ≤ 1,750 mg/kg/day for 4 weeks revealed no treatment-related lesions (ITT Research Institute 1992). While heart weights were significantly increased in female rats given a daily oral dose of 900 mg/kg/day MTBE for 90 days, no accompanying pathological lesions were found in the heart of either sex at doses ≤ 1,200 mg/kg/day (Robinson et al. 1990). 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 per week, histological examination of the heart revealed no treatment-related lesions (Belpoggi et al. 1995).

Gastrointestinal Effects. MTBE appears to be irritating to the gastrointestinal tract of rats as evidenced by diarrhea and histological lesions. Rats treated with daily oral doses ≥ 357 mg/kg/day MTBE had diarrhea by the third day of dosing, which continued throughout the 14-day treatment period (Robinson et al. 1990). Similarly, when MTBE was orally administered daily to rats for 90 days at ≥ 100 mg/kg/day, it produced diarrhea immediately after dosing throughout the exposure period (Robinson et al. 1990). Daily oral administration for 4 weeks of 1,750 mg/kg/day MTBE to rats produced no gross

pathological changes in the duodenum, jejunum, ilium, cecum, colon, rectum, salivary glands, stomach, or tongue (ITT Research Institute 1992). Histopathological changes, however, were seen as submucosal edema, subacute inflammation, epithelial hyperplasia, and ulceration in the stomach of male and female rats given 1,750 mg/kg/day MTBE. 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 per week, histological examination of the oral cavity, salivary glands, tongue, esophagus, stomach (fore and glandular), and intestines (4 levels) revealed no treatment-related lesions (Belpoggi et al. 1995).

Hematological Effects. In some male rats treated with daily oral doses of MTBE for 14 days, elevated red blood cell, hemoglobin, hematocrit, and lymphocyte values were seen at doses $\geq$ 714 mg/kg/day, but monocyte values were significantly reduced at $\geq$ 357 mg/kg/day (Robinson et al. 1990). Male rats given an oral dose of 440 mg/kg/day MTBE for 4 weeks had significantly increased mean red blood cell counts, while female rats given 90 and 1,750 mg/kg/day had significantly increased MCH (ITT Research Institute 1992). 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. In a 90-day oral administration study, male rats had increased monocytes and decreased MCV at 1,200 mg/kg/day (Robinson et al. 1990). Female rats given 1,200 mglkglday MTBE showed elevated levels of erythrocytes, hemoglobin, and hematocrit, while leukocyte counts were significantly reduced. This hemoconcentration might have resulted from the clinical dehydration condition associated with diarrhea observed in these animals instead of being a direct effect of MTBE.

Musculoskeletal Effects. Daily oral administration of $\leq$ 1,750 mg/kg MTBE to rats for 4 weeks did not produce gross pathology in the femur, sternum (bone marrow) or bone marrow smear, or skeletal muscle, nor histopathological changes in skeletal muscle or in the sternum (femur and bone marrow smears were not examined microscopically) (ITT Research Institute 1992). 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 per week, histological examination of the cranium (5 levels) revealed no treatment-related lesions (Belpoggi et al. 1995).

Hepatic Effects. Relative, but not absolute, liver weights were increased in rats given a daily large oral dose (1,428 mg/kg/day) of MTBE for 14 days (Robinson et al. 1990). Although this treatment did not produce histopathological lesions in the liver, it significantly increased SGOT and lactic dehydrogenase levels in male rats at 1,071 mg/kg/day and significantly decreased BUN levels in female rats at 1,428 mg/kg/day. In the 90-day study, relative liver weights were significantly increased in female rats at

900 mg/kg/day and in male rats at 900 and 1,200 mg/kg/day (Robinson et al. 1990). BUN levels were significantly decreased in both males and females at ≥ 100 mg/kg/day. Serum lactic dehydrogenase was significantly elevated in females only at 300 mg/kg/day, and SGOT was significantly elevated in males at 300 and 1,200 mg/kg/day. However, no treatment-related histopathological lesions were found in the liver in either the 14-day study or the 90-day study. Liver weight was increased in rats following daily oral doses of 1,750 mg/kg/day for 4 weeks of MTBE in rats (ITT Research Institute 1992). No treatment- related gross or microscopic lesions were seen in the liver. 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 per week, histological examination of the liver revealed no treatment-related lesions (Belpoggi et al. 1995). Based on the LOAEL of 100 mg/kg/day for decreased BUN levels in male and female rats in the 90-day study (Robinson et al. 1990), an intermediate-duration oral MRL of 0.3 mg/kg/day was calculated as described in the footnote to Table 2-2.

Renal Effects. Large daily oral doses of MTBE for 14 days increased relative but not absolute kidney weight in rats at 1,428 mg/kg/day (Robinson et al. 1990). Serum creatinine was significantly elevated in male rats, while being reduced in females treated with MTBE. The toxicological significance of this change is not clear. Daily oral treatment with 1,428 mg/kg/day of MTBE for 14 days also resulted in a renal tubular disorder characterized by increased hyaline droplets in male rats (Robinson et al. 1990). Similar results were obtained following an oral dose of 440 mg/kg/day of MTBE for 4 weeks (ITT Research Institute 1992). Severe tubular changes, which consisted of mild increases in cytoplasmic hyaline droplets in proximal tubular cells and small numbers of intratubular granular casts at the junction of the outer and inner stripe of the outer medulla, were seen in male rats, but not female rats, following gavage doses of 1,200 mg/kg/day MTBE, but not ≤ 900 mg/kg/day (Robinson et al. 1990). However, 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 per week, histological examination of the kidneys and urinary bladder revealed no treatment-related lesions (Belpoggi et al. 1995).

Endocrine Effects. Daily oral administration of MTBE for 14 days neither affected adrenal gland weights nor did it produce histopathological lesions in the adrenals (Robinson et al. 1990). On the other hand, female rats given 1,200 mg/kg/day MTBE for 90 days showed significantly elevated relative adrenal gland weight, but no histopathological lesions were observed in males or females at ≤ 1,200 mg/kg/day (Robinson et al. 1990). In a 4-week study, a dose of 1,750 mg/kg/day in rats resulted in a significant increase in relative adrenal weight in males, but no gross or histopathological lesions were found in the adrenal gland, pancreas, parathyroids, pituitary, or thyroid (ITT Research Institute 1992). Similarly, 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 per week, histological examination of the pituitary gland, Zymbal gland, thyroid, parathyroid, pancreas, and adrenal glands revealed no treatment-related lesions (Belpoggi et al. 1995).

Dermal Effects. No gross or histopathological lesions were found on the skin of rats dosed orally with 1,750 mg/kg/day MTBE for 4 weeks (ITT Research Institute 1992). Similarly, 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 per week, histological examination of the skin and subcutaneous tissues revealed no treatment-related lesions (Belpoggi et al. 1995).

Ocular Effects. No gross lesions were found in the eyes, exorbital lachrymal glands, or Harderian glands, and no histopathological lesions were found in the eyes (glands not examined microscopically) in rats dosed orally with $\leq$ 1,750 mg/kg/day MTBE for 4 weeks (ITT Research Institute 1992).

Body Weight Effects. High daily oral doses of MTBE for 14 days caused significant decreases in body weight of female rats and in weight gain of males (Robinson et al. 1990); however, the percentage decrease can not be determined from the data. These results may be related to the significant decrease in food intake in treated rats, secondary to the hypoactivity induced by MTBE (see Section 2.2.2.4). In contrast, daily oral doses of up to 1,750 mg/kg/day for 4 weeks (ITT Research Institute 1992) or 1,200 mg/kg/day for 90 days (Robinson et al. 1990) did not cause any body weight change in treated rats. In addition, no changes in body weight were found in mice given $\leq$ 1,000 mg/kg/day MTBE by gavage 5 days per week for 3 weeks (Ward et al. 1994). Similarly, in a 104-week study, in which male and female rats were given gavage doses of 250 or 1,000 mg/kglday, 4 days per week, no treatment-related differences between treated and control rats were found for water and food consumption or body weight changes (Belpoggi et al. 1995).

Other Systemic Effects. Elevated cholesterol levels were consistent findings in rats dosed orally with 714 mg/kg/day (females) or 1,428 mg/kg/day (males) for 14 days (Robinson et al. 1990), 1,750 mg/kglday for 4 weeks (ITT Research Institute 1992), and 100 mg/kg/day (females) or 900 mg/kg/day (males) for 90 days (Robinson et al. 1990).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans after oral exposure to MTBE.

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 males (Robinson et al. 1990). This treatment did not produce histopathological lesions in the spleen or thymus. Similar results were obtained following 90 days treatment with an oral daily dose of 100 to 1,200 mg/kg MTBE (Robinson et al. 1990). Also, rats given ≤ 1,750 mg/kg/day MTBE orally for 4 weeks had no pathology in the bone marrow, mesenteric lymph nodes, mandibular lymph nodes, spleen, or thymus (ITT Research Institute 1992). This treatment did not produce microscopic histopathological changes in all tissues examined (i.e., mesenteric lymph nodes, spleen, or thymus). However, 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 per week, an increased incidence of dysplastic proliferation of lymphoreticular tissues (hyperplastic lymphoid tissues, at various body sites, consisting of atypical lymphoid cells, usually lymphoimmunoblasts) was observed in females at both doses (Belpoggi et al. 1995). 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 2.2.2.8), 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. The highest NOAEL values and the LOAEL value for lymphoreticular effects in rats in each study in each duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to MTBE.

The immediate acute effect of orally administered MTBE is on the central nervous system. Acute oral doses caused marked central nervous system depression, ataxia, tremors, labored breathing, and loss of righting reflex in rats at doses ≥ 4,080 mg/kg, loss of righting reflex and ataxia at 3,160 mg/kg, ataxia at 2,450 mg/kg and slight to marked central nervous system depression at 1,900 mg/kg (ARCO 1980). Onset of neurological signs was rapid, but they disappeared or were markedly reduced within 24 hours. Another study in rats reported drowsiness, which subsided within 24 hours after a single oral dose of 400 mg/kg

(Bioresearch Labs 1990b). A NOAEL of 40 mg/kg for neurological effects for acute oral exposure was identified in the Bioresearch Labs (1990b) study. Based on this NOAEL value, an acute oral MRL of 0.4 mg/kg/day was calculated as described in the footnote to Table 2-2. Large daily oral doses of 1,428 mg/kg/day MTBE for l-14 days in rats also resulted in profound anesthesia soon after treatment, with subsequent recovery of motor and sensory functions within 2 hours of dosing (Robinson et al. 1990). Male rats given 1,428 mg/kg/day for 14 days had significantly reduced brain weights, while female rats given the same dose had significantly increased relative brain weights. However, no histopathological lesions were found in the brain. Anesthesia was also produced in rats immediately after dosing with 1,200 mg/kg/day MTBE every day in a 90-day oral administration study, followed by recovery in approximately 2 hours (Robinson et al. 1990). This treatment did not significantly affect brain weight nor did it produce histopathological lesions in the brain. Daily oral administration of 90, 440, and 1,750 mg/kg/day MTBE for 4 weeks caused salivation in treated rats (ITT Research Institute 1992). In addition, hypoactivity and/or ataxia were observed in rats given the two highest doses. Because all signs of neurotoxicity in the 90-day study by Robinson et al. (1990) and in the 4-week study by ITT Research Institute (1992) occurred shortly after dosing, they are considered signs of acute toxicity. No histopathological lesions were seen in the brain, spinal cord, or sciatic nerve at the termination of the 4-week study (ITT Research Institute 1992). 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 per week, no clinical signs of behavioral changes and no histopathological brain lesions were observed (Belpoggi et al. 1995). The NOAEL value and all LOAEL values for neurological effect in rats for the acute-duration category are recorded in Table 2-2 and plotted in Figure 2-2. Although no histopathological effects in the brains were observed in intermediate- and chronic-duration studies, the doses have been associated with clinical signs of neurological effects; therefore, these doses are not presented as NOAEL values for neurological effects in the intermediate- and chronic-duration categories.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to MTBE.

Daily oral administration of 357 to 1,428 mg/kg/day MTBE in rats for 14 days did not affect testicular or ovarian weights, nor did it produce histopathological lesions in the testes or ovaries (Robinson et al. 1990). Treatment of male and female mice with $\leq$ 1,000 mg/kg/day MTBE by gavage for 3 weeks resulted in no effects on the frequency of germ cells in the testes or ovaries (Ward et al. 1994). Daily oral dosing of 90 to 1,750 mg/kg/day MTBE for 4 weeks in rats produced no gross pathology in the epididymides,

mammary gland, prostate, seminal vesicles, testes, uterus, vagina, or ovaries (ITT Research Institute 1992). Histological examination revealed no treatment-related lesions in these organs (vagina not examined microscopically). Also, a 90-day treatment with daily oral doses of 100-1,200 mg/kg/day MTBE in rats had no significant effect on absolute testicular or ovarian weights (Robinson et al. 1990). This treatment did not produce histopathological lesions in the testes or ovaries. 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 per week, histological examination of the prostate, uterus, and gonads revealed no nonneoplastic treatment-related lesions; however, a significantly increased incidence of testicular Leydig cell tumors was found in the high-dose males (see Section 2.2.2.8) (Belpoggi et al. 1995).

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after oral exposure to MTBE.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to MTBE.

MTBE did not cause chromosomal aberrations in the bone marrow of Sprague-Dawley rats that were gavaged with 0.04, 0.13, or 0.4 ml/kg/day (30, 96, or 296 mg/kg/day) once or for 5 days (ARCO 1980). Similarly, no significant induction of chromosome aberrations in spleen lymphocytes and no evidence of a dose-related increase in hypoxanthine-guanine phosphoribosyl transferase (*hprt*) mutant frequency in spleen lymphocytes were found in mice treated with $\leq$ 1,000 mg/kg/day MTBE by gavage for 3 weeks (Ward et al. 1994). Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to MTBE.

In a 104-week carcinogenicity study, in which male and female rats were given MTBE in oil by gavage at 250 or 1,000 mg/kg/day, 4 days per week, a dose-related increased incidence of lymphomas and leukemia was observed in female rats (2 of 60 in controls, 6 of 60 at 250 mg/kg/day, 12 of 60 at 1,000 mg/kg/day) (Belpoggi et al. 1995). When expressed as the incidence in the female rats alive at 56 weeks of age, when

the first leukemia was observed, these incidences were 2 of 58 (3.4%) in controls, 6 of 51 (11.8%) at 250 mg/kg/day, and 12 of 47 (25.5%) at 1,000 mg/kg/day. The increased incidence was statistically significant ($p<0.0l$) at both dose levels compared with controls. As noted in Section 2.2.2.4, there was also an increased incidence in dysplastic proliferation of lymphoreticular tissues in female rats, which was greater in the low-dose group than in the high-dose group, suggesting that the dysplastic proliferation was preneoplastic. An increase in uterine sarcomas was found in the females only at the low dose (1 of 60 in controls, 5 of 60 at 250 mg/kg/day, and 0 of 60 at 1,000 mg/kg/day). In male rats, there was a statistically significant ($p<0.05$) increased incidence of testicular Leydig cell tumors at the high dose (2 of 60 in controls, 2 of 60 at 250 mg/kg/day, and 11 of 60 at 1,000 mg/kg/day). When expressed as the incidence in male rats alive at 96 weeks of age, when the first Leydig cell tumor was observed, the incidence was 2 of 26 (7.7%) in controls, 2 of 25 (8.0%) at 250 mg/kg/day, and 11 of 32 (34.4%) at 1,000 mg/kg/day.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans following dermal exposure to MTBE.

No deaths occurred in rats exposed to MTBE dermally at $\leq$ 400 mg/kg for 6 hours (Bioresearch Labs 1990b) or in rabbits exposed to MTBE dermally at 10,000 mg/kg for 24 hours (ARCO 1980).

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, hepatic, renal, endocrine, or body weight effects in humans or animals after dermal exposure to MTBE. Studies on dermal and ocular effects in humans and gastrointestinal, dermal, and ocular effects in animals after dermal exposure to MTBE are discussed below. The highest NOAEL values and all LOAEL values from each reliable study in each species and duration category are recorded in Table 2-3.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after dermal exposure to MTBE.

Table 2-3. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
ACUTE EXPOSURE						
Systemic						
Human	1 hr	Dermal	1.7 *ppm*			Cain et al. 1994
		Ocular	1.7 ppm			
Human	1 hr	Dermal	1.39 ppm			Prah et al. 1994
		Ocular	1.39 ppm			
Rat (NS)	4 hr	Ocular		18892 ppm	(eye discharge)	ARCO 1980
Rat (Sprague-Dawley)	9 d 5 d/wk 6 hr/d	Ocular		100 ppm	(lacrimation, conjunctival swelling)	Biodynamics 1981
Rat (Fischer 344)	6 hr	Gastro		40 mg/kg	(light diarrhea)	Bioresearch Labs 1990b
Mouse (CD-1)	10 d Gd 6-15 6 hr/d	Ocular		250 F ppm	(slightly increased lacrimation)	Conaway et al. 1985
Mouse (CD-1)	10 d Gd 6-15 6 hr/d	Ocular	4000 F ppm	8000 F ppm	(increased lacrimation *and periocular* encrustation)	Tyl and Neeper-Bradley 1989

Table 2-3. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
Gn Pig (Hartley)	once	Dermal		0.5 mL (1% soln MTBE) (local irritation and increased erythema)		ARCO 1980
Rabbit (New Zealand)	24 hr	Dermal		0.5 mL (slight to severe erythema, acanthosis, and necrosis)		ARCO 1980
Rabbit (New Zealand)	24 hr	Dermal		10000 mg/kg (erythema, skin thickening, edema, and blanching)		ARCO 1980
Rabbit (New Zealand)	once	Ocular		0.1 mL (corneal opacities, chemosis, conjunctival redness, and discharge)		ARCO 1980
Rabbit (NS)	once	Ocular		0.05 mL (congestion of conjunctival, palpebral thickening, and hypersecretion)		Snamprogetti 1980
Immunological/Lymphoreticular						
Human	1 hr		1.7 ppm			Cain et al. 1994
INTERMEDIATE EXPOSURE						
Systemic						
Rat (Sprague-Dawley)	16-28 wk 5-7 d/wk 6 hr/d	Dermal	2500 ppm			Biles et al. 1987
		Ocular	2500 ppm			

Table 2-3. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
Rat (Fischer 344)	13 wk 5 d/wk 6 hr/d	Dermal	8000 ppm			Dodd and Kintigh 1989
		Ocular	8000 ppm			
Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Dermal	1000 ppm			Greenough et al. 1980
		Ocular	1000 ppm			
Rat (Sprague-Dawley)	14-19 wk 5-7 d/wk 6 hr/d	Ocular	3000 ppm	8000 ppm	(periocular encrustation and ocular discharge)	Neeper-Bradley 1991
Immunological/Lymphoreticular						
Gn Pig (Hartley)	3 wk 3-4 x/wk 1 x/d		0.5 mL (1% soln), then 0.1 mL			ARCO 1980
CHRONIC EXPOSURE						
Systemic						
Rat (Fischer 344)	24 mo 5 d/wk 6 hr/d	Dermal	8000 ppm			Chun et al. 1992
		Ocular	400 M ppm 8000 F ppm	3000 M ppm	(swollen periocular tissue)	

Table 2-3. Levels of Significant Exposure to Methyl tert-Butyl Ether (MTBE) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL		Reference
				Less Serious (ppm)	Serious (ppm)	
Mouse (CD-1)	18 mo 5 d/wk 6 hr/d	Dermal	8000 ppm			Burleigh-Flayer et al. 1992
		Ocular	8000 ppm			

Bd Wt = body weight; d = day(s); F = female; Gastro = gastrointestinal; Gd = gestational day; Gn Pig = guinea pig; hr = hour(s); LOAEL = lowest-observable-adverse-effect level; M = male; mo = month(s); NOAEL = no-observable-adverse-effect level; NS = not specified; soln = solution; wk = week(s)

In a pharmacokinetic study, in which MTBE was applied dermally to the dorsal flank of rats via an occluded chamber, the rats developed slight diarrhea at a dermal dose of 40 mg/kg (Bioresearch Labs 1990b). Since the dermally applied MTBE was protected by occlusion, it is unlikely that oral uptake via grooming contributed to the absorbed dose.

Dermal Effects. Because inhalation exposure to chemicals may result in dermal effects due to direct contact of the vapor on the skin, relevant inhalation studies for dermal effects are presented briefly in Section 2.2.1.2, but are more fully discussed here. In a double-blind study, human volunteers were exposed sequentially to 1.7 ppm MTBE for 1 hour on 1 day, to uncontaminated air for 1 hour 2 days later, and to 7.1 ppm of a 17-component mixture of VOCs for 1 hour 2 days later (or the reverse sequence) (see Respiratory Effects in Section 2.2. I .2) (Cain et al. 1994). Statistical analysis of the results of questionnaires administered every 10 minutes during the various exposure conditions revealed no differences in reporting of skin rash or dry skin. In a similar study, 19 healthy men and 18 healthy women were exposed for 1 hour to clean air and 1.39 ppm MTBE in separate sessions separated by at least 1 week (Prah et al. 1994). The order of exposure was randomly selected, but because of the odor of MTBE, it is likely that the subjects were aware of the exposure conditions. Analysis of the results of the questionnaires administered prior to exposure, immediately upon entering the exposure chamber, after 30 minutes of exposure, and during the last 5 minutes of exposure revealed no differences for skin rash or dry skin.

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 2.2.3.4), 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).

It appears that direct application of liquid MTBE is required to produce these effects since exposure of the skin to MTBE vapors in air during inhalation studies did not result in dermal effects. Histological examination of skin of rats exposed to airborne MTBE at concentrations $\leq$ 8,000 ppm for 13 weeks revealed no treatment-related lesions (Dodd and Kintigh 1989; Greenough et al. 1980). Alopecia was commonly observed in rats exposed to $\leq$ 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, in which rats (Chun et al. 1992) and mice (Burleigh-Flayer et al.

1992) were exposed intermittently by inhalation to 400, 3,000, or 8,000 ppm MTBE, histological examination of the skin revealed no treatment-related lesions.

Ocular Effects. Because inhalation exposure to chemicals may result in ocular effects due to direct contact of the vapor to the eyes, relevant inhalation studies for ocular effects are briefly presented in Section 2.2.1.2, but are more fully discussed here. Eye irritation was among the symptoms reported by motorists or gas station workers during oxygenated fuel programs in which MTBE had been added to gasoline to reduce carbon monoxide emissions. In the preliminary investigation to determine whether symptoms were occurring, whether symptoms occurred in a consistent pattern, and whether symptoms could be related to exposure to oxyfuel in Fairbanks, Alaska, eye irritation was reported by 1 of 4 (25%) taxi drivers, 9 of 26 (35%) health-care workers, and 3 of 15 (20%) students who met the case definition (4 of 12 taxi drivers, 26 of 90 health-care workers, and 15 of 101 students) (Beller and Middaugh 1992). In the preliminary investigation in Anchorage, Alaska, eye irritation was reported by 8 of 12 (67%) taxi drivers and 13 of 36 (36%) health-care workers who met the case definition (12 of 25 taxi drivers and 36 of 137 health-care workers) (Chandler and Middaugh 1992).

In the more definitive CDC study in Fairbanks, Alaska, the frequency of eye irritation in the Phase I subjects, when the oxyfuel program was in full effect, was 12 of 18 for occupationally exposed workers (CDC 1993a; Moolenaar et al. 1994). In the Phase II subjects, after the oxyfuel program was terminated, the frequency of eye irritation was 2 of 28. In the CDC study in Stamford, Connecticut, when the oxyfuel program was in full effect (no Phase II in Stamford), the eye irritation was reported by 10 of 48 (20.8%) mechanics and gas station attendants, 4 of 57 (7%) professional drivers, 2 of 12 (16.7%) “other” workers, and 11 of 59 (18.6%) commuters (CDC 1993b; White et al. 1995).

In the study conducted in Albany, New York, although eye irritation was considered a key symptom and was included in the questionnaire, the percentage of subjects who reported eye irritation was not reported (CDC 1993c). However, it was noted that key symptoms were slightly more prevalent and the presence of 2 or more key symptoms was 2-3 times more prevalent in Group 2 (48 police officers, toll booth worker, and parking garage attendants) and Group 3 (182 office workers and college students) than in Group 1 (34 automobile repair shop workers and service station attendants), but the increases were not statistically significant. Thus, no increase in health complaints among people with higher gasoline exposures was detected.

In the cross-sectional cohort study of self-reported symptoms of garage workers in the state of New Jersey exposed to high (115 workers in northern New Jersey during the wintertime oxyfuel program) and low (122 workers in southern New Jersey 10 weeks after the phase-out date for oxyfuel program) MTBE concentration environments, the results of the questionnaire regarding eye irritation over the last 30 days showed no significant difference (22% in the northern workers and 27% in the southern workers, p=0.39) (Mohr et al. 1994).

In the telephone interview survey conducted in metropolitan Milwaukee, Wisconsin, metropolitan Chicago, Illinois (use of reformulated gasoline was required in both of these areas), and in the rest of Wisconsin exclusive of metropolitan Milwaukee (where the use of reformulated gasoline was not required), prevalence of eye irritation associated with MTBE exposure was statistically higher in Milwaukee than in either Chicago or Wisconsin; prevalence was not different between Chicago and Wisconsin for any symptom (Anderson et al. 1995). As discussed in the Respiratory Effects in Section 2.2.1.2, these results suggest that factors other than reformulated gasoline use, such as knowledge about reformulated gasoline and the likely awareness of potential negative effects, significantly contributed to the differences in symptom prevalence between Milwaukee and the other two areas.

In the double-blind study in humans exposed sequentially to 1.7 ppm MTBE for 1 hour on 1 day, to uncontaminated air for 1 hour 2 days later, and to 7.1 ppm of a 17-component mixture of VOCs for 1 hour 2 days later (or the reverse sequence), subjects were examined for ocular effects (eye redness, tear-film break-up time, epithelial damage to the eye, and staining of tear fluid for total number of cells and differential numbers of polymorphonuclear neutrophilic leukocytes, epithelial cells, monocytes, eosinophils, and lymphocytes) (Cain et al. 1994). The subjects were also administered questionnaires regarding subjective symptoms of eye irritation (dry, itching, or irritated eyes; tired or strained eyes; burning eyes). Statistical analysis revealed no increase in eye irritation due to MTBE exposure for the objective and subjective tests. In a similar study, 19 healthy men and 18 healthy women were exposed for 1 hour to clean air and 1.39 ppm MTBE in separate sessions separated by at least 1 week (Prah et al. 1994). The order of exposure was randomly selected, but because of the odor of MTBE, it is likely that the subjects were aware of the exposure conditions. Analysis of the results of the questionnaires administered prior to exposure, immediately upon entering the exposure chamber, after 30 minutes of exposure, and during the last 5 minutes of exposure revealed no differences for irritation of the eyes, tired or strained eyes, or burning eyes. In addition to the subjective reporting of ocular effects, tear film breakup time (the time from a blink to the appearance of discontinuities of the projected pattern) was determined pre- and postexposure using a keratoscope that projects a white-light pattern on concentric

rings onto the cornea. Hyperemia was determined using color slides of the temporal and nasal conjunctiva obtained pre- and postexposure. For increased ocular inflammation, the levels of mRNA coding for inflammatory mediators present in the conjunctival epithelial cells and the number of inflammatory cells present on the conjunctiva were assessed. No measures approached significance. (See Respiratory Effects in Section 2.2.1.2 for a more complete discussion of the human studies regarding protocols, limitations, and conclusions.)

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 the eyes were washed after exposure or not (ARCO 1980). ARCO MTBE, however, was more irritating than the commercial MTBE. ARCO MTBE induced cornea1 opacities, chemosis, and conjunctival redness, while commercial MTBE caused slight conjunctival redness and some discharge, but no cornea1 opacities. In a similar study, delayed and reversible congestion of the conjunctivae, palpebral thickening, and hypersecretion were observed in the eyes of rabbits (Snamprogetti 1980). Direct exposure of the eyes to MTBE vapors during inhalation exposure has also resulted in ocular effects in animals. A 4-hour exposure to commercial MTBE vapors at concentrations $\geq$ 18,892 ppm resulted in ocular discharge in rats, while rats exposed to ARCO MTBE displayed varying degrees and rapidity of onset of eye irritation (ARCO 1980). Inhalation exposure for 9 days to concentrations $\geq$ 100 ppm MTBE caused higher incidences of lacrimation and conjunctival swelling in exposed rats than in controls (Biodynamics 1981). Gross and histological examination of the eyes, however, revealed no lesions. Pregnant mice exposed to 250-2500 ppm MTBE on gestational days 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 was observed in 1 of 30 mouse dams at 4,000 ppm and 30 of 30 dams at 8,000 ppm (Tyl and Neeper-Bradley 1989). Periocular encrustations were also observed at 8,000 ppm. No ocular effects, as determined histologically or by ophthalmoscopy, were found in rats treated for 13 days (Dodd and Kintigh 1989) or for 13 weeks with $\leq$ 8,000 ppm MTBE (Dodd and Kintigh 1989; Greenough et al. 1980). At longer exposure durations, (16-28 weeks), lacrimation was among the most common effects observed in adult rats exposed to $\leq$ 2,500 ppm (Biles et al. 1987). This sign may not have been the result of this particular exposure, since it was seen at similar incidences in controls. Parental F_1 rats exposed to 8,000 ppm MTBE for 10 weeks before breeding and throughout the 19-day gestational period exhibited ocular discharges and periorbital encrustation (Neeper-Bradley 1991). Blepharospasm was observed in parental F_0 male and female rats at 3,000 and 8,000 ppm, but this is probably a neurological effect due to inhalation of MTBE.

In chronic-duration studies, in which rats (Chun et al. 1992) and mice (Burleigh-Flayer et al. 1992) were exposed intermittently by inhalation to 400, 3,000, or 8,000 ppm MTBE, no treatment-related lesions were observed in eyes. Blepharospasm was observed in both sexes of both species at 3,000 and/or 8,000 ppm, but, as noted above, this is probably a neurological effect due to inhalation of MTBE. Increased incidences of swollen periocular tissue were also observed in male rats exposed to 3,000 and 8,000 ppm (Chun et al. 1992).

2.2.3.3 Immunological and Lymphoreticular Effects

In the experimental double-blind study in humans exposed sequentially to 1.7 ppm MTBE for 1 hour on 1 day, to uncontaminated air for 1 hour 2 days later, and to 7.1 ppm of a 17-component mixture of VOCs for 1 hour 2 days later (or the reverse sequence) (see Respiratory Effects in Section 2.2.1.2), tear fluid from the eyes was stained for the total numbers of cells and differential counts for polymorphonuclear neutrophilic leukocytes, epithelial cell, monocytes, eosinophils, and lymphocytes (Cain et al. 1994). No notable changes between pre- and postexposure values were observed for exposure to MTBE or air.

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 tenth 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. The NOAELs for immunological effects in the eyes of humans and for dermal sensitization in guinea pigs are recorded in Table 2-3.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to MTBE.

The only information regarding neurological effects in animals after dermal exposure to MTBE is that rabbits cried out and were hyperactive for 3-5 minutes after 6,800 or 10,200 mg/kg MTBE was applied dermally to the occluded skin (IBT Labs 1969). It should be noted that the results of studies from IBT are often suspected as being unreliable.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to MTBE.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to MTBE.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after dermal exposure to MTBE. Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to MTBE.

2.3 TOXICOKINETICS

Information is available regarding blood levels of MTBE and *tert*-butanol in motorists, gas stations workers, and experimental subjects after inhalation exposure. The data indicate that MTBE is rapidly absorbed by the lungs during exposure, that MTBE is metabolized to *tert*-butanol, the levels of which also increase in blood after exposure to MTBE, and that levels of MTBE in blood decline rapidly after exposure ceases. Limited information regarding distribution, metabolism, and excretion of MTBE by humans was available for patients who received MTBE via intracystic infusion for the dissolution of gallstones. In these patients, MTBE and the metabolite, *tert*-butanol, were detected in blood, abdominal wall fat, and breast milk. Methanol was also found in the urine. Excretion of MTBE in the urine of these patients was nearly complete by 12-18 hours after treatment, but *tert*-butanol levels in urine declined very little during this time.

Information regarding absorption, distribution, metabolism, and excretion of MTBE in animals after inhalation, oral, or dermal exposure was available only for rats. Information regarding the rate and extent

of absorption of MTBE after inhalation exposure was limited to data on the peak plasma concentration and time to peak plasma concentrations of MTBE. These data indicated that absorption by the lungs is relatively rapid and extensive. Absorption by the gastrointestinal tract was also rapid, with peak plasma concentrations achieved within 15 minutes of dosing. Absorption factors were calculated to be 58% for male rats and 81% for female rats for gastrointestinal absorption, compared with 23-39% for male rats and 9.6-16% for female rats for dermal absorption. A greater percentage of a high dermal dose was absorbed than of a low dermal dose.

MTBE showed little tendency to accumulate in tissues. One inhalation study in rats, however, indicated that MTBE and its metabolite *tert*-butanol were distributed to the brain at concentrations similar to blood concentrations, which may account for the central nervous system toxicity of MTBE.

Metabolic pathways appear to be similar regardless of route of exposure. Unchanged MTBE was the major component in expired air. *tert*-Butanol was expired to a lesser extent, and very little carbon dioxide was formed as a result of MTBE metabolism. Urinary metabolites include 2-methyl-1, 2-propanediol, α-hydroxyisobutyric acid, formaldehyde, methanol, and formic acid. A metabolic pathway was proposed whereby demethyiation results in equimolar amounts of *tert*-butanol and formaldehyde, and *tert*-butanol is oxidized to 2-methyl-1, 2-propanediol, which is in turn oxidized to α-hydroxyiscbutyric acid. According to the pathway, formaldehyde can then be reduced to methanol or oxidized to formic acid. A small amount of carbon dioxide may arise from further metabolism of formic acid.

After inhalation and oral dosing with radiolabeled MTBE, the major routes of excretion were via the lungs and kidneys. Urinary excretion of radioactivity predominated at low doses, while respiratory excretion predominated at high doses. This shift in relative percentages of radioactivity excreted by these routes may be due to shifts in metabolic pathways as metabolizing enzymes become saturated at high doses. No such dose-dependent shift was observed after dermal exposure, presumably because dermal absorption of the higher dose was not great enough to result in enzyme saturation. Very little radioactivity was excreted in the feces after exposure to MTBE by any route. Excretion by lungs was more rapid than by the kidneys after inhalation, oral, and dermal exposure, with excretion by lungs nearly complete in 6 hours and excretion by the kidneys nearly complete by 36 hours. Complete excretion after dermal exposure took somewhat longer.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

As MTBE is a volatile, lipophilic molecular of small molecular size, it is expected to be rapidly and extensively absorbed from the respiratory tract. Information on blood levels of MTBE and its metabolite, *tert*-butanol, in motorists, gas station attendants and mechanics, and in experimentally exposed humans after inhalation of MTBE indicates that MTBE is well absorbed from the lungs. During the oxygenated fuel program in Fairbanks, Alaska, in which MTBE (15% by volume) was added to gasoline to reduce emissions of carbon monoxide, the median preshift blood concentration of MTBE in occupationally exposed workers (mechanics, gas station attendants, and people who spent most of their workdays in motor vehicles) was 1.15 μg/L (range, 0.1-27.8 μg/L) (CDC 1993a; Moolenaar et al. 1994). The median postshift level was 1.80 μg/L (range 0.2-37.0 μg/L). The median 8-hour TWA concentrations of MTBE in the workplace air of service stations and garages were 0.10 ppm with a range of 0.01-0.81 ppm during the program. A strong correlation was found between the air levels of MTBE and the difference in preshift and postshift MTBE blood levels during the program ($r = 0.9$; $p = 0.0001$). When blood levels were again monitored a few months after the oxygenated fuel program had ended, the preshift blood level was 0.20 μg/L (range 0.05-4.35 μg/L) and the postshift level was 0.24 μg/L (range 0.05-l .44 μg/L). The median workplace air level of MTBE was 0.04 ppm, with a range from nondetectable to 0.14 ppm. Blood levels of MTBE in nonoccupationally exposed motorists were 0. 18 μg/L before they left their homes and 0.83 μg/L upon arrival at the workplace after their morning commute during the oxygenated fuel program. After the program had ended, the pre- and post-commute MTBE blood levels were 0.09 μg/L and 0.10 μg/L, respectively.

In May 1993, CDC and New York state health officials investigated whether exposure to MTBE in fuels was measurable in occupationally and nonoccupationally exposed people in Albany, where MTBE is used in small concentrations (CDC 1993c). Group 1 consisted of 34 automobile repair shop workers and service station attendants exposed to gasoline fumes; Group 2 consisted of 48 police officers, toll booth workers, and parking garage attendants exposed to automobile exhaust; and Group 3 consisted of 182 office workers and college students. In both smokers and nonsmokers, subjects in Group 1 had higher levels of MTBE (mean, 0.46 μg/L; range, 0.09-l .5 μg/L for smokers; mean, 0.38 μg/L, range, nondetectable to 0.58 μg/L for nonsmokers) and *tert*-butanol (mean, 5.47 μg/L, range, 1.12-13.77 μg/L for smokers; mean, 3.58 μg/L; range, 1.08 to 5.34 μg/L for nonsmokers) in their blood when compared to subjects in Groups 2 and 3. In Group 2, MTBE blood levels averaged 0.08 μg/L for smokers and

0.05 μg/L for nonsmokers and ranged from nondetectable to 0.11 μg/L for smokers and nondetectable to 0.15 μg/L for nonsmokers; *tert*-butanol blood levels averaged 1.17 μg/L for smokers and 1.16 μg/L for nonsmokers and ranged from 1.14 to 1.53 μg/L for smokers and 0.69 to 2.24 μg/L for nonsmokers. In Group 3, no MTBE was detectable in the blood of smokers or nonsmokers, but *tert*-butanol blood levels averaged 1.36 μg/L in smokers and 1.10 μg/L for nonsmokers and ranged from 1.03 to 1.69 μg/L for smokers and 0.23 to 3.05 μg/L for nonsmokers.

In a similar study conducted in Stamford, Connecticut, during an oxygenated fuel program, the approximate median blood levels of MTBE were 0.1 μg/L in commuters, 2 μg/L in car repairers, 0.1 μg/L in "other" workers (such as meter readers), and 15 μg/L in gas station attendants (CDC 1993b; White et al. 1995). Thus, the blood levels were about an order of magnitude higher in mechanics and two orders of magnitude higher in gas station attendants than in commuters and "other" workers. Blood levels of *tert*-butanol were higher than blood levels of MTBE, but showed the same relative differences among the groups. Workroom air levels and personal breathing zone levels of MTBE, which were considered to underestimate the exposure levels of mechanics and gas station workers, ranged from <0.03 to 12.04 ppm for breathing zones and from 0.001 to 0.429 ppm for workroom air.

In a group of 2 healthy men and 2 healthy women experimentally exposed to 1.7 ppm MTBE for 1 hour, mean blood levels of MTBE rose steeply from a level of 0.83 μg/L preexposure to 17.14 μg/L at the end of the 1-hour exposure, followed by a decline to an average level of 9.74 μg/L at 40 minutes postexposure and 6.32 μg/L at 60 minutes postexposure (Cain et al. 1994). *tert*-Butanol was also monitored but the levels were highly variable. The mean blood level of *tert*-butanol was 2.79 μg/L preexposure and increased during exposure, but remained in the vicinity of 10-15 μg/L from 30 minutes during exposure to 90 minutes postexposure, indicating continued metabolism of MTBE to *tert*-butanol. In a similar study in 1 healthy man and 1 healthy woman experimentally exposed to 1.39 ppm MTBE for 1 hour, the blood level of MTBE rose rapidly to 8.2 μg/L and 14.7 μg/L in the man and woman, respectively (Prah et al. 1994). The blood level of *tert*-butanol rose gradually to a plateau of 7-l 0 μg/L.

Studies in rats indicate that MTBE is also readily absorbed by the pulmonary tract in animals, as it is in humans. Data regarding the excretion of radioactivity in expired air, urine, and feces in Fischer 344 rats exposed nose-only to 400 and 8,000 ppm ^{14}C-MTBE for 6 hours provides information on the rate and extent of absorption (Bioresearch Labs 1990d). Samples were collected at various times for up to 48 hours after exposure. Excretion of radioactivity in feces was minor, with only 0.74-0.76% recovered after exposure to 400 ppm and 0.75-1.06% recovered after exposure to 8,000 ppm. The relative percentages of

radioactivity recovered in the urine and expired air were dose-dependent. At 400 ppm, 64.7 and 65.4% of the total radioactivity was recovered in the urine 48 hours after termination of exposure of males and females, respectively, while 21.2 and 21.9% of radioactivity was recovered in the expired air of males and females, respectively. At 8,000 ppm, radioactivity recovery in urine accounted for 41.6% in males and 35% in females, while radioactivity recovery in expired air accounted for 53.6% in males and 59% in females. The values indicate that at least 85.9-87.3% of the low dose and at least 94-95.2% of the high dose were absorbed via the pulmonary tract in 48 hours. Similar percentages of radioactivity in expired air and urine were obtained in rats exposed nose-only to 400 ppm unlabeled MTBE for 6 hours per day for 14 days and then to 400 ppm ^{14}C-MTBE for 6 hours on day 15.

Data on the peak plasma concentrations and time to peak plasma concentrations in rats provide some information on the rate of pulmonary absorption of MTBE. In rats exposed nose-only to MTBE for 6 hours, plasma concentrations of MTBE and its metabolite *tert*-butanol peaked at 4-6.5 hours during exposure (Bioresearch Labs 1990c). Plasma *tert*-butanol increased more gradually than plasma MTBE concentrations. At an exposure concentration of 400 ppm, plasma MTBE concentrations peaked at 4-6 hours (about 15 μg/mL), while plasma *tert*-butanol concentrations peaked at 6-6.5 hours (about 39 μg /mL). At a concentration of 8,000 ppm MTBE, plasma MTBE levels peaked at 4-6 hours (about 560 μg /mL), while plasma *tert*-butanol levels peaked at 6-6.5 hours (536 μg /mL for males and 245 μg /mL for females).

Blood MTBE levels were generally related to inhaled dose in rats exposed to 50, 100, or 300 ppm, 6 hours per day, 5 days per week for 2-l5 weeks (Savolainen et al. 1985). At the 2 lower exposure concentrations, peak blood MTBE levels of 11 and 24 nmol/g, respectively (about 1.3 μg /mL 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. 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.

2.3.1.2 Oral Exposure

No studies were located regarding absorption of MTBE in humans after oral exposure. However, as MTBE is a volatile, lipophilic molecular of small molecular size, it is expected to be rapidly and extensively absorbed from the gastrointestinal tract of humans after oral exposure, as confirmed in studies in animals.

A peak blood concentration of 5.9 µg /mL MTBE was reached in 0.9 hours in rats given a single oral dose of 0.379 mg/kg MTBE (Li et al. 1991). In rats given a single oral dose of 40 or 400 mg/kg of MTBE in water, MTBE and *tert*-butanol were detected in plasma of both treatment groups (Bioresearch Labs 1990a). The maximum plasma concentrations of MTBE in both males and females at both doses were achieved within 15 minutes, suggesting rapid absorption. The oral bioavailability of MTBE was determined to be 58% in males and 81% in females. In rats given 40 or 400 mg/kg of ^{14}C-MTBE, a total of 80.2-86.3% of the dose of radioactivity was recovered in urine, feces, and volatile radioactive components in the expired air in 48 hours (Bioresearch Labs 1990b). Most of the radioactivity was recovered in the expired air and the urine. Recovery in expired air was 48.5% (males) and 54.4% (females) after a dose 40 mg/kg, and 65.3% (males) and 68.7% (females) after a dose of 400 mg/kg. Recovery in urine was 36.2% (males) and 39% (females) after a dose of 40 mg/kg and 16% (males), and 10.8% (females) after a dose of 400 mg/kg. Recovery of radioactivity in feces was only about 1% or lower in the dosed groups, and recovery in the tissues/carcass was $\leq$ 2% at 48 hours after dosing. These data indicate that as much as 80% of an oral dose of MTBE was absorbed from the gastrointestinal tract of rats within 48 hours.

In rats given a single oral or intravenous dose of 40 mg/kg MTBE, blood samples were collected for up to 240 minutes after dosing (Exxon 1988). Maximum blood levels of MTBE in the orally treated rats occurred within 2.5 to 10 minutes. From a comparison of the mean areas under the curves (AUC) for blood clearance for the intravenous dose group and the oral dose group, an oral bioavailability of 37.4% was obtained. However, the total elimination of MTBE in expired air was 50-55% of the administered dose by 3 hours after dosing, indicating that at least 50-55% of MTBE was absorbed from the gastrointestinal tract. Furthermore, the MTBE in this study was not radiolabeled, precluding the measurement of any radiolabeled metabolites that may have been formed from radiolabeled MTBE. Analysis of total radioactivity would have provided a more accurate estimate of the extent of MTBE absorption by the gastrointestinal tract. In addition, the body weights of the rats used in these experiments varied widely, which could effect the individual values of blood levels obtained.

2.3.1.3 Dermal Exposure

No studies were located regarding absorption of MTBE in humans after dermal exposure, but it is probably less well absorbed by the skin than by the pulmonary or gastrointestinal tract.

In rats, MTBE is absorbed to a lesser extent by the skin than by the gastrointestinal tract. In rats administered 400 mg/kg MTBE to the shaved skin via a dermal chamber to prevent evaporation and cross-exposure by inhalation or ingestion due to grooming, MTBE and *tert*-butanol were detected in plasma within 10 minutes (Bioresearch Labs 1990a). However, measurable MTBE and *tert*-butanol in plasma were barely detectable this soon in rats similarly treated with 40 mg/kg. The peak plasma concentration of MTBE was achieved in 2-6.5 hours after the low dose and 2-4 hours after the high dose. The bioavailability of the dermal dose of MTBE compared with the oral dose (see Section 2.3.1.2) was 23-39% in male rats and 9.5-16% in female rats. In a mass balance study in male and female rats similarly treated with 40 or 400 mg/kg ^{14}C-MTBE, analysis of radiolabeled carbon dioxide, MTBE, and metabolites in urine, feces, and expired air, tissues/carcass, and chamber washings yielded 69.4-76.9% recovery of the dose in 48 hours (Bioresearch Labs 1990b). Most of the radioactivity was excreted in the expired air and urine. Recovery in expired air was 6.05% (males) and 9.67% (females) after a dose of 40 mg/kg and 19.6% (males) and 23.2% (females) after a dose of 400 mg/kg. Recovery in urine was 6.49% (males) and 6.12% (females) after a dose of 40 mg/kg and 12.4% (males) and 10.7% (females) after a dose of 400 mg/kg. Recovery of radioactivity in feces was only about 0.2% or lower in the dosed groups, and recovery in the tissues/carcass and application site was $\leq$ 1 % at 48 hours after dosing. Recovery of radioactivity from the dermal chambers was 56.9% (males) and 59.8% (females) after the low dose and 38.2% (males) and 34.6% (females) after the high dose, representing unabsorbed MTBE. Thus, the dermal absorption of MTBE was higher for the high-dose group than the low-dose group. In a similar mass balance study in rats, recovery of radioactivity in the various components was measured for up to 168 hours after dosing and in selected tissues at 7 days post-dosing (Bioresearch Labs 1991). Total recovery of radioactivity was 88.8% for the 40 mg/kg dose and 99.9% for the 400 mg/kg dose. At 168 hours after dosing, 76.8% of the low-dose and 69.5% of the high dose were recovered in the chamber washes, indicating that dermal absorption was <50% in both dose groups. In the low-dose group, 6.29, 7.58, and 0.25% of the dose was recovered in the urine, expired air, and feces, respectively, at 168 hours. In the high-dose group these recoveries were 15.9, 18.9, and 0.39%, respectively. Therefore, about 14.5 and 35.7% of the low and high dose, respectively, were absorbed dermally. The greater percentage of the dose absorbed after the high dose in this study (Bioresearch Labs 1991) is consistent with that in the other mass balance study (Bioresearch Labs 1990b).

2.3.2 Distribution

As MTBE is a small molecular weight, volatile, lipophilic compound, it is expected to readily cross biological membranes during it 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 (see Section 2.3.2.4).

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution of MTBE in humans after inhalation exposure.

The levels of MTBE and *tert*-butanol in samples of blood, cerebral hemispheres, and perirenal fat were monitored in Wistar rats sacrificed at 2, 6, 10, or 15 weeks of exposure to 50, 100, or 300 ppm MTBE, 6 hours per day, 5 days per week (Savolainen et al. 1985). Blood MTBE levels were generally related to exposure concentration. At the 2 lower exposure concentrations, peak blood MTBE levels of 11 and 24 nmol/g, respectively (about 1.3 μg /mL 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. Blood *tert*-butanol levels peaked at 6 weeks for all concentrations tested (38, 82, and 151 nmol/g or 3.3, 7.2, and 13 μg /mL, respectively) and then decreased sharply at week 10 (11, 34, and 89 nmol/g or 0.97, 3.0, or 7.8 μg /mL, respectively). Brain MTBE and *tert*-butanol levels followed a similar course as blood levels. At the 300 ppm exposure concentration, brain and blood MTBE levels were approximately similar. MTBE levels were considerably higher in perirenal fat than in blood and brain tissues at all exposure concentrations. At 2 weeks, the perirenal fat concentration of MTBE was 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. No trace levels of *tert*-butanol were found in perirenal fat at any exposure concentration and any duration.

The tissue retention of MTBE after acute- and intermediate-duration inhalation exposure has been studied in Fischer 344 rats. Rats exposed nose-only to ^{14}C-MTBE at 400 or 8,000 ppm for 6 hours or to 400 ppm unlabeled MTBE for 6 hours per day for 14 days, and then to 400 ppm ^{14}C-MTBE on day 15 were sacrificed at 48 hours post-exposure (Bioresearch Labs 1990d). Blood, liver, kidney, lungs, heart, brain,

gonads, bone (femur), fat (perirenal), muscle (leg adductor) and skin were removed for analysis of radioactivity. For the single 6-hour exposure, the percentages of total radioactivity recovered from tissues/carcass were as follows: 13.4% (male) and 11.9% (female) after exposure to 400 ppm; 4.07% (male) and 5.02% (female) after exposure to 8,000 ppm. The higher percentage of radioactivity in the tissues after the low dose compared with the high dose may be due to shifts in metabolic and elimination pathways as enzyme systems become saturated at high doses (see Section 2.3.3). In the repeated exposure study, the percentages of total radioactivity recovered from tissues/carcass were 10.9% (males) and 10.4% (females), which were essentially the same as in the single exposure study at 400 ppm. In both experiments, except for the skin, mean radioactivity recoveries in tissues were very low (<1% of the total), indicating that MTBE or its metabolites do not accumulate in tissues after acute exposure. The relatively high radioactivity in the skin of the rats may have been due to contamination.

2.3.2.2 Oral Exposure

No studies were located regarding distribution of MTBE in humans after oral exposure.

In rats given a single oral dose of 40 mg/kg MTBE, the level of MTBE in perirenal fat was determined to be 53.75 μg /g at 30 minutes, 35.51 μg /g at 60 minutes, 25.87 μg /g at 90 minutes, 38.67 μg /g at 120 minutes, 7.86 μg /g at 240 minutes, and 3.43 μg /g at 300 minutes after dosing (Exxon 1988). Thus, the highest levels were found in the first samples at 30 minutes. A time-related decrease in the MTBE fat levels occurred thereafter; however, the plateau of MTBE levels in fat at 90 and 120 minutes was unexplainable. However, the body weights of the rats used in these experiments varied widely, which could effect the individual values of fat levels obtained. Blood clearance curves were fitted to a 2-compartment model with instantaneous distribution and first-order elimination. The volume of distribution for the central compartment was calculated to be 521 mL.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of MTBE in humans after dermal exposure.

Studies in animals did not follow the course of MTBE distribution during exposure, but tissues levels of radioactivity were measured at $\geq$ 2 days after exposure to ^{14}C-MTBE ceased. These studies generally indicate that MTBE does not tend to accumulate in tissues of the rat body. In rats to which 40 or 400 mg/kg ^{14}C-MTBE was applied to the shaved skin via a dermal chamber for 6 hours, the percentages of

radioactivity in tissues, carcass, and site of application at 48 hours after exposure were 0.28% (males) and 0.67% (females) at the high dose, and 0.67% (males) and 1.02% (females) at the low dose (Bioresearch Labs 1990b). In rats similarly treated, radioactivity in selected tissues was measured at 7 days post-dosing (Bioresearch Labs 1991). Except for detectable radioactivity at the application site in both dose groups (0.0 1-0.12% of the dose), no radioactivity was detected in tissues (liver, kidneys, lungs, heart, brain, gonads, femur, perirenal fat, muscle, and skin) or carcass at 7 days post-dosing.

2.3.2.4 Other Routes of Exposure

Limited information regarding distribution of MTBE was available for humans who received MTBE via intracystic infusion for dissolution of gallstones. Among a group of 113 patients who received MTBE (1-15 mL instilled 3-6 times per minute and reaspirated) by this route, fatty tissue from the abdominal wall was collected from 9 patients at the end of treatment (Leuschner et al. 199 I). Breast milk was sampled from one patient after treatment. The mean MTBE concentration in fatty tissue was 0.135 mg/g after a mean treatment time of 9.6 hours. MTBE and *tert*-butanol were present in breast milk in concentrations only slightly less than in blood.

In Charles River CD (Sprague-Dawley) rats that received a single intraperitoneal dose of 232 mg/kg ^{14}C-MTBE (radiolabeled on the methyl and central carbon of the *tert*-butyl group), 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 (Biodynamics 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). High levels of radioactivity were no longer found in mesenteric fat at 6 and 24 hours, but 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 formic acid and methanol were present in the liver and kidney, but quantification of the metabolites was not possible. In Fischer 344 rats given a single intravenous dose of 40 mg/kg ^{14}C-MTBE, the percentages of the radioactive dose in tissues/carcass were 1.69% in males and 1.13% in females at 48 hours (Bioresearch Labs 1990b). When samples were collected 7 days after treatment, <1% of the dose was recovered in the liver, kidneys, fat, skin, and remaining carcass.

2.3.3 Metabolism

No studies were located regarding the metabolism of MTBE in humans after oral or dermal exposure. In a group of 4 nonsmoking, healthy subjects (2 men and 2 women) experimentally exposed to 1.7 ppm MTBE for 1 hour, the mean blood concentration of *tert*-butanol was 2.79 μg /L preexposure, which was 3 times higher than the preexposure mean MTBE blood level (Cain et al. 1994). However, postexposure blood levels of *tert*-butanol exceeded those measured before exposure to MTBE, indicating metabolism of MTBE to *tert*-butanol. Mean blood levels of *tert*-butanol, although highly variable, remained in the vicinity of l0-15 μg /L from 30 minutes during exposure to 90 minutes postexposure, indicating continued metabolism of MTBE to *tert*-butanol. In a similar study in one healthy man and one healthy woman experimentally exposed to 1.39 ppm MTBE for 1 hour, the blood level of *tert*-butanol rose gradually to a plateau of 7-10 μg /L, where it remained for up to 7 hours postexposure, again indicating continued metabolism of MTBE to *tert*-butanol (Prah et al. 1994). In 27 patients who received MTBE via intracystic infusion for dissolution of gallstones, blood and urine samples were collected before treatment, immediately after treatment, and up to 18 hours after treatment for the determination of blood and urine levels of MTBE, methanol, *tert*-butanol, formic acid and formaldehyde (Leuschner et al. 1991). Mean blood levels of *tert*-butanol were 0.04 mg/mL immediately after and 5 hours after treatment and fell only to 0.025 mg/mL at 12-18 hours. Mean urinary levels of MTBE were about 0.018 mg/mL at 5 hours after treatment and <0.005 mg/mL at 12-l 8 hours. Mean urinary levels of *tert*-butanol were higher than those of MTBE, with about 0.036 mg/mL at 5 hours and 0.03 mg/mL at 12-l 8 hours after treatment. Methanol was found in only 3 patients, while no formaldehyde or formic acid were found. *tert*-Butanol was also detected in the abdominal wall fat of 9 patients and breast milk of 1 patient.

MTBE is also metabolized to *tert*-butanol in rats. The metabolism of ^{14}C-MTBE has been studied in Fischer 344 rats exposed by the inhalation (Bioresearch Labs 1990d), oral (Bioresearch Labs 1990b), dermal (Bioresearch Labs 1990b, 1991), and intravenous (Bioresearch Labs 1990b, 1991) routes. Respiratory and urinary metabolites were generally similar following exposure of Fischer 344 rats by all routes, indicating that metabolic pathways are not route dependent. After exposure by all routes, most of the exhaled radioactivity was due to unchanged MTBE and *tert*-butanol, with MTBE predominating. Only a small amount of ^{14}C-carbon dioxide was detected. MTBE and *tert*-butanol were generally not found in the urine, but four urinary metabolites were isolated, with two identified as α-hydroxyisobutyric acid and 2-methyl-1,2-propanediol. The two other metabolites remained unidentified. After inhalation and oral exposure, there was a larger fraction of exhaled *tert*-butanol in low-dose rats than in high-dose rats,

probably due to less extensive metabolism at the high dose as metabolizing enzyme systems become saturated. The proportion of α-hydroxyisobutyric acid increased relative to 2-methyl-1,2-propanediol with increasing sampling time, suggesting that the diol is an intermediate to the formation of the acid. The results are consistent with a metabolic pathway whereby MTBE is biotransformed by demethylation to *tert*-butanol followed by further oxidation to 2-methyl-1,2-propanediol and then to α-hydroxyisobutyric acid. No differences in biotransformation were observed with respect to gender or duration of exposure.

In inhalation experiments, rats were exposed to ^{14}C-MTBE at 400 or 8,000 ppm for 6 hours or to 400 ppm unlabeled MTBE for 6 hours per day for 14 days then to 400 ppm ^{14}C-MTBE on day 15 (Bioresearch Labs 1990d). *tert*-Butanol accounted for 25 and 30% of the recovered radioactivity in expired air at 3 hours after the single and repeated low exposure concentration, respectively. Exposure to the higher concentration for 6 hours resulted in a lower fraction recovered (7-10%) due to *tert*-butanol. At 3-6 hours after exposure, *tert*-butanol represented 72-80% of the radioactivity at the low dose in the single and repeated exposure experiments and 43-54% at the high dose.

In rats treated by gavage with ^{14}C-MTBE at 40 or 400 mg/kg, and in rats to which 40 or 400 mg/kg ^{14}C-MTBE was applied to the shaved skin via a dermal chamber for 6 hours, only 0.01-0.2% of the dose was recovered as ^{14}C-carbon dioxide in expired air (Bioresearch Labs 1990b). After oral dosing, MTBE predominated in expired air during the first 3 hours, with the ratio of *tert*-butanol to MTBE increasing during the next 3 hours. Elimination of *tert*-butanol by the lungs represented 3.1% of the low dose and 1.4% of the high dose. Similarly, another study also found that, in Fischer 344 rats given a single oral dose of 40 mg/kg, pulmonary elimination of *tert*-butanol for 5 hours after exposure represented 2% of the administered MTBE dose (Exxon 1988). After dermal exposure, a greater percentage of the dose was recovered in the expired air (6.05% in males, 9.67% in females at 40 mg/kg and 19.6% in males, 23.2% in females at 400 mg/kg) than in the urine (6.12-6.49% at 40 mg/kg and 10.7-l 2.4% at 400 mg/kg) (Bioresearch Labs 1990b). MTBE predominated in expired air. Only 0.63% to 0.93% of the dermal dose was eliminated in expired air as *tert*-butanol at the low dose, and 1.0% to 1.3% of the dose as *tert*-butanol at the high dose. The reason that the percentage of *tert*-butanol in expired air was not greater after the low dermal dose than after the high dose is probably that MTBE is less well absorbed after dermal exposure than after oral or inhalation exposure. Thus, the amount absorbed after the high dermal dose was probably not great enough to saturate metabolizing enzymes. When samples were collected for up to 168 hours after dermal dosing, 11.6% of the dose was exhaled as MTBE and 0.39% as *tert*-butanol in rats given

400 mg/kg (Bioresearch Labs 1991). Expired air levels of MTBE and *tert*-butanol were below detection limits in rats given 40 mg/kg dermally.

Results of dosing Fischer 344 rats with ^{14}C-MTBE intravenously were similar to those obtained by the inhalation, oral, and dermal routes (Bioresearch Labs 1990b). In Charles River CD (Sprague-Dawley) rats injected with a single intraperitoneal dose of 232 mg/kg ^{14}C-MTBE, blood, tissue, expired air, urine, and feces were sampled at various times up to 48 hours (Biodynamics 1984). At 6 hours, about 92% of the radioactive dose was eliminated in expired air, 99.1% of which was unchanged MTBE. An average of 7.38% of the administered dose was expired as radiolabeled carbon dioxide. Although not specified, apparently no *tert*-butanol was found in expired air. Urinary radioactivity accounted for an average of 2.95% of the administered dose. Analysis of the urine revealed that radiolabeled formic acid accounted for 96.6% of the urinary radioactivity. The remaining radioactivity in the urine was assumed to be radiolabeled methanol and formaldehyde. Methanol and formic acid were also detected in plasma, kidney, and liver. No *tert*-butanol was found in the urine. The reason for differences in metabolites found by Biodynamics (1984) compared with those found by Bioresearch Labs (1990b, 1990d, 1991) is not clear, except that these studies used different strains of rats and different analytical methods for identification of the metabolites.

Some aspects of the *in vitro* metabolism of MTBE have been studied using microsomes prepared from Sprague-Dawley rats (Brady et al. 1990). Incubation of MTBE with microsomes prepared from phenobarbital-pretreated rats resulted in equimolar amounts of *tert*-butanol and formaldehyde. In comparing the metabolism of MTBE using microsomes from phenobarbital- and acetone-pretreated rats and untreated rats, a 4-fold increase in V_{max} value with acetone-induced microsomes and a 5.5-fold increase in the V_{max} value with phenobarbital-induced microsomes was found compared with the control max value. These results indicated that cytochrome P-4502B1, which is inducible by phenobarbital, and cytochrome P-4502E1, which is inducible by acetone, play a role in the demethylation of MTBE. Inclusion in the incubation of monoclonal antibody against cytochrome P-4502E1, the major acetoneinducible isoenzyme, resulted in a 35% inhibition of the demethylation of MTBE, indicating that cytochrome P-4502El only partially contributes to the demethylation. Microsomes prepared from rats pretreated with MTBE yielded a 47-fold induction of liver microsomal pentoxyresorufin O-dealkylase, with no change in N-nitrosodimethylamine demethylase activity. These results are consistent with an elevation of cytochrome P-4502B 1 activity with no change in cytochrome P-4502El activity, which was confirmed by immunoblot analysis.

The metabolism of MTBE to formaldehyde was studied using liver microsomes from control and phenobarbital-pretreated rats (strain not specified) (Snyder 1979). Phenobarbital-pretreatment approximately doubled in formation of formaldehyde from MTBE. The author postulated that formaldehyde and *tert*-butanol are the first metabolites of MTBE formed as a result of o-demethylation reaction by the hepatic mixed function oxidases. Further metabolism of formaldehyde would yield methanol and/or formic acid with the probable enzymes and cofactors being alcohol dehydrogenase and NADH for the formation of methanol and aldehyde dehydrogenase and NAD for the formation of formic acid.

To account for the results in the *in vivo* and *in vitro* studies in rats discussed above, a proposed metabolic scheme for MTBE is presented in Figure 2-3.

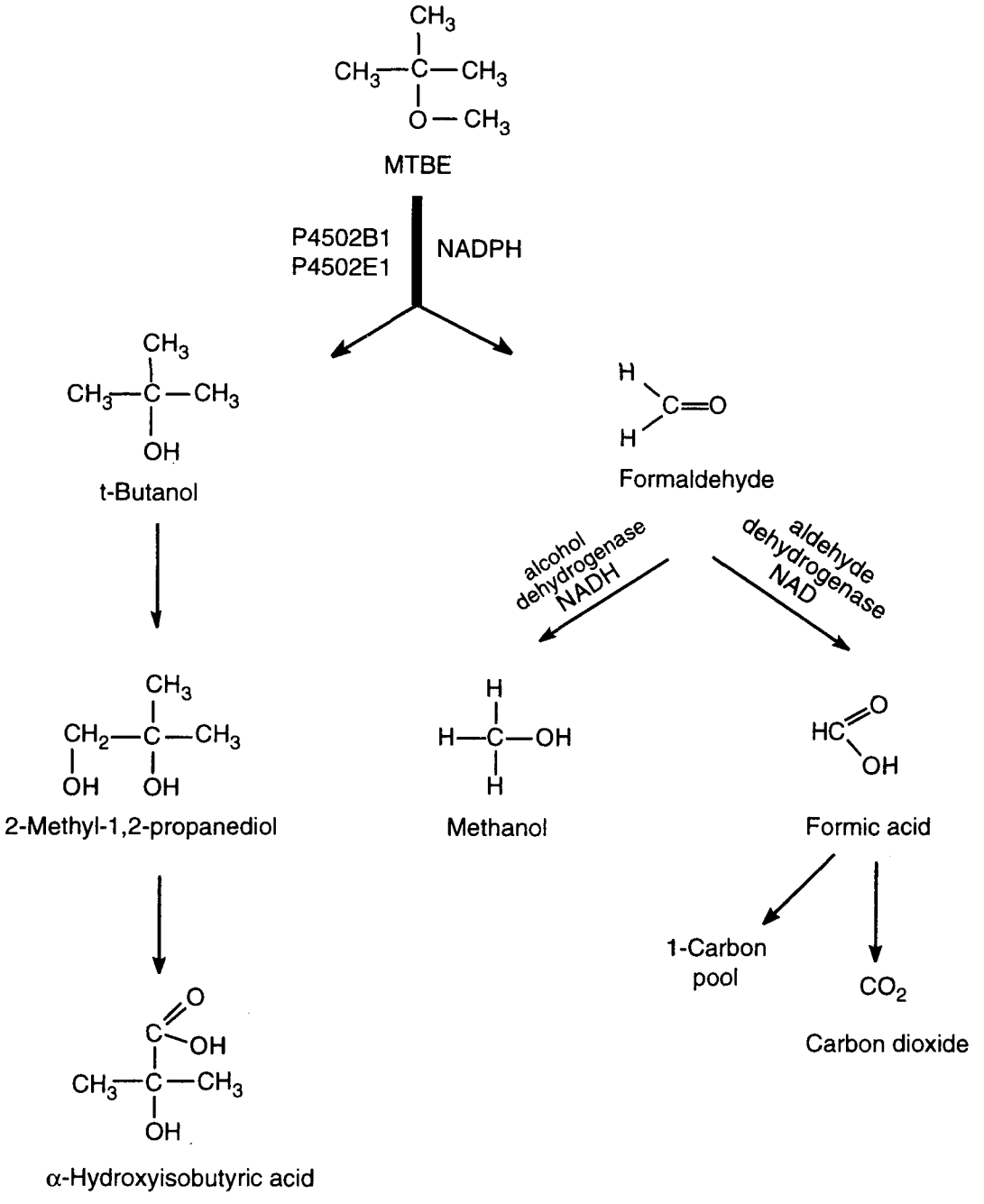
2.3.4 Excretion

2.3.4.1 Inhalation Exposure

In a group of 2 healthy men and 2 healthy women experimentally exposed to 1.7 ppm MTBE for 1 hour, the mean blood level of MTBE rose steeply from a level of 0.83 μg /L preexposure to 17.14 μg /L at the end of the 1-hour exposure, followed by a decline to an average level of 9.74 μg /L at 40 minutes postexposure and 6.32 μg /L at 60 minutes postexposure (Cain et al. 1994). At 90 minutes postexposure, the average blood level of MTBE was 7.42 μg /L, indicating that the rapid elimination phase lasted about 60 minutes, but that levels did not drop to preexposure levels within the 60-90 minute postexposure period. Blood levels were not measured beyond the 90-minute postexposure period. In a similar study in one healthy man and one healthy woman experimentally exposed to 1.39 ppm MTBE for 1 hour, the blood level of MTBE rose rapidly to 8.2 μg /L and 14.7 μg /L in the man and woman, respectively (Prah et al. 1994). Immediately after exposure, the blood levels of MTBE started to decline rapidly with clearance half-times of 36 and 37 minutes, respectively. At the end of the 7-hour sampling period, 0.2 and 0.6 μg/L MTBE were detected in the blood of the man and woman, respectively. The blood level of *tert*-butanol rose gradually to a plateau of 7-10 μg /L, where it remained for up to 7 hours postexposure.

The excretion of radioactivity in expired air, urine, and feces was studied in Fischer 344 rats exposed noseonly to 400 and 8,000 ppm ^{14}C-MTBE for 6 hours (Bioresearch Labs 1990d). Samples were collected at various times for up to 48 hours after exposure. Excretion of radioactivity in feces was minor, with only 0.74-0.76% recovered after exposure to 400 ppm and 0.75-l.06% recovered after exposure to 8,000 ppm.

Figure 2-3. Proposed Metabolic Pathway for Methyl *tert*-Butyl Ether (MTBE) in Rats



Source: Biodynamics 1984; Bioresearch Labs 1990b, 1990c, 1990d, 1991; Brady et al 1990; Snyder et al. 1979

The relative percentages of radioactivity recovered in the urine and expired air were dose-dependent. At 400 ppm, 64.7 and 65.4% of the total radioactivity was recovered in the urine 48 hours after termination of exposure of males and females, respectively, while 21.2 and 21.9% of radioactivity was recovered in the expired air of males and females, respectively. At 8,000 ppm, radioactivity recovery in urine accounted for 41.6% in males and 35% in females, while radioactivity recovery in expired air accounted for 53.6% in males and 59% in females. The shift in excretion route from urine to expired air with increase in dose was probably due to shifts in metabolic pathways as metabolizing enzymes become saturated at high doses. 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. Urinary excretion was slower than excretion in expired air, but was faster after exposure to 400 ppm than after exposure to 8,000 ppm. At the low exposure concentration, the percentages of recovered radioactivity excreted in the urine were 27-30% by 6 hours, 65% by 12 hours, 81-85% at 24 hours, and 94-96% by 36 hours. At the high exposure concentration, the percentages were 8-10% by 6 hours, 38-47% by 12 hours, 82-88% by 24 hours, and 95-98% by 36 hours. Similar percentages and rates of excretion of radioactivity in expired air and urine were obtained in rats exposed nose-only to 400 ppm unlabeled MTBE for 6 hours per day for 14 days and then to 400 ppm ^{14}C-MTBE for 6 hours on day 15.

2.3.4.2 Oral Exposure

No studies were located regarding the excretion of MTBE or its metabolites in humans after oral exposure.

The excretion of radioactivity in expired air, urine, and feces was studied in Fischer 344 rats given single gavage doses of 40 or 400 mg/kg ^{14}C-MTBE (Bioresearch Labs 1990b). Samples were collected at various times up to 48 hours after dosing. As after inhalation exposure, the major routes of excretion are expired air and urine, with dose-dependent shifts in the relative percentages excreted by these routes, and some significant gender differences. After the 40 mg/kg dose, total recovery of radioactivity in expired air was 45.8% in males and 54.4% in females. After the 400 mg/kg dose, this recovery was 65.3 and 68.7% in males and females, respectively. The total recovery of radioactivity in urine was 36.2 and 29% of the dose in males and females, respectively, at the low dose and 16 and 10.8% in males and females, respectively, at the high dose. As with inhalation exposure, the shift in excretion route from urine to expired air with increase in dose was probably due to shifts in metabolic pathways as metabolizing enzymes become saturated at high doses**.** Feces contained only 0.87-l. 19% of the radioactivity after the low dose and 0.26-0.28% after the high dose. Excretion of radioactivity was rapid, with complete elimination in expired air and urine occurring within 6 and 36 hours, respectively. Excretion in expired air was 92-93%

complete at 3 hours and 98% complete at 6 hours after the low dose. After the high dose, excretion in expired air at 3 hours accounted for less radioactivity (85-86%) than after the low dose. At 6 hours after dosing with 400 mg/kg, however, excretion in expired air was 98% complete. At 6 hours after either dose, very little radioactivity was recovered in urine (5-7%), but urinary excretion of radioactivity at 12 hours was 41-46% complete at 40 mg/kg and 41-50% complete at 400 mg/kg. Recovery of urinary radioactively continued to increase to almost complete excretion by 36 hours.

In another study in Fischer 344 rats treated with a single oral dose of 40 mg/kg, mean blood concentrations of MTBE were 5.37 μg /mL at 1 minute, 15.01 μg /mL at 2.5 minutes, 15.83 μg /mL at 5 minutes, 14.22 μg /mL at 7.5 minutes, 12.68 μg /mL at 10 minutes, 9.81 μg /mL at 20 minutes, 5.56 μg /mL at 40 minutes, 3.83 μg /mL at 60 minutes, 2.25 μg /mL at 90 minutes, 1.46 μg /mL at 120 minutes, 0.70 μg /mL at 180 minutes, and 0.26 μg /mL at 240 minutes, with a half-life of blood clearance of 56.7 minutes (Exxon 1988). Thus, after the initial absorptive phase, blood MTBE levels declined sharply from 10 minutes to about 40 minutes, followed thereafter by a slower decline. The time course of elimination of MTBE in expired air revealed a rapid elimination of unmetabolized MTBE, with >30 and 40% of the administered dosed expired by 45 and 90 minutes, respectively, and <10% expired between 120 and 300 minutes. Total expiration of MTBE by 3 hours after dosing represented 50-55% of the administered dose. In contrast to MTBE, the elimination of *tert*-butanol in expired air remained fairly constant over the time period, with a maximum amount of expired *tert*-butanol over 5 hours post dosing of 2% of the administered MTBE dose.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of MTBE or its metabolites in humans after dermal exposure to MTBE.

The excretion of radioactivity in expired air, urine, and feces was studied in Fischer 344 rats administered 40 or 400 mg/kg MTBE to the shaved skin via a dermal chamber for 6 hours (Bioresearch Labs 1990b). Samples were collected at various times up to 48 hours after exposure. As after inhalation and oral exposure, the major routes of excretion are expired air and urine with some significant gender differences. However, in contrast with the observed shifts after inhalation and oral dosing, no dose-dependent shifts in the relative percentages excreted by lungs and kidneys were found for the dermal route. Since MTBE is less well absorbed after dermal exposure than after oral or inhalation exposure, the amount absorbed after the high dermal dose was probably not great enough to saturate metabolizing enzymes, thus accounting for lack of shift. A greater percentage of the dose was recovered in the expired air (6.05% in males, 9.67% in

females) at 40 mg/kg and (19.6% in males, 23.2% in females) at 400 mg/kg than in the urine. Recovery of radioactivity in urine was 6.49 and 6.12% in males and females, respectively, at 40 mg/kg and 12.4 and 10.7% in males and females, respectively, at 400 mg/kg. Only 0.13-0.14% of the dose was recovered from the feces after the low dose and 0.11-0.19% after the high dose. At 3 hours after dosing with 40 mg/kg, 48-49% of the recovered radioactivity in expired air was excreted. At 6 hours, excretion of radioactivity in expired air was 58-60% complete. At 3 hours after dosing with 400 mg/kg, excretion of radioactivity in expired air was 70% complete, and at 6 hours after dosing, recovery was 78-80% complete. At 6 hours after dosing with either dose, excretion of radioactivity in urine was only l-5% complete. After dosing with 40 mg/kg, urinary excretion was 14% (males) and 26% (females) complete at 12 hours, 55-56% complete at 24 hours, and 75-77% complete at 36 hours. After dosing with 400 mg/kg, urinary excretion was 16-17% (both sexes) complete at 12 hours, 72% (males) and 61% (females) complete at 24 hours, and 87% (males) and 80% (females) complete at 36 hours.

2.3.4.4 Other Routes of Exposure

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 up to 18 hours after treatment for the determination of levels of MTBE, methanol, *tert*-butanol, formic acid, and formaldehyde. Mean urinary levels of MTBE were about 0.018 mg/mL at 5 hours after treatment and < 0.005 mg/mL at 12-l8 hours. Mean urinary levels of *tert*-butanol were higher than levels of MTBE, with about 0.036 mg/mL at 5 hours and 0.03 mg/mL at 12-18 hours after treatment. Methanol was found in only 3 patients, while no formaldehyde or formic acid were found.

The excretion of radioactive MTBE after intraperitoneal (Biodynamics 1984) and intravenous (Bioresearch Labs 1990b, 1991; Exxon 1988) dosing with ^{14}C-MTBE has been studied in rats. In general, results are similar to those obtained after inhalation and oral dosing of rats (Bioresearch Labs 1990b, 1990d; Exxon 1988). Since intraperitoneal and intravenous routes of exposure are not environmentally relevant, and since information is available for the inhalation, oral, and dermal routes, details of the results of studies by the other routes are not provided.

The only toxicokinetic information for MTBE in mice involves pulmonary excretion after intraperitoneal dosing (Yoshikawa et al. 1994). The mice were injected with 50, 100, or 500 mg/kg. The pulmonary eliminations were very similar for each dose. MTBE concentrations in air increased rapidly and reached

maximum concentrations within 35-45 minutes after dosing. The peak air concentrations of MTBE were approximately 8 ppm at the dose of 50 mg/kg, 30 ppm at the dose of 100 mg/kg, and 200 ppm at the dose of 500 mg/kg. Thereafter, the MTBE concentrations decreased exponentially, with an initial rapid decrease (α-phase; 40-200 minutes) followed by a slow decrease (β phase; 200-360 minutes) for the 100 and 500 mg/kg doses. No β phase was observed for the 50 mg/kg dose because the measurement of MTBE could not be continued after 2 10 minutes. The mean pulmonary elimination rate constants of the α phase were 0.0166 per minute for the 50 mg/kg dose, 0.0159 per minute for the 100 mg/kg dose, and 0.0148 per minute for the 500 mg/kg dose. The mean pulmonary elimination constants for the β phase were 0.0086 per minute at the 100 mg/kg dose and 0.0089 per minute at the 500 mg/kg dose. Thus, the half-time for the α phase was approximately 45 minutes, and the half-time for the β phase was approximately 80 minutes. The amount of MTBE exhaled was dose-dependent: the mice exhaled 23.2% of the 50 mg/kg dose, 37.6% of the 100 mg/kg dose, and 69.0% of the 500 mg/kg dose, reflecting the low solubility of MTBE in blood. Greater than 90% of the amount excreted in air was eliminated within 3 hours.

2.3.5 Physiologically Based Pharmacokinetic (PBPK) Model

A physiologically based pharmacokinetic (PBPK) model for MTBE and *tert*-butanol in male Fischer 344 rats is being 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 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 obtained in the studies by Bioresearch Labs (1990a, I990b, 1990c, 1990d) and for humans in the study by Cain et al. (1994). The model described MTBE metabolism as occurring via two saturable pathways and predicted gas uptake data up to 2,000 ppm initial concentrations. The model was also able to predict MTBE blood concentrations in rats exposed by inhalation to 400 or 8,000 ppm for 6 hours, in rats injected intravenously with 40 mg/kg, and in rats treated orally at 40 or 400 mg/kg in the Bioresearch Labs (1990a, 1990b, 1990c, 1990d) studies. 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 are needed to refine the model.

2.4 MECHANISMS OF ACTION

As MTBE is a volatile, lipophilic molecule of small molecular size (see Chapter 3) it is rapidly and extensively absorbed from the respiratory and gastrointestinal tracts (Bioresearch Labs 1990a, 1990b, 1990c, 1990d). Dermal absorption is less extensive (Bioresearch Labs 1990a, 1990b). Absorption is probably by passive diffusion. Much of the absorbed MTBE is rapidly excreted as unchanged MTBE in the expired air. The remainder is demethylated to *tert*-butanol and formaldehyde via cytochromes P-4502El and P-4502B1 activities (Brady et al. 1990). Formaldehyde may be further reduced to methanol and oxidized to formic acid (Snyder 1979), which may be further metabolized to carbon dioxide. *tert*-Butanol is further metabolized to 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid, which are excreted in the urine (Bioresearch Labs 1990b). Unchanged MTBE and *tert*-butanol are the main respiratory excretion products. MTBE and its metabolites show little tendency to accumulate in tissues (Bioresearch Labs 1990b). An inhalation study in rats indicated that MTBE and its metabolite *tert*-butanol were distributed to the brain at concentrations similar to blood concentrations (Savolainen et al. 1985). In addition, the perirenal fat concentration of MTBE was higher than the blood concentration of MTBE, but no levels of *tert*-butanol were found in perirenal fat.

Neurological Effects. The presence of MTBE and/or *tert*-butanol in the brain may account for the central nervous system toxicity of MTBE. Numerous studies have shown that exposure to MTBE results in profound central nervous system depression in animals (ARCO 1980; Bioresearch Labs 1990d; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Gill 1989; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 1991; Robinson et al. 1990; Tyl 1989). The central nervous system depression has been characterized by ataxia, hypoactivity, drowsiness, anesthesia, prostration, lack of startle response, and lack of righting reflex. The exact mechanism by which MTBE and *tert*-butanol exert their central nervous system depression is not known, but may involve changes in membrane fluidity.

Hepatic Effects. MTBE is minimally toxic to the liver of animals, resulting in increased liver weights (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; ITT Research Institute 1992; Neeper-Bradley 1991; Robinson et al. 1990; Tyl 1989). This increase in liver weight may be due to induction of microsomal enzymes. Intraperitoneal injection of rats with MTBE resulted in a 47-fold induction of pentoxyresorufin O-dealkylase activity, an activity associated with cytochrome P-4502B1 (Brady et al. 1990). Cytochrome P-4502B1 is also involved in the demethylation of MTBE; thus, MTBE appears to induce its own metabolism. Mice exposed to MTBE by inhalation had an

increased incidence of hepatocellular hypertrophy (which could be related to enzyme induction) after exposure for intermediate (Chun and Kintigh 1993) and chronic (Burleigh-Flayer et al. 1992) durations and hepatocellular adenoma after exposure for chronic durations (Burleigh-Flayer et al. 1992), but no liver lesions were found in rats similarly exposed chronically (Chun et al. 1992). Mice are particularly susceptible to chemically induced hepatic tumors. The results of cell proliferation evaluations of hepatocytes from male and female mice exposed for 5 or 23 exposures revealed statistically significant increased uptake of 5-bromo-2'-deoxyuridine in the nuclei of hepatocytes after 5 exposures, but not after 23 exposures (Chun and Kintigh 1993). The authors proposed that MTBE initially places an increased metabolic demand on liver cells resulting in a compensatory increase in hepatocellular proliferation that eventually leads to hepatocellular hypertrophy. As noted in Section 2.5 for Genotoxic Effects, the available data indicate that MTBE has little or no genotoxic activity. The mechanism by which MTBE induces liver tumors in mice is a topic of ongoing studies, which indicate that the mechanism may involve the modulation of hormones, specifically estrogen, but that MTBE did not show tumor-promoting activity (see Section 2.10.3).

Suggestive clinical evidence of hepatocellular necrosis (increased SGPT or SGOT, serum lactic dehydrogenase) was found in rats given MTBE orally for 14 or 90 days (Robinson et al. 1990), but histological evidence of hepatocellular necrosis was not found in any study (Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 1991; Robinson et al. 1990).

Renal Effects. MTBE produces renal effects in male rats that are consistent with the α_{2u}-globulin syndrome, including large hyaline droplets in proximal tubular cells (Dodd and Kintigh 1989; Robinson et al. 1990); intratubular granular casts at the junction of the outer and inner stripe of the outer medulla (Robinson et al. 1990); increases in α_{2u}-globulin accumulation in kidney tissues (Swenberg and Dietrich 1991); increased incidence and severity and earlier onset of chronic progressive nephropathy, accompanied by fibrous osteodystrophy, hyperplasia in the parathyroid glands, and mineralization in numerous tissues; and an increased incidence of renal tubular adenoma and carcinoma (Chun et al. 1992). However, the evidence that the renal effects observed in male rats exposed to MTBE are associated with α_{2u}-globulin accumulation has been questioned, since the increase in α_{2u}-globulin accumulation was not dose-related, and α_{2u}-globulin positive proteinaceous casts at the junction of the proximal tubules and thin limb of Henle were not observed, unlike the classical lesions of other α_{2u}-globulin inducing agents (Swenberg and Dietrich 1991). Furthermore, a study specifically designed to determine whether MTBE induces α_{2u}-globulin accumulation in male rats found no evidence, based on immunostaining with an

antibody to α_{2u}-globulin (Chun and Kintigh 1993). However, an increased accumulation of protein in male renal proximal convoluted tubule epithelium associated with increased epithelial cell proliferation was found as determined by Mallory's Heidenhain technique. Chun and Kintigh (1993) suggested that a mechanism other than α_{2u}-globulin accumulation (perhaps the accumulation of another unknown protein unique to male rats) may be involved. It is possible that the hypothetical protein is also unique to male rats and acts in the same way as α_{2u}-globulin. Since the female rats did not have increased epithehal cell proliferation in the renal tubules, the female rat kidney tissues were not examined for protein accumulation.

α_{2u}-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 α_{2u}-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 α_{2u}-globulin is relatively slow compared with that of other proteins (Swenberg et al. 1989). Chemicals that bind to α_{2u}-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 α_{2u}-globulin complex causes lysosomal overload and necrosis of the tubule cells, with subsequent proliferative regeneration. If exposure to the chemical is chronic, accumulation, necrosis, and subsequent cellular proliferation continues, and can lead to a carcinogenic response. α_{2u}-Globulin nephropathy is a condition specific to male rats; that is, it has not been found in female rats or males and females of any other species, including humans.

The increased incidence, increased severity, and earlier onset of chronic progressive nephropathy observed in male rats exposed by inhalation to MTBE may involve the accumulation of α_{2u}-globulin or a similarly acting unknown other protein unique to male rats. Although chronic progressive nephropathy is an agerelated phenomenon common in rats, chemicals that induce α_{2u}-globulin (or by extension, the unknown protein) accumulation may exacerbate chronic progressive nephropathy in male rats. However, chronic progressive nephropathy was also enhanced in female rats (which do not produce α_{2u}-globulin in the liver), but at higher doses of MTBE, with lesser severity, and at later onset than in males (Chun et al. 1992). Thus, an additional unknown mechanism may also be involved in the enhancement of chronic progressive nephropathy by MTBE.

Lymphoreticulur Effects. Although most studies in animals exposed to MTBE indicate that the lymphoreticular system is not a target of MTBE toxicity, a 13-week inhalation study indicated a higher

incidence of lymphoid hyperplasia in the submandibular lymph nodes in male rats (Dodd and Kintigh 1989), and a chronic oral study indicated an increased incidence of dysplastic proliferation of lymphoreticular tissues and of leukemia and lymphoma in female rats (Belpoggi et al. 1995). Since the increased incidence of leukemia and lymphoma was dose-related, but the increased incidence of dysplastic proliferation of the lymphoreticular tissues was higher at the low dose than at the high dose, 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 (Belpoggi et al. 1995). Although the mechanism by which MTBE produced lymphoreticular effects and leukemia is not known, the authors discussed the possibility that formaldehyde, a known metabolite of MTBE (see Section 2.3.3), is involved, since formaldehyde increased the incidence of lymphomas and leukemias in male and female rats in other studies from their laboratories.

Reproductive Effects. Although reproductive studies in animals indicate that MTBE exposure does not affect reproductive performance or result in nonneoplastic histopathological lesions in reproductive organs, a dose-related increased incidence of interstitial cell adenoma in the testes was observed in Fischer 344 male rats in a chronic inhalation study (Chun et al. 1992). Fischer 344 rats commonly develop testicular tumors, and the incidences in MTBE-exposed groups were in the range of historic controls, while the incidence in the concurrent control group was lower than the incidence in historic controls. However, in a chronic oral study of Sprague-Dawley rats, an increased incidence of Leydig interstitial cell tumors of the testes was found (Belpoggi et al. 1995). As noted in Section 2.5 for Genotoxic Effects, the available data indicate that MTBE has little or no genotoxic activity. The mechanism by which MTBE induces testicular tumors in rats is not known, but ongoing or future studies investigating the relationship between endocrine modulation and the mechanism of MTBE-induced toxicity may provide some insight (see Section 2.10.3).

Developmental Effects. MTBE exposure of pregnant rats and rabbits did not result in developmental effects in the offspring (Conaway et al. 1985; Tyl 1989). MTBE did produce developmental effects in mice, including increased incidence of fused sternebrae, increased number of nonviable implantations per litter, increased late resorptions, reductions in the number of viable implantations, reduced fetal body weight, significantly increased incidences of cleft palate, skeletal defects, and reduced skeletal ossification, and a significantly reduced incidence of partial fetal atelectasis (Tyl and Neeper-Bradley 1989). The mechanism by which MTBE resulted in most of these development effects is not known, but the increased incidence of cleft palate was considered to be related to maternal stress with possible associated increased endogenous levels of corticosterone.

2.5 RELEVANCE TO PUBLIC HEALTH

Until recently (1980), commercial production of MTBE was relatively low; however, MTBE is currently used extensively as a gasoline additive to increase octane levels and to reduce the levels of carbon monoxide emissions. This widespread use has increased the possibility of general population exposure. In addition to inhalation risks while fueling motor vehicles, MTBE is emitted to the ambient air, primarily from pre-combustion volatilization. These atmospheric pathways are of most concern for the general population; however, more localized risks can arise if MTBE becomes a groundwater or soil contaminant from major production sites, large tank batteries, transfer terminals, active or abandoned waste sites, leaking underground storage tanks, or from gasoline spills. Thus, while inhalation exposure is the major route of concern, exposure from contaminated drinking water or dermal contact with contaminated soil can also occur. MTBE is also used to dissolve gallstones by injecting it into the bile duct or gallbladder, but side effects of this procedure are of concern only for individuals undergoing this therapy.

MTBE is a volatile, lipophilic molecule of small molecular size, which is rapidly and extensively absorbed from the respiratory and gastrointestinal tracts. Dermal absorption is less extensive. Absorption is probably by passive diffusion. Much of the absorbed MTBE is rapidly excreted as unchanged MTBE in the expired air. The remainder is metabolized to *tert*-butanol, formaldehyde, methanol, formic acid, carbon dioxide, 2-methyl-l,2-propanediol, and α-hydroxyisobutyric acid. Unchanged MTBE and *tert*-butanol are the main respiratory excretion products. MTBE and its metabolites distribute to tissues, but show little tendency to accumulate. MTBE and its metabolite *tert*-butanol may distribute to the brain at concentrations similar to blood concentrations. The presence of MTBE and *tert* -butanol in the brain may account for the transient central nervous system depression, which appears to be the most sensitive effect of acute exposure.

Information regarding health effects of MTBE exposure in humans was located in studies of motorists, gas station attendants, and mechanics, in experimental studies in subjects exposed by inhalation to MTBE for one hour, and in clinical studies of patients receiving MTBE therapy for the dissolution of gallstones. In some situations, motorists and workers have reported symptoms of coughing, burning sensations in the nose and throat, headache, nausea or vomiting, dizziness, and feelings of spaciness and disorientation that may been associated with MTBE exposure. In some studies, it was determined that the subjective reporting of these symptoms declined when the oxyfuel programs were terminated. Other studies failed to detect symptoms associated with MTBE exposure. Whether the symptoms reported for MTBE in gasoline are directly due to MTBE, to mixtures of the components in the oxygenated gasoline, or to atmospheric

degradation products of MTBE (e.g., formaldehyde, *tert*-butanol, or *tert*-butyl formate), to all of these factors, or not even MTBE-related are current topics of debate and research. In addition, such factors as cloud cover, the condition of individual automobiles, dose-time factors, and the role of weather and sunlight in modulating the amounts of the atmospheric degradation products of MTBE may partly explain why some individuals experience these effects and other don't and why some individuals experience effects on some days, but not on other days.

In patients receiving MTBE for gallstone dissolution, effects include typical signs of transient central nervous system depression, gastrointestinal irritation, evidence of necrosis in the liver and gallbladder, transient cardiovascular effects such as hyper- or hypotension and palpitations, and transient leukocytosis. These side effects have occurred from overflow of MTBE from the gallbladder lumen or extravasation into the systemic circulation. In most cases, the patients who experienced these side effects recovered with no residual effects.

The central nervous system effects are characteristic of ether anesthesia and ethyl alcohol intoxication and include lacrimation, ataxia, loss of righting reflex, hyperpnea, labored breathing, incoordination, prostration, drowsiness, hypoactivity, decreased startle and pain reflexes, decreased muscle tone, anesthesia, blepharospasm, and stereotypy. These effects have been observed in animals exposed for acute-, intermediate-, and chronic-durations by the inhalation and/or oral routes, and probably can also occur after dermal exposure. Other effects noted in animals include increased liver and kidney weights, induction of liver and kidney microsomal enzymes, respiratory irritation after inhalation exposure, gastrointestinal irritation and decreased BUN levels after oral exposure, dermal irritation after dermal exposure, and ocular irritation after direct contact of the liquid or vapor with the eyes. Histological hepatic effects have been observed only in mice and consisted of increased incidences of hepatocellular hypertrophy and hepatocellular adenoma after chronic-duration inhalation exposure. Renal effects have been observed in rats, and are suggestive of accumulation a α_{2u}-globulin or another protein unique to male rats, which has no relevance to human health, and exacerbation of chronic progressive nephropathy in male and female rats, which may have relevance to human health. In addition, male rats developed renal tubule cell carcinoma and adenoma, the development of which may be related to α_{2u}-globulin or other unique protein accumulation in the proximal renal tubule cells. However, the evidence that the kidney tumor development in male rats is related to α_{2u}-globulin or another protein accumulation is limited, making the relevance to human health uncertain. In a chronic oral study, female rats were reported to develop dysplastic proliferation of lymphoreticular tissues (possibly preneoplastic) and lymphoma and leukemia, while male rats were reported to develop testicular Leydig cell tumors.

MTBE has been well studied for reproductive effects in animals exposed by the inhalation routes for acute, intermediate, and chronic durations. The results indicate that MTBE is not a reproductive toxicant in animals. MTBE has also been studied for developmental effects in rats, rabbits, and mice. No developmental effects were found in rabbits and the only effect in rats was decreased body weight of F_1 and F_2 pups. MTBE did produce developmental effects in mice, including increased incidence of fused sternebrae, increased number of nonviable implantations per litter, increased late resorptions, and reductions in the number of viable implantations. In addition, reduced fetal body weight, significantly increased incidences of cleft palate, skeletal defects, and reduced skeletal ossification, and a significantly reduced incidence of partial fetal atelectasis were observed in mouse fetuses. Whether the offspring of humans exposed to MTBE would develop developmental effects is not known. The available data on the genotoxic potential of MTBE indicate that it may be weakly genotoxic.

Based on toxicity and toxicokinetic data from studies in animals, potentially susceptible humans populations may be people who ingest barbiturates or alcoholic beverages, which induce microsomal enzymes; human embryos and fetuses because of the developmental effects observed in mice; and elderly people or people with pre-existing nephropathy, because of the exacerbation of chronic progressive nephropathy in female rats. In addition, some people may become more chronically sensitive to MTBE as a result of prolonged low level exposure.

Minimal Risk Levels for MTBE

Inhalation MRLs

- An MRL of 2 ppm has been derived for acute-duration inhalation exposure (14 days or less) to MTBE.

The MRL for acute-duration inhalation exposure is based on a NOAEL of 800 ppm for 6 hours for neurological effects in rats in a study by Gill (1989). In this study, groups of 22 male and 22 female Fischer 344 rats were exposed to 0, 800, 4,000, or 8,000 ppm MTBE for 6 hours. Groups of 14 male and 14 female rats were studied for motor activity, and the remaining groups of 8 male and 8 female rats were given a functional observation battery of tests at 1, 6, and 24 hours after exposure. Concentration-related increases in ataxia and duck-walk gait occurred in both males and females at 4,000 and 8,000 ppm (Gill 1989). Other effects noted in high-dose 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, indicating the transient nature. The time course of changes in motor activity corresponded with the functional observation battery findings, and supported the exposure-related central nervous system depression. No neurological effects were observed at the NOAEL of 800 ppm for 6 hours. The NOAEL of 800 ppm is supported by another study, which found only increased motor activity in female rats, but no other neurological effects in rats at 800 ppm, 6 hours per day, 5 days per week for 13 weeks (Dodd and Kintigh 1989). In this study, hypoactivity at 4,000 ppm and hypoactivity and ataxia at 8,000 ppm were observed daily after the 6-hour per day exposure, thus representing effects of acute exposure. A number of acute-duration inhalation studies in rats, mice, and rabbits have described similar clinical signs of neurotoxicity at MTBE concentrations ≥ 2,000 ppm (ARCO 1980; Biodynamics 1981; Bioresearch Labs 1990d; Chun and Kintigh 1993; Dodd and Kintigh 1989; Tyl 1989; Tyl and Neeper-Bradley 1989).

- An MRL of 0.7 ppm has been derived for intermediate-duration inhalation exposure (15-364 days) to MTBE.

The MRL for intermediate-duration inhalation exposure is based on a NOAEL of 400 ppm for clinical signs of neurotoxicity in a reproductive study in male and female rats (Neeper-Bradley 1991). In this study, groups of 25 male and 25 female rats were exposed to 0, 400, 3,000, or 8,000 ppm MTBE for 6 hours per day, 5-7 days per week. Exposure for 10 weeks prior to mating and through day 19 of gestation to the concentration of 8,000 ppm MTBE resulted in salivation and hypoactivity in F_0 and F_1 parental rats. F_0 and F_1 parental groups also showed hypoactivity and lack of startle response, as well as blepharospasm, at 3,000 ppm. In a 28-day study, rats and mice had similar effects at exposure levels of ≥ 3,000 ppm, but not at 400 ppm (Chun and Kintigh 1993). In a 13-week study, rats were exposed to 0, 800, 4,000, or 8,000 ppm MTBE 6 hours per day, 5 days per week (Dodd and Kintigh 1989). At 4,000 ppm, the rats were hypoactive, had elevated body temperature, and decreased hind limb grip strength. At 8,000 ppm, both ataxia and hypoactivity were observed. Some hyperactivity occurred in female rats at 800 ppm, but no signs were observed in males at 800 ppm.

- An MRL of 0.7 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to MTBE.

The MRL for chronic-duration inhalation exposure to MTBE is based on a NOAEL of 400 ppm for chronic progressive nephropathy in female rats in a study by Chun et al. (1992). In this study, groups of 50 male and 50 female Fischer 344 rats were exposed to 0, 400, 3,000, or 8,000 ppm MTBE 6 hours per day, 5 days per week for up to 24 months. Male rats exposed to $\geq$ 400 ppm had increased mortality and decreased mean survival time due to chronic progressive nephropathy. End points monitored were clinical signs, body weight, organ weight, hematological parameters, corticosterone evaluation, and comprehensive gross and histological examination. Increased absolute and relative liver and kidney weights were observed in females at $\geq$ 3,000 ppm. No gross or histopathological lesions were found in the liver of either sex, but concentration-related increased incidence and severity of chronic progressive nephropathy, accompanied by osteodystrophy, hyperplasia of the parathyroids, and mineralization in numerous tissues was found in males at all exposure levels and in females at $\geq$ 3,000 ppm. No evidence of renal effects was found in the female rats at 400 ppm. 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 α_{2u}-globulin or another unknown protein unique to male rats. Because the enhancement of chronic progressive nephropathy, which led to increased mortality and decreased survival time in males, may be associated with the accumulation of α_{2u}-globulin or other protein unique to male rats, these end points in male rats are not considered for MRL derivation. However, since female rats also had enhanced chronic progressive nephropathy not associated with accumulation of a male rat specific protein, the chronic inhalation MRL of 0.7 ppm was calculated based on the NOAEL of 400 ppm for renal effects in female rats. The chronic-duration inhalation NOAEL for renal effects is also a NOAEL for clinical signs of neurotoxicity in rats in this study. The NOAEL of 400 ppm for chronicduration inhalation exposure to MTBE is supported by a similar study in male and female mice similarly exposed to the same concentrations for 18 months (Burleigh-Flayer et al. 1992). In this study, absolute and relative liver weights were increased at $\geq$ 3,000 ppm, and absolute and relative kidney weights were increased at 8,000 ppm. Comprehensive histological examination of organs and tissues revealed an increased incidence of hepatocellular hypertrophy and hepatocellular adenoma in female mice at 8,000 ppm. The NOAEL for liver effects in this study was 400 ppm, which was also a NOAEL for neurotoxicity in mice in this study.

Oral MRLs

- An MRL of 0.4 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to MTBE.

The acute-duration oral MRL is based on a NOAEL of 40 mg/kg for neurological effects in a pharmacokinetic study in rats (Bioresearch Labs 1990b). In this study, groups of 6 male and 6 female Fischer 344 rats were treated by gavage with MTBE in water at doses of 0, 40, and 400 mg/kg. The rats given 400 mg/kg showed signs of drowsiness. Although this study was designed as a pharmacokinetic study rather than a toxicity study, the observation of drowsiness is consistent with observations of central nervous system depression in animals exposed to MTBE by the inhalation and oral routes, and the study provides the highest NOAEL below which there is no LOAEL. In other acute-duration oral studies, rats had mild central nervous system depression at 1,900 mg/kg and ataxia at 2,450 mg/kg (ARCO 1980), salivation at 90 mg/kg and hypoactivity and/or ataxia at 440 mg/kg (ITT Research Institute 1992), and profound but transient anesthesia at 1,200 mg/kg or 1,428 mg/kg/day for 14 days (Robinson et al. 1990).

- An MRL of 0.3 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to MTBE.

The intermediate-duration oral MRL of 0.3 mg/kg/day is based on a minimal LOAEL of 100 mg/kg/day for hepatic effects in rats exposed by gavage to MTBE for 90 days (Robinson et al. 1990). In this study, significantly decreased BUN levels were observed in both male and female rats exposed to the lowest dose tested. Significantly decreased BUN levels were also observed in female rats given 1,428 mg/kg/day orally for 14 days (Robinson et al. 1990). In the 90-day study, relative liver weights were significantly increased in female rats at 900 mg/kg/day and in male rats at 900 and 1,200 mg/kg/day. Serum lactic dehydrogenase was significantly elevated in females only at 300 mg/kg/day, and SGOT was significantly elevated in males at 300 and 1,200 mg/kg/day. However, no treatment-related histopathological lesions were found in the liver in either the 14-day study or the 90-day study.

An MRL was not derived for chronic-duration oral exposure to MTBE because in the only chronic oral study (Belpoggi et al. 1995), increased mortality occurred in female rats at the lowest dose tested (250 mg/kg/day). Furthermore, the dose of 250 mg/kg/day was associated with dysplastic proliferation of lymphoreticular tissues and an increased incidence of lymphoma and leukemia in female rats.

Death. No information was located regarding death in humans exposed to MTBE by any route. The data in animals indicate that very high inhalation concentrations, oral doses, or derrnal doses are required to cause death after acute exposure. Acute inhalation LC_{50} values range from 33,700 ppm for 4 hours in rats (ARCO 1980) to 180,000 ppm for 10 minutes in mice (Snamprogetti 1980). Acute oral LD_{50} values were 3,866 mg/kg in rats (ARCO 1980) and 4,000 mg/kg in mice (Little et al. 1979). No deaths occurred in rats exposed to $\leq$ 8,000 ppm for 6 hours (Bioresearch Labs 1990d; Gill 1989). Repeated inhalation and oral dosing of rats, mice, and rabbits even with high doses generally has resulted in no deaths in acute- or intermediate-duration studies. The following inhalation concentrations did not result in death: $\geq$ 8,000 ppm for 6 hours per day for 1 or 2 days (Vergnes and Chun 1994; Vergnes and Morabit 1989) or for 5 days per week for $\leq$ 13 weeks (Biodynamics 1981; Chun and Kintigh 1993; Dodd and Kintigh 1989; Greenough et al. 1980); $\leq$2,500 ppm for 16-28 weeks (Biles et al. 1987); and 80,000 for 5-10 minutes per day, 5 days per week for 30 days (Snamprogetti 1980). Oral doses $\geq$ 1,750 mg/kg/day for $\leq$ 4 weeks (Bioresearch Labs 1990a; ITT Research Institute 1992; Robinson et al. 1990; Ward et al. 1994) and $\geq$ 1,200 mg/kg/day for 90 days (Robinson et al. 1990) also did not result in death. Similarly, no deaths occurred in rats, mice, or rabbits exposed to 8,000 ppm during gestation (Conaway et al. 1985; Tyl 1989; Tyl and Neeper-Bradley 1989). In addition, no deaths occurred after dermal exposure of rats at $\leq$ 400 mg/kg for 6 hours (Bioresearch Labs 1990b) or rabbits at 10,000 mg/kg for 24 hours (ARCO 1980). However, chronic inhalation exposure resulted in increased mortality due to enhanced chronic progressive nephropathy in male rats at $\geq$ 400 ppm (Chun et al. 1992) and to obstructive uropathy in male mice at 8,000 ppm (Burleigh-Flayer et al. 1992). Mortality was not increased at 400 or 3,000 ppm in male mice or at any concentration in female rats or mice. In a chronic oral study, female rats, but not male rats, had a dose-related increase in mortality at $\geq$ 250 mg/kg/day beginning at 16 weeks from the start of the study (Belpoggi et al. 1995).

Lower doses produced death in animals after exposure routes that are not environmentally relevant (e.g., intravenous, intrahepatic), but humans have been treated with MTBE via intracystic infusion for the dissolution of gallstones. Significant numbers of rats died after a dose of only 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, 2 of 5 rats died after dosing with 3,705 mg/kg (Brady et al. 1990), but intraperitoneal LD_{50} values of 1,249 mg/kg in rats and 1,010 mg/kg in mice were determined (Snamprogetti 1980). 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 6 rabbits that received 1,782 mg/kg through the bile duct died (Adam et al. 1990).

Based on the information in animals, it is unlikely that levels of MTBE in occupational settings, the ambient environment, and at hazardous waste sites would be high enough to cause death of humans. Exposure to MTBE at high levels would be expected only in the case of accidental spills.

Systemic Effects

Respiratory Effects. Coughing and a burning sensation of the nose or throat were frequently reported symptoms in motorists, gas station attendants, and mechanics during oxygenated fuel programs in which MTBE was added to gasoline for reducing carbon monoxide emissions (CDC 1993a, 1993b; Moolenaar et al. 1994; White et al. 1995). In addition, a preliminary survey of symptoms in petroleum refinery employees working with gasoline containing MTBE indicated high self-reporting of respiratory symptoms, such as nose irritation, cough, and shortness of breath (Mehlman 1995). However, these and other studies (Anderson et al. 1995; Beller and Middaugh 1992; Chandler and Middaugh 1992) in humans suggest that knowledge about oxygenated fuel programs, including the likely awareness of the potential negative effects of MTBE and the higher cost of oxygenated fuels, may have biased the subjective symptom reporting. In addition, other surveys of humans (CDC 1993c; Fiedler et al. 1994; Mohr et al. 1994) and experimental studies in humans (Cain et al. 1994; Prah et al. 1994) found no increase in health complaints among people with high oxygenated fuel or MTBE exposure. No information was located regarding respiratory effects in humans after exposure to MTBE by the oral or dermal routes. In animals, changes in lung weight (either decreases or increases) have been observed after intermediate-duration inhalation exposure (Greenough et al. 1980) or after oral dosing for 14 or 90 days (Robinson et al. 1990). However, treatment-related gross or histopathological lung or respiratory tract lesions generally have not been observed in rats, mice, or rabbits exposed by inhalation or orally for any duration (ARCO 1980; Belpoggi et al. 1995; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; ITT Research Institute 1992; Neeper-Bradley 1991 ; Robinson et al. 1990; Tyl 1989). However, a high incidence of increased severity of chronic inflammation of the nasal mucosa and trachea was observed in rats exposed to 1,000 or 3,000 ppm for 9 days (Biodynamics 1981), indicative of irritation at the portal of entry and exit, as much unchanged MTBE and the metabolite *tert*-butanol are eliminated in the expired air. An RD_{50} (50% decrease in respiratory rate) of 4,604 ppm, indicative of sensory irritation, was found in mice exposed by inhalation for 1 hour (Tepper et al. 1994). Four-hour inhalation exposures of rats to very high concentrations of MTBE ($\geq$ 18,892 ppm) resulted in such effects as hyperpnea, tachypnea, nasal discharge, and respiratory failure (ARCO 1980); labored breathing was observed in mouse dams exposed to 8,000 ppm MTBE during gestation (Tyl and Neeper-Bradley 1989); and labored respiration was observed

in rats given high oral doses (≥ 4,080 mg/kg) (ARCO 1980). These effects may be related to the central nervous system effects of MTBE (see Neurological Effects, below).

Respiratory effects have also been found in animals after exposure routes that are not environmentally relevant (e.g., intravenous, intrahepatic, intraperitoneal), but humans have been treated with MTBE intracystically for the dissolution of gallstones. While a number of clinical studies of patients receiving MTBE therapy have recorded side effects, no studies were located that reported respiratory effects. 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 mglkg 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. 1985a).

Based on the subjective reports of symptoms of coughing and respiratory irritation in motorists, gas station attendants, and mechanics during oxygenated fuel programs, levels of MTBE in occupational settings, the ambient environment, and at hazardous waste sites might be high enough to cause respiratory tract irritation and breathing difficulties in humans. Based on animal studies, effects on the lungs might conceivably occur in patients receiving MTBE for gallstone dissolution if leakage from the gallbladder occurred, but none of clinical studies located documented respiratory effects.

Cardiovascular Effects. No information was located regarding cardiovascular effects in humans after exposure to MTBE by the inhalation, oral, or dermal routes or in animals exposed by the dermal route. However, inhalation studies in which rats and/or mice were exposed to MTBE for acute, intermediate, and chronic durations (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980) or orally for intermediate or chronic durations (Belpoggi et al. 1995; ITT Research Institute 1992; Robinson et al. 1990) indicated that MTBE produced no treatment-related histopathological lesions in the heart or aorta.

Although no studies were located regarding cardiovascular effects in humans exposed to MTBE by environmentally relevant routes, humans have been treated with MTBE intracysticafly for the dissolution of gallstones. A number of clinical studies of patients receiving MTBE therapy have recorded side effects, among which are transient hypertension in 1 of 10 patients (Murray et al. 1988) and hypotension in 2 of 29, palpitations in 1 of 29, and angina in 1 of 29 patients (Neoptolemos et al. 1990) given MTBE via nasobiliary catheter. Vasovagal reactions were found in 4 of 24 patients given MTBE via the percutaneous transhepatic route to the gallbladder (Eidsvoll et al. 1993). A number of studies have been conducted in animals to determine possible side effects of MTBE therapy for gallstone dissolution. 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).

Levels of MTBE in occupational settings, the ambient environment, and at hazardous waste sites are probably not high enough to cause cardiovascular effects in humans. Based on animal studies and clinical studies in humans, effects on the cardiovascular system might occur in patients receiving MTBE for gallstone dissolution.

Gastrointestinal Effects. Nausea or vomiting, which may be related to neurological symptoms, were reported symptoms in motorists, gas station attendants, and mechanics during oxygenated fuel programs in which MTBE was added to gasoline for reducing carbon monoxide emissions (CDC 1993a, 1993b; Moolenaar et al. 1994; White et al. 1995). In addition, a preliminary survey of symptoms in petroleum refinery employees working with gasoline containing MTBE indicated high self-reporting of nausea (Mehlman 1995). However, these and other studies (Anderson et al. 1995; Beller and Middaugh 1992; Chandler and Middaugh 1992) in humans suggest that knowledge about oxygenated fuel programs, including the likely awareness of the potential negative effects of MTBE and the higher cost of oxygenated fuels, may have biased the subjective symptom reporting. In addition, other humans surveys (CDC 1993c; Fiedler et al. 1994; Mohr et al. 1994) and experimental studies in humans (Cain et al. 1994; Prah et al. 1994) found no increase in health complaints among people with high oxygenated fuel or MTBE exposure. No information was located regarding gastrointestinal effects in humans exposed to MTBE by the oral or dermal routes. The gastrointestinal tract was not affected in rats exposed to MTBE by inhalation for any duration or in mice exposed chronically, as determined by histological examination (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980; Neeper-Bradley 1991). However, MTBE appears to be irritating to the gastrointestinal tract of

rats exposed orally as evidenced by diarrhea and histological lesions. Effects included diarrhea in rats treated with ≥ 357 mg/kg/day MTBE for 14 days or ≥ 100 mg/kg/day for 90 days (Robinson et al. 1990), and submucosal edema, subacute inflammation, epithelial hyperplasia, and ulceration in the stomach of male and female rats given 1,750 mg/kg/day MTBE (ITT Research Institute 1992). As with irritation of the respiratory tract observed in animals after inhalation exposure, these gastrointestinal effects represent irritation at the portal of entry. However, in a study in which MTBE was applied dermally to the dorsal flank of rats via an occluded chamber, the rats developed slight diarrhea at a dermal dose of 40 mg/kg (Bioresearch Labs 1990b). Since the MTBE was applied under occlusion, it is unlikely that oral exposure via grooming contributed to the exposure.

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. 1985b; Bonardi et al. 1986; Brandon et al. 1988; DiPadova et al. 1986; Eidsvoll et al. 1993; Hellstem et al. 1990; Ho11 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). The irritation occurs due to leakage from the gallbladder into the gastrointestinal tract. A number of studies have been conducted in animals to determine possible side effects of MTBE therapy for gallstone dissolution. Effects noted in animals after administration of MTBE by other routes include light diarrhea in rats injected intravenously (Bioresearch Labs 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. 1985a).

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 mucusol weight (Zakko et al. 1995).

Levels of MTBE inhaled by motorists, in occupational settings, the ambient environment, and at hazardous waste sites are probably not high enough to cause gastrointestinal effects unrelated to

neurological symptoms in humans. Based on clinical studies in humans, effects on the gastrointestinal tract are likely in patients receiving MTBE for gallstone dissolution.

Hematological Effects. No information was located regarding hematological effects in humans after exposure to MTBE by the inhalation, oral, or dermal routes or in animals after exposure by the dermal route. Some changes in hematological parameters have been found in animals after inhalation (Dodd and Kintigh 1989; Greenough et al. 1980) and oral exposure (ITT Research Institute 1992; Robinson et al. 1990) to MTBE, but it is difficult to discern a typical pattern. In one oral study, female rats had changes indicative of hemoconcentration (elevated levels of erythrocytes, hemoglobin, and hematocrit, while leukocyte counts were reduced (Robinson et al. 1990). This hemoconcentration may have been due to a clinical dehydration condition associated with diarrhea observed in these animals instead of being a direct effect of MTBE. No treatment-related hematological effects were found in mice or rats in chronic-duration inhalation studies (Burleigh-Flayer et al. 1992; Chun et al. 1992).

Although no studies were located regarding hematological effects in humans exposed to MTBE by environmentally relevant routes, humans have been treated with MTBE intracystically for the dissolution of gallstones. A number of clinical studies of patients receiving MTBE therapy have recorded hematological findings. These include a transient leukocytosis (Allen et al. 1985b; Hellstern et al. 1990; Ho11 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 (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 (DiPadova et al. 1986; Eidsvoll et al. 1993; McNulty et al. 1991; Uchida et al. 1994). 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. 1985a; Peine et al. 1990) or pigs (Vergunst et al. 1994) received MTBE via gallbladder catheter, and no hematological effects were found.

Hematological effects do not seem to be a concern for humans exposed to MTBE in occupational settings, the ambient environment, and at hazardous waste sites. Based on clinical studies in humans,

hematological effects might occur in patients receiving MTBE for gallstone dissolution if accidental overflow of MTBE or bile leakage occurs during the procedure.

Musculoskeletal Effects. No information was located regarding musculoskeletal effects in humans after any route of exposure or in animals after dermal exposure. MTBE does not appear to have effects on skeletal muscle or bone of animals except for fibrous osteodystrophy, which was secondary to MTBE- induced exacerbation of chronic progressive nephropathy, in rats exposed chronically by inhalation (Chun et al. 1992). Gross and histological examination of bone and skeletal muscle of rats exposed to MTBE via inhalation for acute (Biodynamics 1981) and intermediate durations (Dodd and Kintigh 1989; Greenough et al. 1980), in mice exposed via inhalation for chronic durations (Burleigh-Flayer et al. l992), and in rats exposed orally for 4 weeks (ITT Research Institute 1992) or 104 weeks (Belpoggi et al. 1995) revealed no treatment-related lesions. MTBE had no effect on muscle succinate dehydrogenase or acetylcholinesterase activities in rats exposed by inhalation (Savolainen et al. 1985). Muscle creatinine kinase activity decreased at 2 weeks, returned to normal levels at week 10, and then significantly increased at 15 weeks. Since muscle creatinine kinase is associated with contractile activity, the changing activity due to MTBE exposure may represent an adaptation of the muscle cell.

The available information indicates that musculoskeletal effects are not a concern for humans exposed to MTBE in occupational settings, the ambient environment, and at hazardous waste sites. Clinical studies of patients receiving MTBE for gallstone dissolution have not described any effects on muscles or bone.

Hepatic Effects. No information was located regarding hepatic effects in humans after exposure to MTBE by the inhalation, oral, or dermal routes or in animals after dermal exposure. MTBE appears to be mildly toxic to the liver of animals exposed by the inhalation or oral routes, producing little or no clinical or histological evidence of hepatotoxicity in most studies. However, increased absolute and/or relative liver weight has been observed in rats exposed to 3,000 ppm for 9 days (Biodynamics 1981), in rats exposed to $\geq$ 4,000 ppm and in mice exposed to $\geq$ 2,000 ppm for 13 days (Dodd and Kintigh 1989), in rabbits exposed to 8,000 ppm during gestation (Tyl 1989), in rats exposed to 800-8,000 ppm for intermediate durations (Dodd and Kintigh 1989; Neeper-Bradley 1991), in rats and mice exposed to $\geq$ 3,000 ppm for intermediate (Chun and Kintigh 1993) and chronic durations (Burleigh-Flayer et al. 1992; Chun et al. 1992), and in rats dosed orally with 900-l,750 mg/kg/day for 14 days to 13 weeks (ITT Research Institute 1992; Robinson et al. 1990). Since the increased liver weight may be due to induction of hepatic microsomal enzymes or to increased hepatocellular proliferation (Chun and Kintigh 1993), and because the incidence of hepatocellular hypertrophy was significantly increased in mice at $\geq$ 3,000 ppm for intermediate (Chun and

Kintigh 1993) and chronic (Burleigh-Flayer et al. 1992) durations, the increased liver weight is considered to be potentially adverse. Exposure of rats to relatively low inhalation concentrations (50-300 ppm) resulted in transient dose-related increases in liver microsomal UDPGT at 2 weeks, but had no effects on rat liver cytochrome P-450 content or the activities of other microsomal enzymes (Savolainen et al. 1985). In addition, intraperitoneal pretreatment of rats with 741 mg/kg MTBE resulted in a 47-fold induction of liver microsomal pentoxyresorufin O-dealkylase, an activity associated with cytochrome P-4502B1 (Brady et al. 1990). Much higher inhalation exposure levels and oral doses were required to result in increased liver weight and hepatocellular hypertrophy.

Some clinical evidence of hepatocellular necrosis was found in rats exposed orally to MTBE, although other than the hepatocellular hypertrophy found in mice exposed chronically by inhalation, no histopathological lesions were found in the liver in any studies. Nevertheless significantly increased levels of SGOT and lactic dehydrogenase were found in rats treated orally for 14 days or 13 weeks (Robinson et al. 1990). In addition, decreased levels of BUN were observed in male and female rats treated orally with $\geq$ 100 mg/kg/day for 13 weeks.

Although no studies were located regarding hepatic effects in humans exposed to MTBE by environmentally relevant routes, humans have been treated with MTBE intracystically for the dissolution of gallstones. 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 transaminases, indicative of hepatocellular necrosis, hematobilia, or increases in serum bilirubin (Allen et al. 1985b, Bonardi et al. 1986; Holl et al. 1991; Janowitz et al. 1993; Kaye et al. 1990; Leuschner et al. 1991, 1994; Neubrandt 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 transaminase levels (Kaye et al. 1990), cholecystitis and pericholecystitis (Schumacher et al. 1990), persistent dilatation of the common bile duct (Tritapepe et al. 1989), and transient, reversible edema and inflammation of the gallbladder mucosa (Eidsvoll et al. 1993; Uchida et al. 1994; vansonnenberg et al. 1991). A number of studies have been conducted in animals to determine possible 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 alkaline phosphatase in dogs (Allen et al. 1985a) and increased serum alkaline phosphatase, SGOT, and SGPT 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.

Levels of MTBE in occupational settings, the ambient environment, and at hazardous waste sites are probably not high enough to cause serious hepatic effects in humans. Based on clinical studies in humans and experimental studies in animals, effects on the liver, gallbladder, and bile duct are, however, likely in patients receiving MTBE for gallstone dissolution.

Renal Effects. No information was located regarding renal effects in humans after exposure to MTBE by the inhalation, oral, or dermal routes or in animals exposed by the dermal route. The kidney is a target for male rats exposed to MTBE by the inhalation and oral routes. In male rats, MTBE exposure resulted in the accumulation of hyaline droplets containing α_{2u}-globulin or possibly another unknown protein unique to male rats in the renal tubule cells, which may have lead to renal tubule cell carcinoma and exacerbation of chronic progressive nephropathy (see Section 2.4). The accumulation of α_{2u}-globulin in hyaline droplets in renal tubules is suggested by studies in male rats exposed by inhalation to MTBE for 13 weeks (Dodd and Kintigh 1989; Swenberg and Dietrich 1991) or orally for 14-90 days (ITT Research Institute 1992; Robinson et al. 1990). The evidence that the renal effects observed in male rats exposed to MTBE are associated with α_{2u}-globulin accumulation has been questioned, however. In a 90-day inhalation study, the increase in α_{2u}-globulin accumulation in male rats was not dose-related, and α_{2u}-globulin positive proteinaceous casts at the junction of the proximal tubules and thin limb of Henle were not observed (Swenberg and Dietrich 1991). This is unlike the classical lesions of other α_{2u}-globulin inducing agents. Furthermore, a study specifically designed to determine whether MTBE induces α_{2u}-globulin accumulation in male rats found no evidence, based on immunostaining with an antibody to α_{2u}-globulin (Chun and Kintigh 1993). However, an increased accumulation of protein in male renal proximal convoluted tubule epithelium associated with increased epithelial cell proliferation was found as determined by Mallory's Heidenhain technique. Chun and Kintigh (1993) suggested that a mechanism other than α_{2u}-globulin accumulation (perhaps the accumulation of another unknown protein unique to male rats) may be involved. It is possible that the hypothetical other protein unique to male rats acts in the same the way as α_{2u}-globulin. In rats given $\geq$ 100 mg/kg/day MTBE orally for 90 days, severe tubular changes were observed (Robinson et al. 1990). These changes consisted of increases in hyaline droplets in

proximal tubular cells and small numbers of intratubular granular casts at the junction of the outer and inner stripe of the outer medulla.

The accumulation of α_{2u}-globulin (or by extension, the hypothetical other protein unique to male rats) can lead to the exacerbation of chronic progressive nephropathy, which is commonly seen in aging rats. Increased incidence and severity of chronic progressive nephropathy, accompanied by fibrous osteodystrophy, hyperplasia in the parathyroid glands, and mineralization in numerous tissues was observed in both male and female rats exposed to MTBE by inhalation for up to 24 months, but the nephropathy occurred at greater incidence and severity, at earlier onset, and at a lower exposure concentration in males than in females (Chun et al. 1992). The production of α_{2u}-globulin (or the other protein) associated with renal tubule nephropathy has not been demonstrated in female rats or in males or females of any other species, including humans. However, because female rats also had enhanced chronic progressive nephropathy, the possibility of the relevance to renal toxicity in humans exposed to MTBE is strengthened.

In addition to the other renal effects noted above, MTBE inhalation and oral exposure of animals has been associated with increased kidney weight, generally in the absence of clinical (increased BUN, creatinine, or albumin levels) or histological evidence of kidney or urinary bladder lesions (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980; Neeper-Bradley 1991). However, increased levels of BUN were reported in male rats exposed to 1,000 ppm for 13 weeks (Greenough et al. 1980), and urinalysis revealed a slight increase in the pH of the urine in male and female mice at 8,000 ppm and a slight increase in the gamma globulin fraction in male mice at 8,000 ppm in an 18-month inhalation study (Burleigh-Flayer et al. 1992). The increases in pH and gamma globulin fraction may have caused the obstructive uropathy in male mice, since a urethra plug composed largely of proteinaceous material is characteristic of the urethral obstruction.

In rats exposed to relatively low concentrations of MTBE (50-300 ppm) the cytochrome P-450 content of kidney microsomes was significantly increased only after 15 weeks of exposure, while UDPGT and NADP-cytochrome c reductase activities were significantly increased only after 2 weeks of exposure (Savolainen et al. 1985). Although induction of kidney microsomal enzymes may be potentially adverse, other studies (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980; Robinson et al. 1990) indicate that microscopic renal

lesions and increased kidney weight due to enzyme induction occur in animals only at much higher exposure levels and longer exposure durations.

Although no studies were located regarding renal effects in humans exposed to MTBE by environmentally relevant routes, humans have been treated with MTBE intracystically for the dissolution of gallstones. 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. 1985b) 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). 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).

Since humans commonly experience age-related nephropathy, the possibility that prolonged exposure to MTBE occupationally, in the ambient environment, or at hazardous waste sites could enhance this process can not be ruled out. Severe renal complications due to acute exposure during MTBE therapy for gallstone dissolution appear to be rare.

Endocrine Effects. No information was located regarding endocrine effects in humans exposed to MTBE by the inhalation, oral, or dermal routes or in animals exposed by the dermal route. Inhalation and oral studies in rats and/or mice have demonstrated that MTBE exposure generally produces no treatment-related histopathological lesions in the adrenals, pancreas, thyroid/parathyroid, or pituitary regardless of exposure concentration or dose and duration of exposure (Belpoggi et al. 1995; Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Dodd and Kintigh 1989; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 1991; Robinson et al. 1990). However, in rats exposed chronically to MTBE by inhalation, hyperplasia of the parathyroid gland, which was secondary to chronic progressive nephropathy, was found at $\geq$ 400 ppm in males and at $\geq$ 3,000 ppm in females (Chun et al. 1992). While most studies found no effects on adrenal weight, other studies reported conflicting effects on adrenal weight. An increase in adrenal weight was found in male and female rats at 8,000 ppm for 13 days (Dodd and Kintigh 1989), in male rats at 8,000 ppm and female rats at $\geq$ 3,000 ppm in a 4-5 week study (Chun and Kintigh 1993); an increase in adrenal weight that was greater in male rats than in female rats exposed to $\geq$ 4,000 ppm for 13 weeks was reported in another study (Dodd and Kintigh 1989), while an increase in adrenal weight in only female rats given 900 mg/kg/day MTBE orally for 90 days was reported in yet another study (Robinson et al. 1990). Results of studies measuring hormone levels are also

contradictory. While an increase in corticosterone levels was reported in rats exposed to 8,000 ppm for 13 weeks (Dodd and Kintigh 1989), a decrease in corticosterone levels was found at 81 weeks in male rats exposed chronically to 8,000 ppm (Chun et al. 1992). Furthermore, mice of both sexes exposed to 8,000 ppm for 18 months were reported to have increased corticosterone levels at 79 weeks, and male mice exposed to 8,000 ppm had increased adrenal gland weight (Burleigh-Flayer et al. 1992). In mice exposed to MTBE for 28 days, no effects on thyroid weight and no histopathological lesions of the thyroid were found (Chun and Kintigh 1993). Special blood chemistry evaluation of total T3, total T4, TSH, total bile acid, and estradiol revealed that an increase in total T4 and TSH occurred in male mice at 8,000 ppm, while decreases in total T4 were found in female mice in the hepatic cell proliferation studies, but not in the main experiment. However, these changes were not considered to be biologically significant or exposure related due to the absence of histological evidence of thyroid lesions and the lack of consistent results in the various experiments.

Although no studies were located regarding endocrine effects in humans exposed to MTBE by environmentally relevant routes, humans have been treated with MTBE intracystically for the dissolution of gallstones. A number of clinical studies of patients receiving MTBE therapy have investigated effects on the pancreas. In one study of 75 patient 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 8 patients 1 year after intraductal administration of MTBE revealed no pancreatic abnormalities (Tritapepe et al. 1989). 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. 1985a) or pigs (McGahan et al. 1988; Vergunst et al. 1994).

The weight of evidence suggests that endocrine effects are not of concern for humans exposed to MTBE under any exposure scenario.

Dermal Effects. The only information located regarding dermal effects in humans exposed to MTBE is that subjects exposed experimentally to 1.7 ppm (Cain et al. 1994) or 1.39 ppm (Prah et al. 1994) MTBE for 1 hour reported no greater frequency of skin rash or dry skin than when exposed to uncontaminated air. MTBE is irritating to the skin of rabbits and guinea pigs. Application of MTBE to the intact or abraded skin of rabbits resulted in slight to severe erythema, blanching, epidermal thickening, acanthosis, or focal necrosis (ARCO 1980). Guinea pigs developed local irritation at the site of intradermal injection of MTBE. Rats and mice have been examined for dermal effects after exposure to MTBE vapors in air. Any

dermal effects in these studies would probably be due to direct contact of the skin with the vapor. However, since histological examination of skin of rats or mice exposed to MTBE vapors revealed no treatment-related lesions (Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980), it appears that direct application of liquid MTBE is required to produce dermal effects. Furthermore, no gross or histopathological lesions were found on the skin of rats dosed orally with 1,750 mg/kg/day MTBE for 4 weeks (ITT Research Institute 1992) or with 1,000 mg/kg/day MTBE for 104 weeks (Belpoggi et al. 1995).

Dermal irritation resulting from direct skin contact with the undiluted ether is likely in humans. However, the presence of MTBE in air would not be expected to cause skin irritation. MTBE in water would probably be too dilute to cause skin irritation. The ability of MTBE in soil to cause skin irritation would depend on the concentration.

Ocular Effects. Eye irritation was among the symptoms reported by motorists or gas station workers during oxygenated fuel programs in which MTBE had been added to gasoline to reduce carbon monoxide emissions (CDC 1993a, 1993b; Moolenaar et al. 1994; White et al. 1995). However, these and other studies (Anderson et al. 1995; Beller and Middaugh 1992; Chandler and Middaugh 1992) in humans suggest that knowledge about oxygenated fuel programs, including the likely awareness of the potential negative effects of MTBE and the higher cost of oxygenated fuels, may have biased the subjective symptom reporting. In addition, other humans surveys (CDC 1993c; Fiedler et al. 1994; Mohr et al. 1994) found no increase in health complaints among people with high oxygenated fuel exposure. Furthermore, an experimental study in humans exposed to 1.7 ppm MTBE for 1 hour found no statistically significant differences for eye redness; tear-film break-up time; epithelial damage to the eye; and total number of cells and differential numbers of polymorphonuclear neutrophilic leukocytes, epithelial cells, monocytes, eosinophils, and lymphocytes in tear fluid than when the subjects were exposed to uncontaminated air (Cain et al. 1994). The subjects also reported no greater frequency of subjective symptoms of eye irritation (dry, itching, or irritated eyes; tired or strained eyes; burning eyes) than when they were exposed to air alone. Similar results were found in a experimental study in which humans were exposed to 1.39 ppm MTBE for 1 hour (Prah et al. 1994). No information was located regarding ocular effects in humans after exposure to MTBE by the oral or dermal routes. MTBE is irritating to the eyes of animals. Direct instillation of MTBE into the eyes of rabbits resulted in ocular irritation (ARCO 1980; Snamprogetti 1980), regardless of whether the eyes were washed after exposure or not (ARCO 1980). Such effects as cornea1 opacities, chemosis, conjunctival redness, delayed and reversible congestion of the conjunctivae, palpebral thickening, and hypersecretion were observed. Direct exposure of the eyes to

MTBE vapors during inhalation exposure has also resulted in ocular effects in animals, such as ocular discharge, eye irritation (ARCO 1980; Neeper-Bradley 1991), lacrimation (Biodynamics 1981; Conaway et al. 1985; Tyl and Neeper-Bradley 1989), conjunctival swelling (Biodynamics 198 I), periocular encrustations (Tyl and Neeper-Bradley 1989), and swollen periocular tissue (Chun et al. 1992). Gross, histological, and ophthalmoscopic examination of the eyes of rats and mice exposed by inhalation, however, revealed no lesions (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980). No gross lesions were found in the eyes, exorbital lachrymal glands, or Harderian glands, and no histopathological lesions were found in the eyes in rats dosed orally (ITT Research Institute 1992).

Direct contact of the eyes with liquid MTBE or MTBE vapor in air, such as could occur in occupational settings, is likely to be irritating to the eyes of humans. Eye irritation has also been reported by motorists and gas station workers during oxygenated fuel programs when MTBE was added to gasoline to reduce carbon monoxide emissions. Concentrations in ambient air would probably not be high enough to cause eye irritation. The possibility that concentrations at hazardous waste sites, especially around storage containers, would be high enough to cause eye irritation cannot be ruled out.

Body Weight Effects. No information was located regarding body weight effects in humans after exposure to MTBE by any route or in animals after dermal exposure. Decreased body weight gains have been observed in some studies in which rats, mice, or rabbits were exposed to MTBE by the inhalation (Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Neeper-Bradley 1991; Tyl 1989; Tyl and Neeper-Bradley 1989) and oral (Robinson et al. 1990) routes. In some cases, the decreases were accompanied by decreases in food consumption, which could be secondary to the hypoactivity induced by MTBE. Decreased body weight does not seem to be of concern to humans exposed to MTBE.

Other Systemic Effects. The only information regarding other systemic effects of inhalation, oral, or dermal exposure of humans or animals is that elevated cholesterol levels were consistent findings in rats dosed orally with MTBE for 14-90 days (ITT Research Institute 1992; Robinson et al. 1990). The relevance of this effect to public health with respect to MTBE exposure is not known.

Immunological and Lymphoreticular Effects. No differences in interleukin-6 levels were found between the morning and evening blood samples collected from volunteers exposed to auto emissions derived from oxygenated fuels (Duffy 1994). In addition, the total number of cells and differential counts for polymorphonuclear neutrophilic leukocytes, epithelial cells, monocytes, eosinophils, and lymphocytes in nasal lavage material and tear fluid from the eyes did not differ significantly in human subjects exposed experimentally to 1.7 ppm MTBE for I hour than when they were exposed to uncontaminated air (Cain et al. 1994). No information was located regarding immunological or lymphoreticular effects in humans exposed to MTBE by the oral or dermal routes. No studies were located that conducted immunological tests in animals exposed to MTBE by inhalation or orally. However, no evidence that MTBE was a dermal sensitizer was found in guinea pigs (ARCO 1980).

Most studies in animals exposed by the inhalation and oral routes indicate that the lymphoreticular system is not a target of MTBE toxicity regardless of the exposure concentration or oral dose or the duration of exposure. In most studies, histological examinations of bone marrow, lymph nodes, spleen (Biodynamics 1981 ; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980; Neeper-Bradley 1991), and thymus (Robinson et al. 1990; ITT Research Institute 1992) revealed no treatment-related lesions. However, exposure for 13 weeks to 8,000 ppm of MTBE was reported to result in a higher incidence of lymphoid hyperplasia in submandibular lymph nodes in male rats (Dodd and Kintigh 1989). In addition, in a chronic oral study, female rats, but not male rats, developed dysplastic proliferation of lymphoreticular tissues (possibly preneoplastic) at 250 mg/kg/day (Belpoggi et al. 1995). Some studies have reported decreases in spleen or thymus weight, in the absence of histopathological lesions. Absolute and relative spleen weights were decreased in rats and mice exposed to 8,000 ppm for about 28 days, but the spleens were not examined histologically (Chun and Kintigh 1993). Absolute spleen weights were decreased in male and female mice exposed to 8,000 ppm for 18 months (Burleigh-Flayer et al. 1992). Similarly, oral administration of MTBE for 14 days significantly reduced absolute spleen weight and absolute and relative thymus weights in female rats but not males (Robinson et al. 1990). These effects on spleen and thymus weights were not found in other studies, and in the absence of other evidence of toxicity to the spleen or thymus, the toxicological significance is unclear. In a study. in which MTBE was infused into the gallbladders of pigs through a catheter, histological examination of the spleens revealed no lesions (McGahan et al. 1988).

The weight of evidence suggests that effects of MTBE on the immunological or lymphoreticular system are not a concern for humans. Clinical studies of patients receiving MTBE for gallstone dissolution have not described any effects on the immunological or lymphoreticular system.

Neurological Effects. Headache was the symptom most frequently reported by motorists or gas station workers during oxygenated fuel programs in which MTBE had been added to gasoline to reduce carbon monoxide emission (CDC 1993a, 1993b; Moolenaar et al. 1994; White et al. 1995). Nausea or vomiting, dizziness, and a feeling of spaciness or disorientation were also reported. In addition, a preliminary survey of symptoms in petroleum refinery employees working with gasoline containing MTBE indicated high self-reporting of these symptoms (Mehlman 1995). However, these and other studies (Anderson et al. 1995; Beller and Middaugh 1992; Chandler and Middaugh 1992) in humans suggest that knowledge about oxygenated fuel programs, including the likely awareness of the potential negative effects of MTBE and the higher cost of oxygenated fuels, may have biased the subjective symptom reporting. In addition, other humans surveys (CDC 1993c; Fiedler et al. 1994; Mohr et al. 1994) found no increase in health complaints among people with high oxygenated fuel exposure. Furthermore, experimental studies in humans exposed to 1.7 ppm (Cain et al. 1994) or 1.39 ppm (Prah et al. 1994) MTBE or air for 1 hour found no statistically significant differences in neurobehavioral tests evaluating symbol-digit substitution, switching attention, and POMS or subjectively reported frequency of headache, difficulty remembering things or concentrating, feelings of depression, unusual tiredness, fatigue or drowsiness, tension, irritability, or nervousness, dizziness or lightheadedness, mental fatigue or "fuzziness," and pain or numbness in the hands or wrists. No information was located regarding neurological effects in humans exposed to MTBE by the oral or dermal routes.

Only one study described neurological effects in animals exposed dermally (IBT Labs 1969), but its reliability is questionable. MTBE exposure of animals by the inhalation and oral routes has produced signs of central nervous system depression. These signs usually occur immediately after high inhalation concentrations or high oral doses, are transient in nature, and resemble effects of ether anesthesia or ethyl alcohol intoxication. Signs include lacrimation, ataxia, loss of righting reflex, hyperpnea, labored breathing, incoordination, prostration, drowsiness, hypoactivity, decreased startle and pain reflexes, decreased muscle tone, anesthesia, blepharospasm, and stereotypy (ARCO 1980; Bioresearch Labs 1990b; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 1991 Robinson et al. 1990; Tyl 1989; Tyl and Neeper-Bradley 1989). In a study in which a functional observation battery of tests was given to rats exposed to up to 8,000 ppm for 6 hours, effects were similar to those described above, and included ataxia, duck-walk gait, labored respiration, decreased muscle tone, decreased performance on a treadmill, increased hind limb splay, decreased hind limb grip strength, and increased latency to rotate on an inclined screen (Gill 1989). All of the these effects were seen only at 1 hour after exposure, not at 6 or 24 hours, confirming the transient nature of the central nervous system depression. In none of the studies conducted

in animals were histopathological lesions found in the brain. Furthermore, neither brain succinate dehydrogenase nor acetylcholinesterase activity was affected by exposure for 15 weeks to 50-300 ppm MTBE (Savolainen et al. 1985). MTBE and its metabolite *tert*-butanol were found to be distributed to the brain of rats at concentrations similar to blood concentrations (Savolainen et al. 1985).

A number of clinical studies of patients receiving MTBE therapy for gallstone dissolution have recorded neurological effects that are typical of transient central nervous system depression and have been described as drowsiness, mild sedation, somnolence, confusion, coma, vertigo, and dizziness (Allen et al. 1985b; Bonardi et al. 1986; Brandon et al. 1988; DiPadova 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; vanSonnenberg et al. 1986). A vasovagal reaction was found in 4 of 24 patients in the study by Eidsvoll et al. (1993). These conditions usually occurred in patients as a result of MTBE overflow from the gallbladder into the systemic circulation. 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. 1985a; Dai et al. 1989; McGahan et al. 1988; Snamprogetti 1980; Tritapepe et al. 1989) are similar to those noted for inhalation and oral exposure.

The subjective reports of headache, dizziness, nausea, and disorientation by motorists and gas station workers indicate that inhalation of MTBE added to gasoline for the reduction of carbon monoxide emissions may have neurological effects of concern to humans exposed to MTBE under any scenario.

Reproductive Effects. No information was located regarding reproductive effects in humans exposed to MTBE by any route or in animals exposed dermally. MTBE appears to have no reproductive effects in animals exposed by inhalation and oral routes. MTBE had no structural effect on the reproductive system or reproductive performance of male and female rats exposed by inhalation in 2-generation (Biles et al. 1987) and 3-generation studies (Neeper-Bradley 1991). In studies on the potential developmental effects of MTBE, inhalation exposure of rat dams (Conaway et al. 1985) rabbit dams (Tyl 1989) or mouse dams (Tyl and Neeper-Bradley 1989) during gestation did not adversely affect the mean number of corpora lutea, uterine implantations, resorptions, or live fetuses, percentage of pre- or postimplantation loss, or sex ratio. However, gravid uterine weights were decreased in mouse dams exposed to 8,000 ppm (Tyl and Neeper-Bradley 1989). In toxicity studies conducted in rats or mice exposed to MTBE by inhalation for all duration categories (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and

Kintigh 1989; Greenough et al. 1980) or orally for all duration categories (Belpoggi et al. 1995; ITT Research Institute 1992; Robinson et al. 1990), gross and histological examination of male and female reproductive organs and tissues revealed no treatment-related nonneoplastic lesions. However, rats exposed chronically to MTBE by the oral route developed testicular Leydig cell tumors (Belpoggi et al. 1995). Although no information was located for humans, the animal data strongly suggest that MTBE would pose no reproductive risk for humans.

Developmental Effects. No information was located regarding developmental effects in humans exposed to MTBE by any route or in animals exposed orally or dermally. In inhalation developmental studies, exposure of pregnant animals to MTBE did not result in adverse structural developmental effects in the offspring of rats or mice at $\leq$ 2,500 ppm (Conaway et al. 1985) or rabbits at $\leq$ 8,000 ppm (Tyl 1989). However, inhalation exposure of mouse dams to 8,000 ppm MTBE during gestation resulted in developmental effects in the offspring, increased number of nonviable implantations per litter, increased late resorptions, reductions in the number of viable implantations, sex ratio changes, significantly increased incidences of cleft palate and skeletal defects, significantly reduced incidence of partial fetal atelectasis, increased incidence of reduced skeletal ossification, and reduced fetal body weight (Tyl and Neeper-Bradley 1989). Signs of toxicity (decreased maternal weight, decreased food consumption, increased incidence of signs of central nervous system depression) were observed in maternal mice at the same exposure levels that resulted in developmental effects in the offspring. The increased incidence of cleft palate was considered to be related to maternal stress with possible associated increased endogenous levels of corticosterone. Long-term exposure of male and female rats during premating and gestational periods of 16-28 weeks, without exposing the litters, had no effects on pup viability, mean pup body weight, and renal pelvises (Biles et al. 1987). The only effect on rat pups in the 3-generation study was a decreased weight gain of developing pups (Neeper-Bradley 1991).

Although no information on developmental effects in humans was located, the developmental findings in mice and rats indicate that the possibility that MTBE exposure could result in developmental effects cannot be ruled out.

Genotoxic Effects. Information regarding genotoxic effects of MTBE indicates that it has little if any genotoxic activity. As seen from Table 2-4, MTBE was negative in the sex-linked recessive lethal assay in *Drosophila melanogaster* (Semau 1989) for chromosomal aberrations in Fischer 344 rats exposed via inhalation (Vergnes and Morabit 1989), and Sprague-Dawley rats (ARCO 1980), and CD-1 mice (Ward et al. 1994) exposed orally, for *hprt* mutant frequency in lymphocytes of CD-1 mice exposed orally (Ward et

Table 2-4. Genotoxicity of Methyl *tert*-Butyl Ether (MTBE) *In Vivo*

Species (test system)	End point	Results	Reference
Drosophila melanogaster	Sex-linked recessive lethal	–	Sernau 1989
Rat (whole body (F344) vapor exposure)	Chromosomal aberrations	–	Vergnes and Morabit 1989
Rat (oral dosing) (Sprague-Dawley)	Chromosomal aberrations	–	ARCO 1980
Mouse (oral dosing) (CD-1)	Micronuclei in bone marrow	–	Vergnes and Kintigh 1993
Mouse (oral dosing) (CD-1)	Chromosomal aberrations	–	Ward et al. 1994
Mouse (oral dosing) (CD-1)	*hrpt* mutant frequency in lymphocytes	–	Ward et al. 1994
Mouse (inhalation exposure) (CD-1)	DNA repair in cultural primary hepatocytes	–	Vergnes and Chun 1994

– = negative result

al. 1994), and for micronuclei formation in erythrocytes (Vergnes and Kintigh 1993) and increases in DNA repair in cultured primary hepatocytes (Vergnes and Chun 1994) of CD-1 mice exposed via inhalation. *In vitro* assays were also generally negative. As seen from Table 2-5, MTBE was negative for reverse mutation in *Salmonella typhimurium* strains TAl535, TA 1537, TA 1538, TA98, and TAl00 (ARCO 1980; Cinelli et al. 1992) and in *Saccharomyces cerevisiae* D4 (ARCO 1980) both in the presence and in the absence of metabolic activation. In addition, negative results were obtained for unscheduled DNA synthesis in primary rat hepatocytes and for gene mutation in Chinese hamster V79 fibroblasts (Cinelli et al. 1992). However, MTBE was positive for forward mutations in mouse lymphoma L5178Y tk^+/tk^- cells in the presence, but not in the absence, of metabolic activation (ARCO 1980). That the positive result was not due to the metabolite, *tert*-butanol, was demonstrated by McGregor et al. (1988), who found that *tert*-butanol was negative for forward mutations in the L5178Y tk^+/tk^- mouse lymphoma cell assay with and without metabolic activation. MTBE produced equivocal results for sister chromatid exchange in Chinese hamster ovary cells in the presence of metabolic activation (ARCO 1980). However, no increase in chromosomal aberrations in Chinese hamster ovary cells was found with or without metabolic activation. In addition, MTBE was predicted to be non-genotoxic in an analysis by a structureactivity relational expert system using results generated by the National Toxicology Program (NTP) for rodent carcinogenicity, *Salmonella* mutagenicity test results, the induction of sister chromatid exchanges and chromosomal aberrations, and structural alerts for genotoxicity (Rosenkranz and Klopman 1991). The weight of evidence indicates that MTBE has little or no genotoxic activity.

Cancer. No information was located regarding cancer in humans exposed to MTBE by any route or in animals exposed dermally. Chronic inhalation exposure of rats to 400, 3,000, or 8,000 ppm MTBE, resulted in increased incidences of renal tubular adenoma and carcinoma in male rats at 3,000 and 8,000 ppm (Chun et al. 1992). These tumors may have resulted from accumulation of α_{2u}-globulin or some other unknown protein unique to male rats in the renal tubular cells (see Section 2.4). In addition, male Fischer 344 rats exposed to the two higher concentrations had increased incidences of interstitial cell testicular adenoma when compared with concurrent controls, but not when compared with historic controls. Furthermore, the incidence in the concurrent control group was low compared with the historic controls, suggesting that the higher incidence of testicular adenoma **in** the MTBE-exposed rats was not exposure related. Male Fischer 344 rats have a high spontaneous rate of testicular tumors. However, chronic oral exposure of male Sprague-Dawley rats to 250 or 1,000 mg/kg/day resulted in testicular Leydig cell tumors (Belpoggi et al. 1995). Chronic inhalation exposure of mice exposed to 400, 3,000, or 8,000 ppm MTBE resulted in an increased incidence of hepatocellular adenoma in female mice and increased incidence of hepatocellular adenoma and carcinoma in male mice at 8,000 ppm (Burleigh-Flayer

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

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Salmonella typhimurium	Reverse mutation	–	–	ARCO 1980; Cinelli et al. 1992
TA1535				
TA1537				
TA1538				
TA98				
TA100				
Saccharomyces cerevisiae D4	Reverse mutation	–	–	ARCO 1980
Rat primary hepatocytes	Unscheduled DNA synthesis	NA	–	Cinelli et al. 1992
Mouse lymphoma cells L51785	Forward mutation	+	–	ARCO 1980
Chinese hamster ovary cells	Sister chromatid exchange	±	–	ARCO 1980
Chinese hamster ovary cells	Chromosomal aberrations	–	–	ARCO 1980
Chinese hamster V79 fibroblasts	Gene mutation	–	–	Cinelli et al. 1992

– = negative result; + = positive result; ± = equivocal result

et al. 1992). Chronic oral exposure of female rats at 250 or 1,000 mg/kg/day resulted in lymphoma and leukemia (Belpoggi et al. 1995). Since the available carcinogenicity studies indicate the MTBE can produce tumors in multiple species of animals, multiple strains of rats, and at multiple sites, the relevance of the carcinogenic effects of MTBE to humans is of concern. However, the NTP, EPA, and International Agency for Research on Cancer (IARC) have not yet classified MTBE as to its carcinogenicity potential for humans. Formaldehyde, a metabolite of MTBE (see Section 2.3.3), is a substance which may reasonably be anticipated to be a carcinogen (NTP 1994). There is sufficient evidence that formaldehyde induces squamous cell carcinomas in the nasal cavity of male and female rats exposed by inhalation and limited evidence that formaldehyde induces nasal or nasopharyngeal cancer in humans exposed by inhalation. However, nasal cavity tumors were not observed in rats (Chun et al. 1992) or mice (Burleigh-Flayer et al. 1992) exposed by inhalation to MTBE at concentrations as high as 8,000 ppm. Therefore, the possibility that metabolism of MTBE to formaldehyde might lead to the development of nasal cancer seems to be unfounded.and not to pose a concern for humans exposed to MTBE. However, the mechanism by which MTBE induced lymphoma and leukemia in female rats may involve formaldehyde, which has resulted in the lymphoma and leukemia in male and female rats in other studies conducted by Belpoggi and co-workers (Belpoggi et al. 1995). The main metabolite of MTBE, *tert*-butanol, was studied for carcinogenicity in rats and mice exposed via drinking water for 2 years (Cirvello et al. 1995). Increased incidences of renal tubule adenoma and carcinoma in male rats, increased incidences of transitional epithelial hyperplasia of the kidney in male and female rats, increased incidences of follicular cell adenoma of the thyroid in female mice, and increased incidences of follicular cell hyperplasia of the thyroid and inflammation and hyperplasia of the urinary bladder in male and female mice were observed. In addition, there was a slight increase in follicular cell adenoma and carcinoma (combined) of the thyroid in male mice, which may be related to exposure to *tert*-butanol. Since increased incidences of renal tubular adenoma and carcinoma in male rats were observed in the inhalation study of MTBE by Chun et al. (1992), it is possible that the metabolite, *tert*-butanol, was responsible for or contributed to the renal tumors seen in the rats in the inhalation study of MTBE.

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to MTBE are discussed in Section 2.6.1.

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 effects caused by MTBE are discussed in Section 2.6.2.

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 2.8, Populations That Are Unusually Susceptible.

2.6.1 Biomarkers Used to Identify or Quantify Exposure to MTBE

As discussed in Section 2.3, much of absorbed MTBE is excreted unchanged in the expired air. In addition, 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 the workroom air levels of MTBE in service station and dealership garages and the difference in blood concentrations of MTBE between

preshift and postshift measurements (r = 0.9, p = 0.0001) in Fairbanks, Alaska, during the oxygenated fuel program in which MTBE (about 15% by volume) was added to gasoline to reduce emission levels of carbon monoxide (CDC 1993a; Moolenaar et al. 1994). The median 8-hour TWA concentration of MTBE in the workplace air was 0.10 ppm, with a range of 0.01-0.81 ppm. The median preshift blood concentration of MTBE was 1.15 μg/L, with a range of 0.1-27.8 μg/L, while the postshift blood concentration of MTBE was 1.80 μg/L, with a range of 0.2-37.0 μg/L. 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 (CDC 1993b; White et al. 1995). For mechanics, TWA concentrations of personal breathing zones levels ranged from <0.03 ppm-12.04 ppm. The median blood concentrations of MTBE were approximately 2 μg/L for car repairers and 15 μg/L for gas station attendants. Median blood concentrations of *tert*-butanol were about 15 μg/L for car repairers and 75 μg/L for gas station attendants. The estimated correlation coefficients were 0.80 between air MTBE and blood MTBE (p = 0.0001) and 0.7 between air MTBE and blood *tert*-butanol (p = 0.0001).

MTBE and *tert*-butanol blood levels were also generally related to exposure concentrations in rats (Savolainen et al. 1985). Urinary metabolites found in rats exposed by the inhalation, oral, and dermal routes were identified as 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid (Bioresearch Labs 1990b, 1990d, 1991), but no studies were located that identified these metabolites in the urine of humans. In addition, another study in which rats were injected with ^{14}C-MTBE intraperitoneally, analysis of the urine revealed that radiolabeled formic acid accounted for 96.6% of the urinary radioactivity (Biodynamics 1984). The remaining radioactivity in the urine was assumed to be radiolabeled methanol and formaldehyde.

In a study of 27 humans treated with MTBE via percutaneous intracystic infusion for the dissolution of gallstones, mean blood levels of *tert*-butanol were 0.04 mg/mL immediately after and 5 hours after treatment and fell only to 0.025 mg/mL at 12-l 8 hours (Leuschner et al. 1991). 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 those of MTBE, with about 0.036 mg/mL at 5 hours and 0.03 mg/mL at 12-18 hours after treatment. Methanol was found in only 3 patients, while no formaldehyde or formic acid were found. *tert*-Butanol was also detected in the abdominal wall fat of 9 patients and breast milk of 1 patient.

In rats exposed to ^{14}C-MTBE by inhalation for 6 hours or to unlabeled MTBE for 6 hours per day for 14 days and to ^{14}C-MTBE for 6 hours on day 15 (Bioresearch Labs 1990d), 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 (Bioresearch Labs 1990d). Similar rates of excretion of radioactivity were found in rats exposed to unlabeled MTBE for 6 hours per day for 14 days and to ^{14}C-MTBE for 6 hours on day 15. 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 (Bioresearch Labs 1990b). Excretion was considerably slower in rats exposed dermally to MTBE. Halflives for MTBE and *tert*-butanol for plasma clearance in rats exposed by the inhalation and oral routes were generally <1 or 2 hours (Bioresearch Labs 1990a, 1990c).

Thus, the presence of MTBE in blood, expired air, or urine of humans would probably be sufficient as a biomarker of exposure. Of the various possible metabolites (*tert*-butanol, methanol, formaldehyde, and formic acid) that might be monitored in humans, *tert*-butanol appears to be the best metabolite for use as a biomarker of exposure because blood levels of *tert*-butanol rise after exposure to MTBE, but any of the metabolites may either be present naturally (formic acid) or arise from the metabolism of other xenobiotic chemicals.

Based on limited information in humans and clearance data in rats, monitoring of expired air, blood, or urine for MTBE or *tert*-butanol in humans could be used for determining very recent exposure to MTBE, but after exposure ceases for a few days, MTBE and its metabolites would be cleared.

No known effects or combination of effects resulting from MTBE exposure are specific for MTBE (see Section 2.6.2); therefore, no known effect or combination of effects can be used as a biomarker to identify or quantify exposure to MTBE specifically.

2.6.2 Biomarkers Used to Characterize Effects Caused by MTBE

MTBE exposure can lead to central nervous system depression characterized by ataxia, hypoactivity, drowsiness, anesthesia, duck-walk gait, decreased muscle tone, prostration, lack of startle response, and lack of righting reflex (ARCO 1980; Bioresearch Labs 1990d; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Gill 1989; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 199 I ; Robinson et al. 1990; Tyl 1989). MTBE exposure can induce hepatic microsomal enzymes (Brady et al. 1990; Savolainen et al. 1985), or lead to elevated levels of SGPT, SGOT, or serum lactic

dehydrogenase (Robinson et al. 1990), and may increase (Greenough et al. 1980) or decrease (Robinson et al. 1990) BUN levels. 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 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 (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.7 INTERACTIONS WITH OTHER CHEMICALS

No studies were located regarding interactions between MTBE and other chemicals agents. However, pretreatment of rats with phenobarbital or acetone enhanced the metabolism MTBE to *tert*-butanol and formaldehyde in liver microsomes, by inducing cytochromes P-4502B1 and P-4502E1, respectively (Brady et al. 1990; Snyder 1979). Thus, acetone and phenobarbital, as well as other inducers of these enzymes, would be expected to enhance the metabolism of MTBE. Whether this enhanced 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 cytochrome P-4502B1 (Brady et al. 1990). Thus, MTBE itself is an inducer of cytochrome P-4502B1, which can lead to the enhanced metabolism and toxicity of a number of chemicals. Since MTBE is a component of gasoline in oxygenated fuels, it is possible that MTBE may interact with other components of gasoline, such as benzene and branched chain alkanes.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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 we expect 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. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

No specific human populations that are unusually susceptible to the toxic effects of MTBE have been identified. As discussed in Section 2.2.1.2, in a study conducted to determine whether symptoms associated with MTBE were reported at an increased rate among subjects with known multiple chemical sensitivities or chronic fatigue syndrome compared to normal control individuals, no significant differences were found among the groups for driving a car or visiting gas stations (Fiedler et al. 1994). The authors concluded that the study did not provide clear evidence to support that an unusually high rate of symptoms or an increase of symptoms occurred uniquely where MTBE exposure was likely.

Studies in rats (Brady et al. 1990; Snyder 1979) suggest that people who are exposed to inducers of cytochromes P-4502Bl and P-4502El may be more susceptible. For example, people who take phenobarbital, an inducer of cytochrome P-4502B1, or people who are exposed to acetone or drink alcoholic beverages, may be more susceptible. Both acetone and ethanol are inducers of cytochrome P-4502E1.

Pharmacokinetic studies in rats indicated some differences between males and females in absorption and elimination kinetics (Bioresearch Labs 1990a, 1990b, 1990c, 1990d, 1991). In general these studies indicated that female rats absorbed more MTBE than did males after inhalation, oral, or dermal exposure, and eliminated it more quickly. Whether or not these relations would operate in humans is **not** known. MTBE was fetotoxic in mice, resulting in skeletal anomalies, reduced fetal weights, increased resorption, and increased incidences of cleft palate (Conaway et al. 1985; Tyl and Neeper-Bradley 1989) and minimally toxic to developing rats, resulting in reduced F_1 and F_2 pup weight (Neeper-Bradley 1991). MTBE exposure of rabbit dams, however, did not result in developmental effects in the offspring (Tyl 1989). Thus, human embryos and fetuses may be susceptible.

MTBE appears to be minimally toxic to the liver, except in mice (Burleigh-Flayer et al. 1992). Mice developed hepatocellular hypertrophy and hepatocellular adenoma and carcinoma at high exposure levels for chronic durations, but mice have a high spontaneous rate of liver tumor development. Whether MTBE would exacerbate preexisting liver disease in humans is not known.

As discussed in Sections 2.4 and 2.5, MTBE exposure of male rats results in renal effects that may be associated with the accumulation α_{2u}-globulin or other protein unique to male rats (Chun and Kintigh 1993; Dodd and Kintigh 1989; Robinson et al. 1990; Swenberg and Dietrich 1991); chronic progressive nephropathy, accompanied by fibrous osteodystrophy, hyperplasia in the parathyroid glands, and mineralization in numerous tissues; and an increased incidence of renal tubular adenoma and carcinoma (Chun et al. 1992). The increased incidence of renal tubular adenoma and carcinoma may have resulted from α_{2u}-globulin or other protein accumulation. Accumulation of α_{2u}-globulin (or by extension, a hypothetical protein unique to male rats) is specific for male rats, and has no relevance to human health. When α_{2u}-globulin accumulation is accompanied by chronic progressive nephropathy in male rats, the chronic progressive nephropathy is believed to be exacerbated by α_{2u}-globulin accumulation. However, female rats, which do not produce a α_{2u}-globulin, also had increased incidence and severity of chronic progressive nephropathy, but at higher doses and later onset than males. The fact that female rats also showed an enhancement of chronic progressive nephropathy indicates that an additional factor other than accumulation of α_{2u}-globulin or another protein unique to male rats may be involved. Since humans often develop age-related nephropathy, elderly people or people with pre-existing nephropathy may be more susceptible to the nephrotoxicity of MTBE.

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.

2.9 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to MTBE. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to MTBE. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.9.1 Reducing Peak Absorption Following Exposure

Human exposure to MTBE may occur by inhalation, ingestion or by dermal contact. Vapors are likely to be irritating to the eyes and upper respiratory tract and once absorbed can cause central nervous system and respiratory depression. Unprotected skin exposure can cause defatting and subsequent dermatitis.

Exposed individuals should be moved to fresh air and administered 100% humidified supplemental oxygen. If skin contact occurred, the skin should be thoroughly irrigated with water.

Following ingestion, the potential risk of aspiration leading to airway and pulmonary damage usually outweighs the potential benefit of administering syrup of ipecac to induce emesis. Aspiration of MTBE is another complication that must be avoided. If patients present with cough following acute ingestion, the possibility of prior aspiration must be considered and evaluated with careful respiratory exam, arterial blood gases and radiography (HSDB 1995). Once in the care of a health professional, gastric lavage can be useful if administered within 1 hour of the exposure to reduce the amount of absorbed solvent.

Following ocular contamination, the eyes should be irrigated with copious amounts of room temperature water or saline, for at least 15 minutes. If irritation, lacrimation, or especially pain, swelling, and photophobia persist after 15 minutes of irrigation, then ophthalmologic consultation should be sought to evaluate potential ocular damage.

2.9.2 Reducing Body Burden

The body does not retain significant amounts of MTBE. Exhaled concentrations of the vapor represent an important means of elimination. There is no currently recognized treatment to enhance detoxification, and orthodox treatment for ingestion is entirely supportive.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

Clinical effects caused by acute MTBE exposure include central nervous system depression and mild liver function abnormalities (Burg 1992). Other effects include malaise, dizziness, fatigue, headache, and lightheadedness, all of which may disappear soon after the exposure ceases. The mechanism of action for the central nervous system effects has not been clearly established, but may be related to solvent effects on cellular membranes. Just as for other VOCs, this is often referred to as an “anesthetic effect.” The usually short duration of such symptoms and the absence of known chronic effects make pharmacological therapy difficult to justify.

In addition to causing acute central nervous system effects similar to solvent exposure, beverage ethanol may compete or enhance the metabolic activation of solvents and can increase the severity of health

effects, particularly liver toxicity. Alcoholic beverages should be avoided following exposure to MTBE and other solvents.

2.10 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of MTBE is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of MTBE.

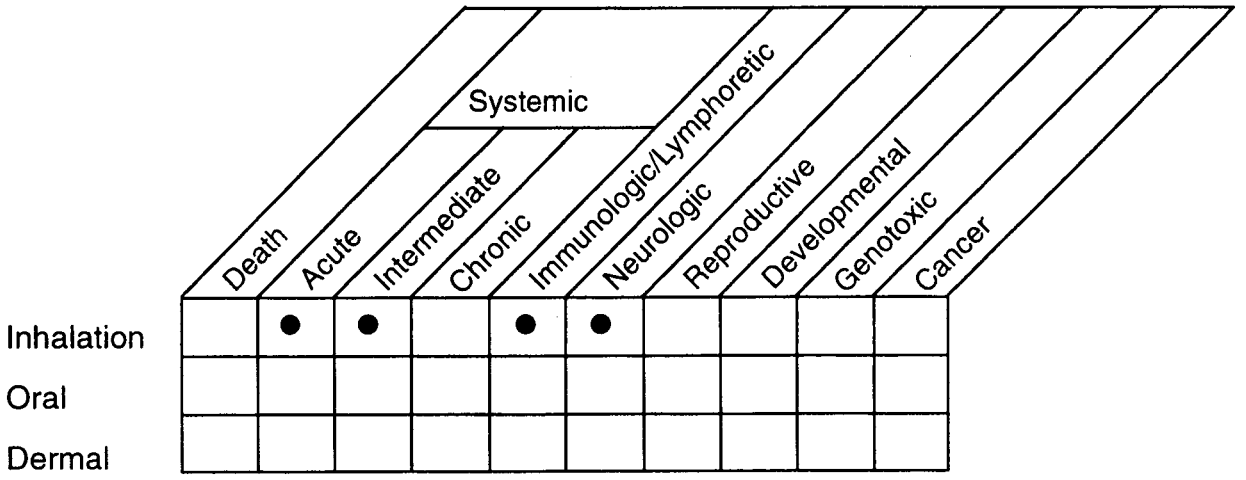
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.10.1 Existing information on Health Effects of MTBE

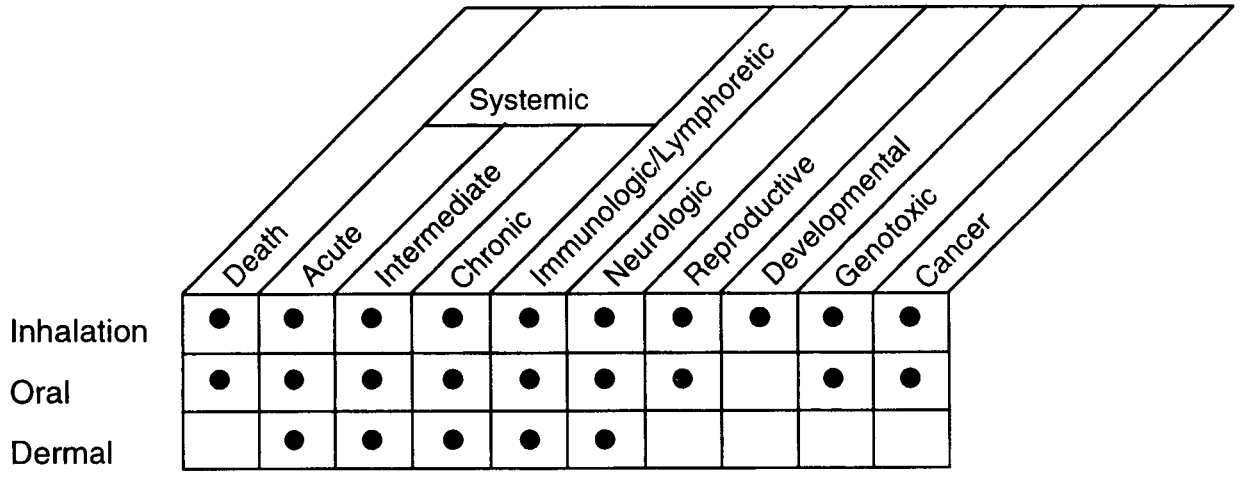
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to MTBE are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of MTBE. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As seen from Figure 2-4, no studies were located regarding health effects in humans after oral or dermal exposure to MTBE. Information on toxic effects of MTBE in humans was available from subjective reports of symptoms (headache, eye irritation, nose and throat irritation, nausea or vomiting, dizziness, confusion, spaciness) in motorists and gas station workers during oxygenated programs in which MTBE

Figure 2-4. Existing Information on Health Effects of Methyl *tert*-Butyl Ether (MTBE)



Human



Animal

● Existing Studies

was added to gasoline for reduction of carbon monoxide emissions. In addition information on toxic effects in humans were available from clinical studies of patients receiving MTBE via percutaneous intracystic infusion or infusion into the gallbladder via catheter for the dissolution of gallstones. While this does not represent an environmentally relevant route, the side effects of this therapeutic treatment are generally consistent with health effects observed in animals exposed to MTBE by the inhalation and oral routes. These side effects include typical signs of transient central nervous system sedation, gastrointestinal irritation, evidence of necrosis in the liver and gallbladder, transient cardiovascular effects, such as hyper- or hypotension and palpitations, and transient leukocytosis. These side effects have occurred from overflow of MTBE from the gallbladder lumen or extravasation into the systemic circulation. In most cases, the patients who experienced these side effects recovered with no residual effects.

Figure 2-4 shows that MTBE has been well studied for health effects by the inhalation route in animals. Since MTBE is a volatile liquid used as an additive to gasoline, inhalation exposure is the major route of concern. Data on death, systemic effects, lymphoreticular, neurological, reproductive, developmental, and genotoxic effects, and cancer are available in animals for the inhalation route. For the oral route of exposure, data on lethal levels, systemic effects, lymphoreticular, neurological, genotoxic, and reproductive effects and cancer are available. No developmental studies conducted by the oral route in animals were located. The dermal route of exposure is the least well studied. No lethality data were located for the dermal route. Data for acute-duration dermal exposure concerns irritation of the skin and eyes from direct application of the liquid to the skin or eyes, respectively, or irritation to the eyes from exposure to vapor during inhalation experiments. All of the intermediate- and chronic-duration data concerns irritation to the eyes from vapor contact during inhalation exposure. In a study performed by IBT Labs, dermally applied MTBE was reported to cause neurological effects, but results of studies from IBT are often suspected as being unreliable.

2.10.2 Identification of Data Needs

Acute-Duration Exposure. No studies were located regarding systemic effects in humans after oral or dermal exposure to MTBE for acute durations. People exposed to MTBE while operating or fueling motor vehicles or people occupationally exposed (such as mechanics, service station workers) have reported subjective symptoms of headache; nausea or vomiting, irritation of eyes, nose, and throat; dizziness; and disorientation (Beller and Middaugh 1992; CDC 1993a, 1993b; Chandler and Middaugh 1992; Moolenaar et al. 1994; White et al. 1995). However, two experimental studies in humans exposed

to ≤ 1.7 ppm for 1 hour did not find any of these effects (Cain et al. 1994; Prah et al. 1994). In animals, information on acute-duration inhalation exposure includes LC_{50} values for rats (ARCO 1980) and mice (Snamprogetti 1980). Information on systemic effects includes clinical signs of respiratory irritation in rats exposed acutely (ARCO 1980); a 5-day intermittent exposure study that found increased hepatocellular proliferation in mice and increased renal tubule cell epithelial proliferation in rats (Chun and Kintigh 1993); a 9-day intermittent exposure study in rats that examined respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, and body weight effects in rats and identified the nasal mucosa and trachea and the liver (increased liver weight) as targets (Biodynamics 1981); a developmental study that found no effects on the liver or body weight of rat or mouse dams (Conaway et al. 1985); a developmental study in mouse dams that reported labored breathing and reduced maternal weight gain (Tyl and Neeper-Bradley 1989); a developmental study in rabbit dams that found increased liver weight and reduced maternal weight gain (Tyl 1989); a 13-day intermittent exposure study in rats and mice that found no effects on lungs but decreased body weight gains of rats (Dodd and Kintigh 1989), an intermediate-duration study that found decreased body weight gain in rats during the first 2 weeks (Chun and Kintigh 1993). Some of these studies also provided information on the immunological/ lymphoreticular system, on neurological effects, on reproductive toxicity, and developmental toxicity (see separate data needs). An acute inhalation neurotoxicity study (Gill 1989) provided the NOAEL used for the MRL of 2 ppm for acute-duration inhalation exposure to MTBE. Information on acute-duration oral exposure includes LD_{50} values for rats (ARCO 1980) and mice (Little et al. 1979). Information on systemic effects includes a report of labored respiration in rats given a single high oral dose (ARCO 1980); a single dose study in rats that reported diarrhea; and a 14-day gavage study in rats that provided information on all systemic end points and found gastrointestinal, hematological, hepatic, renal, body weight, and other (elevated cholesterol) effects (Robinson et al. 1990). Acute-duration oral studies also provided information on the immunological/lymphoreticular, neurological, and reproductive systems (see separate data needs). An acute oral study that described neurological effects in rats provided the NOAEL used for the MRL of 0.4 mg/kg/day for acute-duration oral exposure to MTBE (Bioresearch Labs 1990b). Acute-duration dermal studies reported diarrhea in rats (Bioresearch Labs 1990b) and dermal irritation in rabbits and guinea pigs (ARCO 1980). Data on ocular irritation was also available for direct application of liquid MTBE into the eyes (ARCO 1980; Snamprogetti 1980) and exposure of the eyes to MTBE vapors in inhalation studies (ARCO 1980; Biodynamics 1981; Conaway et al. 1985; Dodd and Kintigh 1989; Tyl and Neeper-Bradley 1989). Thus, information on acute-duration inhalation and oral exposure was sufficient to identify the central nervous system as the most sensitive target system from which to derive acute MRLs. Because absorption of MTBE by the dermal route is substantially lower than absorption by the inhalation and oral routes, the concern for systemic effects of dermal exposure to MTBE is probably

less important than the concern for systemic effects of inhalation or oral exposure to persons who might be exposed to MTBE for acute-duration periods around hazardous waste sites. However, dermal or ocular exposure would be of concern for irritation at the site of contact.

Intermediate-Duration Exposure. No studies were located regarding systemic effects in humans after oral or dermal exposure to MTBE for intermediate durations. People exposed to MTBE while operating or fueling motor vehicles or people occupationally exposed (such as mechanics, service station workers) have reported subjective symptoms of headache; nausea or vomiting; irritation of eyes, nose, and throat; dizziness; and disorientation (Beller and Middaugh 1992; CDC 1993a, 1993b; Chandler and Middaugh 1992; Moolenaar et al. 1994; White et al. 1995). Several intermediate-duration inhalation studies in animals were located that provided information on respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, and body weight effects in rats (Biles et al. 1987; Chun and Kintigh 1993; Dodd and Kintigh 1989; Greenough et al. 1980; Neeper-Bradley 1991; Savolainen et al. 1985; Swenberg and Dietrich 1991) and hepatic effects in mice (Chun and Kintigh 1993). In addition to central nervous system toxicity (see Neurotoxicity, below), the kidney (Chun and Kintigh 1993; Dodd and Kintigh 1989; Greenough et al. 1980; Swenberg and Dietrich 1991) and liver (Neeper-Bradley 1991) have been identified as target organs. In addition, effects have been observed on hematological and endocrine end points (Dodd and Kintigh 1989). These studies also provided information on immunological or lymphoreticular, neurological, reproductive, and developmental effects (see separate data needs). An intermediate-duration inhalation study that described neurological effects in rats provided the NOAEL used for the MRL of 0.7 ppm for intermediate-duration inhalation exposure to MTBE (Neeper-Bradley 1991). Two oral studies in rats (ITT Research Institute 1992; Robinson et al. 1990) were located that provided information on all aforementioned systemic end points. Target organs and systems identified consisted of the gastrointestinal tract, the liver, the kidneys, other (serum cholesterol) (ITT Research Institute 1992; Robinson et al. 1990), and hematological (Robinson et al. 1990). These studies also provided information on immunological/lymphoreticular, neurological, and reproductive effects (see separate data needs). An MRL of 0.3 mg/kg/day for intermediate-duration oral exposure to MTBE was derived based on a LOAEL for hepatic effects in rats (Robinson et al. 1990). Intermediate-duration dermal studies were not located. The available information concerns dermal and ocular effects of direct contact of the skin or eyes with MTBE vapors during inhalation studies. Because absorption of MTBE by the dermal route is substantially lower than absorption by the inhalation and oral routes, the concern for systemic effects of dermal exposure to MTBE is probably less important than the concern for systemic effects of inhalation or oral exposure to persons who might be exposed to MTBE for

intermediate-duration periods around hazardous waste sites. However, information on dermal or ocular exposure is needed regarding irritation at the site of contact.

Chronic-Duration Exposure and Cancer. No studies were located regarding systemic effects or cancer in humans after inhalation, oral, or dermal exposure to MTBE. Two chronic-duration inhalation studies, one in rats (Chun et al. 1992) and one in mice (Burleigh-Flayer et al. 1992), were located. The study in rats monitored clinical signs, mortality, body weight, organ weights, hematology, corticosterone levels, and performed comprehensive gross and histological examination. The study in mice monitored all of the above, and also monitored water consumption and performed urinalysis. Mortality was increased in male rats and male mice. The cause of death of male rats was chronic progressive nephropathy. The cause of death for male mice was obstructive uropathy. In rats, the kidney was the major target with secondary involvement of the bone and parathyroid. In addition, liver weights were increased and body weights decreased. In mice, the liver was the major target (increased weight and hepatocellular hypertrophy). Mice also had increased kidney weights and decreased body weight gains. Based on the NOAEL for chronic progressive nephropathy in female rats, a chronic-duration inhalation MRL of 0.7 ppm was derived for MTBE. One study was located regarding effects in rats after oral exposure to MTBE for chronic duration (Belpoggi et al. 1995). The chronic oral study monitored mortality, clinical signs, food and water consumption, and body weight changes, and performed comprehensive histological examination. No effects on food or water consumption or body weight, and no nonneoplastic tissue lesions were found. However, increased mortality and dysplastic proliferation of lymphoreticular tissues (possibly preneoplastic) were observed in the female rats at the lowest dose tested (250 mg/kg/day). Comprehensive studies by the oral route in rats at lower doses and in mice are needed to provide further information on target organs of chronic-oral exposure and define thresholds that could be used to determine a chronic oral MRL. No chronic dermal studies were located. The available information concerns dermal and ocular effects of direct contact of the skin or eyes with MTBE vapors during inhalation studies. Because absorption of MTBE by the dermal route is substantially lower than absorption by the inhalation and oral routes, the concern for systemic effects of dermal exposure to MTBE is probably less important than the concern for systemic effects of inhalation or oral exposure to persons who might be exposed to MTBE for chronic-duration periods around hazardous waste sites. However, information on dermal or ocular exposure is needed for irritation at the site of contact.

In the chronic-duration inhalation studies in rats (Chun et al. 1992) and mice (Burleigh-Flayer et al. 1992), MTBE was studied for potential carcinogenicity. In male rats exposed to the 2 highest concentrations, there was a concentration-related increased incidence of renal tubule cell adenoma and carcinoma (Chun et

al. 1992). The development of these tumors may have been related to the accumulation of α_{2u}-globulin in male rat renal tubule cells, which has no relevance to human health. However, the evidence that the renal effects observed in male rats exposed to MTBE are related to α_{2u}-globulin accumulation has been questioned. The accumulation of α_{2u}-globulin was not dose related, and α_{2u}-globulin positive proteinaceous casts at the junction of the proximal tubules and thin limb of Henle were not observed, unlike the classical lesions of other α_{2u}-globulin inducing agents (Swenberg and Dietrich 1991). Furthermore, another study found no evidence of α_{2u}-globulin accumulation in male rats, but did find an accumulation of protein in the renal proximal convoluted tubule associated with epithelial cell proliferation in male rats (Chun and Kintigh 1993). Since no epithelial cell proliferation was found in the renal tubules of the female rats, the authors did not look for protein accumulation in the female rat. The existence of another unknown protein unique to male rats that accumulates in the renal proximal convoluted tubule in male rats exposed to MTBE was proposed. Thus, further studies on the mechanism by which MTBE leads to nephropathy and renal tumors in male rats are needed to provide information on whether these effects have any relevance to humans. In female mice exposed to the highest concentration of MTBE, there was an increased incidence of hepatoceilular adenoma (Burleigh-Flayer et al. 1992). Mice are especially susceptible to chemical-induced hepatic tumors. In the chronic oral study in rats (Belpoggi et al. 1995), female rats developed lymphoma and leukemia at both doses (250 and 1,000 mg/kg/day) and male rats developed testicular Leydig cell tumors at the high dose. If a chronic oral study in mice were conducted, comprehensive histological examination of organs and tissues would reveal whether MTBE is carcinogenic in mice by the oral route.

Genotoxicity. Information regarding genotoxic effects of MTBE is limited. No studies were located regarding genotoxic effects in humans exposed to MTBE by any route of exposure. MTBE was negative in the sex-linked recessive lethal assay in *D. melanogaster* (Sernau 1989), for chromosomal aberrations in Fischer 344 rats exposed via inhalation (Vergnes and Morabit 1989) and Sprague-Dawley rats (ARCO 1980) and CD-1 mice (Ward et al. 1994) exposed orally, for *hprt* mutant frequency in lymphocytes of CD-1 mice exposed orally (Ward et al. 1994), and for micronuclei formation in erythrocytes (Vergnes and Kintigh 1993) and increases in DNA repair in cultured primary hepatocytes (Vergnes and Chun 1994) of CD-1 mice exposed via inhalation. *In vitro* assays were negative in *S. typhimurium* (ARCO 1980; Cinelli et al. 1992) and *S. cerevisiae* for reverse mutations, negative for unscheduled DNA synthesis in primary rat hepatocytes and for gene mutations in Chinese hamster V79 fibroblasts (Cinelli et al. 1992), negative for chromosomal aberrations in Chinese hamster ovary cells (ARCO 1980), positive for forward mutations in mouse lymphoma cells with metabolic activation (ARCO 1980), and equivocal for sister chromatid exchange in Chinese hamster ovary cells with activation (ARCO 1980). A structure-activity analysis

predicted that MTBE would not be genotoxic (Rosenkranz and Klopman 1991). While the available data indicate that MTBE has little if any genotoxic activity, an extensive battery of *in vivo* or *in vitro* studies are needed to provide confirmation.

Reproductive Toxicity. No studies were located regarding reproductive effects in humans after inhalation, oral, or dermal exposure to MTBE or in animals after dermal exposure. MTBE has been tested in two inhalation reproduction studies in rats (Biles et al. 1987; Neeper-Bradley 1991) with negative results. MTBE has also been tested for developmental effects by inhalation in rat dams (Conaway et al. 1985), rabbit dams (Tyl 1989), and mouse dams (Tyl and Neeper-Bradley 1989) during gestation without evidence of reproductive effects in the dams. In toxicity studies conducted in rats or mice exposed to MTBE by inhalation for all duration categories (Biodynamics 1981; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980) or orally for all duration categories (Belpoggi et al. 1995; ITT Research Institute 1992; Robinson et al. 1990), gross and histological examination of male and female reproductive organs and tissues revealed no treatment-related nonneoplastic lesions. However, in the chronic oral study, male rats developed testicular Leydig cell tumors (Belpoggi et al. 1995). Although no information was located for humans, the animal data strongly suggest that MTBE would pose no reproductive risk for humans.

Developmental Toxicity. No studies were located regarding developmental effects in humans after inhalation, oral, or dermal exposure to MTBE or in animals exposed orally or dermally. In inhalation developmental studies, MTBE did not cause structural developmental effects in the offspring of rat (Conaway et al. 1985) and rabbit (Tyl 1989) dams exposed during gestation. However, MTBE was a developmental toxicant in the offspring of mouse dams exposed by inhalation (Conaway et al. 1985; Tyl and Neeper-Bradley 1989). No developmental effects, other than decreased weight gain of developing pups, were observed in the multigeneration reproductive inhalation studies in rats (Biles et al. 1987; Neeper-Bradley 1991). Although no developmental studies were conducted by the oral route, pharmacokinetic data are similar for the inhalation and oral routes, suggesting no need for oral studies other than to determine dose-responses.

Immunotoxicity. No indication was found that MTBE exposure increased interleukin-6 levels in volunteers exposed to auto emissions derived from oxygenated fuels (Duffy 1994) or increased polymorphonuclear neutrophilic leukocytes in nasal lavage material or tear liquid from eyes of humans exposed by inhalation to 1.7 ppm MTBE for 1 hour (Cain et al. 1994). No studies were located regarding immunological or lymphoreticular effects in humans after oral or dermal exposure. No studies were

located that conducted immunological tests in animals exposed to MTBE by the inhalation or oral routes. No evidence that MTBE was a dermal sensitizer was found in guinea pigs (ARCO 1980). Most studies in animals exposed by the inhalation and oral routes indicate that the lymphoreticular system is not a target of MTBE toxicity regardless of the exposure concentration or oral dose or the duration of exposure (Biodynamics 1981 ; Burleigh-Flayer et al. 1992; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 1991; Robinson et al. 1990). However, MTBE was reported to result in a higher incidence of lymphoid hyperplasia in submandibular lymph nodes in male rats exposed by inhalation (Dodd and Kintigh 1989) and dysplastic proliferation of lymphoreticular tissue (possibly preneoplastic) in female rats exposed orally (Belpoggi et al. 1995). Some studies have reported decreases in spleen or thymus weight, in the absence of histopathological lesions (Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Robinson et al. 1990). A battery of immune tests in animals exposed by inhalation are needed to determine or rule out whether MTBE has immunological effects. Since inhalation is the most likely route of exposure, and since pharmacokinetic data suggest similar kinetic and metabolic behavior regardless of route, testing by the oral route seems unwarranted.

Neurotoxicity. No studies were located regarding neurological effects in humans after oral or dermal exposure to MTBE. People exposed to MTBE while operating or fueling motor vehicles or people occupationally exposed (such as mechanics, service station workers) have reported subjective symptoms of headache, nausea or vomiting, dizziness, and disorientation (Beller and Middaugh 1992; CDC 1993a, 1993b; Chandler and Middaugh 19921; Moolenaar et al. 1994; White et al. 1995). The central nervous system is without question the most sensitive target of acute exposure to MTBE. MTBE exposure of animals by the inhalation and oral routes has produced signs of central nervous system depression. These signs usually occur immediately after inhalation concentrations or oral doses, are transient in nature, and resemble effects of ether anesthesia or ethyl alcohol intoxication (ARCO 1980; Bioresearch Labs 1990b; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 1991; Robinson et al. 1990; Tyl 1989; Tyl and Neeper-Bradley 1989). MTBE has been tested in a functional observation battery of tests in . rats exposed by inhalation (Gill 1989) with evidence of transient central nervous depression. Additional testing by the oral route appears to be unwarranted, since there is no reason to believe that the effects are route specific. MTBE and *tert*-butanol were found to be distributed to the brain of rats at concentrations similar to blood concentrations (Savolainen et al. 1985), which probably accounts for the sensitivity of this end point. The acute- and intermediate-duration inhalation MRLs of 2 and 0.7 ppm, respectively, are based on NOAELs for neurological effects. Likewise, the acute-duration oral MRL of 0.4 mg/kg/day is based on a NOAEL for neurological effects.

Epidemiological and Human Dosimetry Studies. No studies were located regarding health effects in humans exposed to MTBE by oral or dermal routes. Survey studies in motorists and workers at gas stations, automobile dealerships, and people whose occupations require extensive use of motor vehicles have been conducted in Anchorage and Fairbanks, Alaska, and Stamford, Connecticut, during oxygenated fuel programs, in which MTBE (15% by volume) was added to gasoline for the reduction of carbon monoxide emissions (Beller and Middaugh 1992; CDC 1993a, 1993b; Chandler and Middaugh 1992; Moolenaar et al. 1994; White et al. 1995). A preliminary survey of petroleum refinery employees working with gasoline containing MTBE (Mehlman 1995) provided no detail other than the percentages of workers with self-reported symptoms. In the CDC studies, blood levels of MTBE were analyzed in some of the mechanic and gas station workers and were to correlate with workroom or personal breathing zone levels, but exposure levels were considered to be underestimated because the content of the air a worker actually breathed may have been substantially different from the air level measured by the monitoring devices, many of the windows a.nd doors were open due to the mildness of the weather, or higher exposure levels of MTBE probably occurred while the workers were close to the gasoline tank (CDC 1993a; CDC 1993b). In addition, these and other studies (Anderson et al. 1995) in humans suggest that knowledge about oxygenated fuel programs, including the likely awareness of the potential negative effects of MTBE and the higher cost of oxygenated fuels, may have biased the subjective symptom reporting. Furthermore, other human surveys (CDC 1993c; Fiedler et al. 1994; Mohr et al. 1994) found no increase in health complaints among people with high oxygenated fuel exposure. Experimental studies, in which volunteers were exposed to 1.7 ppm (Cain et al. 1994) or 1.39 ppm (Prah et al. 1994) MTBE for 1 hour, found no indication of adverse effects. However, whether multiple exposures, higher exposure levels, or exposures of longer duration, which are more relevant for real-life exposure of motorists to MTBE, could cause cumulative effects could not be resolved. Thus, the relationship between the experimental studies and the field studies is not clear. Information on toxic effects of MTBE in humans was available from clinical studies of patients receiving MTBE via percutaneous intracystic infusion or infusion into the gallbladder via catheter for the dissolution of gallstones. While this does not represent an environmentally relevant route, the side effects of this therapeutic treatment (central nervous system sedation, liver effects, leukocytosis) are generally consistent with health effects observed in animals exposed to MTBE by the inhalation and oral routes. However, doses associated with effects for this treatment route are not predictive of environmental exposure levels. Experimental studies of volunteers exposed to more realistic exposure levels for longer durations are needed to establish the threshold for irritation and mild central nervous system effects. Since MTBE is widely used as a gasoline additive, more studies of workers involved in the gasoline industry and of people who work at or live near gasoline filling stations are needed to provide more reliable information on atmospheric levels that produce effects, especially signs of

irritation and central nervous system depression, and to eliminate biases in the subjective symptom reporting. People who live in areas where the air, groundwater, or soil is contaminated from major production sites, large tank batteries, transfer terminals, active or abandoned waste sites, and gasoline leaks should also be studied for adverse health effects. More data are needed regarding the possibility that some persons are or can become more chronically sensitive to MTBE, that a combustion product of MTBE may be playing a role in causing symptoms, and that such factors as cloud cover that may contribute to reported adverse effects of exposed humans.

Biomarkers of Exposure and Effect.

Exposure. The amount of MTBE in blood or expired air appears to be the most useful biomarker of exposure because much of the absorbed MTBE is excreted unchanged in the expired air (Bioresearch Labs 1990b, 1990d). In addition, expired air or blood levels of its metabolite *tert*-butanol can be useful indicators of MTBE exposure. Recently, investigations of motorists and workers in Alaska and Connecticut showed that ambient or personal breathing zone levels of MTBE were strongly correlated with human blood levels of MTBE (CDC 1993a, 1993b; Moolenaar et al. 1994; White et al. 1995). MTBE and *tert*-butanol, as well as the metabolite methanol, were measured in the blood of rats, and MTBE blood levels were generally related to exposure concentration (Savolainen et al. 1985). Based on limited information in humans and based on clearance data in rats, monitoring of expired air, blood, or urine for MTBE or *tert*-butanol in humans could be used for determining very recent exposure to MTBE. However, after exposure ceases for a few days, MTBE and its metabolites would be cleared. The development of an alternative biomarker does not seem necessary or possible, because MTBE and its metabolites are cleared rapidly and no evidence of interactions with biological macromolecules was located.

Effect. MTBE exposure can lead to central nervous system depression characterized by ataxia, hypoactivity, drowsiness, anesthesia, duck-walk gait, decreased muscle tone, prostration, lack of startle response, and lack of righting reflex (ARCO 1980; Bioresearch Labs 1990d; Burleigh-Flayer et al. 1992; Chun and Kintigh 1993; Chun et al. 1992; Dodd and Kintigh 1989; Gill 1989; Greenough et al. 1980; ITT Research Institute 1992; Neeper-Bradley 1991; Robinson et al. 1990; Tyl 1989). Exposure can induce hepatic microsomal enzymes (Brady et al. 1990; Savolainen et al. I985), or lead to elevated levels of SGPT, SGOT, or serum lactic dehydrogenase (Robinson et al. 1990), and may increase (Greenough et al. 1980) or decrease BUN (Robinson et al. 1990) levels. 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 exposure to MTBE specifically. The central

nervous system depression appears to be the most sensitive and immediate effect of MTBE exposure, and may be due to solvent effects on neuronal membranes. The development of a biomarker to detect the effect on cell membranes would not be practical given the transient and short-lived nature of the anesthetic effect.

Absorption, Distribution, Metabolism, and Excretion. The absorption, distribution, metabolism, and excretion of MTBE has been well studied in rats after inhalation, oral, dermal, and intravenous exposure (Biodynamics 1984; Bioresearch Labs 1990a, 1990b, 1990c, 1990d, 1991; Brady et al. 1990; Snyder 1979). These studies provided information on rates and extent of absorption, retention in tissues, metabolism, and rates and extent of excretion of relatively low and high doses of MTBE. Based on shifts in elimination pathways following exposure to high compared to low doses, saturation of metabolizing enzymes appears to occur, but does not appear to influence the overall elimination of MTBE from the body. Information on respiratory and urinary metabolites and results of *in vitro* studies with rat liver microsomes have provided sufficient information to propose a plausible metabolic pathway. Metabolism does not appear to be route specific. The studies in rats provided some pharmacokinetic parameters, and a preliminary physiologically based pharmacokinetic model has recently been developed by Borghoff et al. (1996). Further refinement of the model in rats is needed for extrapolating pharmacokinetic, toxicity, and dose-response data to humans.

Comparative Toxicokinetics. Comprehensive information on toxicokinetics of MTBE is available only for rats (see data need for Absorption, Distribution, Metabolism, and Excretion). The only toxicokinetic information for MTBE in mice involves pulmonary excretion after intraperitoneal dosing (Yoshikawa et al. 1994). Information on side effects in patients receiving MTBE therapeutically for the dissolution of gallstones indicates that central nervous system depression is also the most sensitive end point in humans (Allen et al. 1985b; Bonardi et al. 1986; Brandon et al. 1988; DiPadova et al. 1986; 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; vansonnenberg et al. 1986). Studies in motorists and workers occupationally exposed to MTBE (CDC 1993a, 1993b; Moolenaar et al. 1994; White et al. 1995), and an experimental inhalation study in humans (Cain et al. 1994) indicated that MTBE is well absorbed from lungs and metabolized to *tert*-butanol. Limited information regarding distribution, metabolism, and excretion of MTBE was available for humans who received MTBE via percutaneous intracystic infusion for dissolution of gallstones (Leuschner et al. 1991). 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. Toxicokinetic studies in other species are needed to determine if the disposition of MTBE is similar across species. As discussed in Absorption, Distribution, Metabolism, and Excretion above, the studies in rats provided some pharmacokinetic parameters, and a preliminary physiologically based pharmacokinetic model has recently been developed by Borghoff et al. (1996). Further refinement of the model in rats is needed for extrapolating pharmacokinetic, toxicity, and dose-response data to humans.

Methods for Reducing Toxic Effects. MTBE is rapidly absorbed, distributed, metabolized, and excreted. The most immediate systemic effect is central nervous system depression, which is transient. Because MTBE is absorbed rapidly by passive diffusion, gastric lavage can be useful only if performed within one hour of exposure to reduce the amount absorbed in the case of ingestion. Reducing absorption after inhalation exposure is not feasible. Thorough washing of the skin with water could reduce dermal absorption. MTBE and its metabolites are not retained in the body for any substantial period of time, so there is little need to reduce body burden. The mechanism of action of the central nervous system effects has not been clearly established, but is probably related to solvent effects on cell membranes. The short duration of the central nervous system symptoms and the absence of known chronic effects make pharmacological therapy difficult to justify.

MTBE is rapidly absorbed, distributed, metabolized, and excreted. The most immediate effect is central nervous system depression, which is transient. Because MTBE is absorbed rapidly by passive diffusion, gastric lavage can be useful only if performed within one hour of exposure to reduce the amount absorbed in the case of ingestion. Reducing absorption after inhalation exposure is impractical. Thorough washing of the skin would reduce dermal absorption. MTBE and its metabolites are not retained in the body for any substantial period of time, obviating the need to reduce body burden. The mechanism of action of the central nervous system effects has not been clearly established, but is probably related to solvent effects on cell membranes. The short duration of the central nervous system symptoms and the absence of known chronic effects make pharmacological therapy difficult to justify.

2.10.3 Ongoing Studies

ATSDR is funding a study on the genotoxicity of MTBE entitled "Genotoxic Effects of a Widely Used Gasoline Additive" to be performed by Dr. Norman Kado of the University of California at Davis, who will test MTBE in a modified Ames assay using a microsuspension procedure for volatile compounds, in a micronucleus assay in pregnant mice and their fetuses, and in an assay for induction of mutations at the lacZ gene in kidneys of transgenic mice. Information regarding ongoing research on the genotoxicity of MTBE was also found in an abstract.

As reported in an abstract, Choi et al. (1993) injected MTBE into the liver parenchyme, the peritoneum, the tail vein, the portal vein, or the inferior vena cava of rats. In the portal vein and inferior vena cava groups, 90% of the rats died. Necropsy revealed localized areas of congestion, hemorrhage, and interstitial edema in the lung and degenerative changes and necrosis in the liver. In the other groups, all rats survived and showed only mild lung changes upon necropsy after sacrifice.

In an abstract of a report presented at the International Congress of Toxicology - VII, July 2-6, 1995, Johanson et al. (1995) reported that in 10 healthy male volunteers exposed to MTBE by inhalation at 5, 25, or 50 ppm for 2 hours during light physical exercise, low uptake (32-41% of inhaled amount), high postexposure exhalation (18-34% of uptake), low blood clearance, and low recovery of *tert*-butanol in urine were found. The half-life of *tert*-butanol was 8-10 hour in blood and urine. The pharmacokinetic data are being used to develop a physiologically based pharmacokinetic (PBPK) model for MTBE. The subjects reported minimal or no subjective symptoms (discomfort, irritation, central nervous system effects), and eye and nose acoustic rhinometry revealed minimal or no effects.

In another abstract and poster presentation at a meeting of the International Society for Environmental Epidemiology in Durham, North Carolina, by these authors, Nihlen et al. (1994) reported that the preliminary analysis postexposure decay curve of MTBE in blood of the 10 healthy male volunteers exposed to MTBE by inhalation at 5, 25, or 50 ppm for 2 hours during light physical exercise indicated three half-times in the blood of about 1 minute, 0.6 hour, and 7 hours. The preliminary half-time of *tert*-butanol in urine was about 4 hours. These values were reported in the abstract. In the poster, however, it appeared that the 3 half-times of MTBE in the blood were about 7 minutes, 47 minutes, and 6.2-7.2 hours. Two half-times of MTBE in the urine were 16-22 minutes and 3-3.1 hours. The half-time of *tert*-butanol in the urine was 10.7 hours for the 25 ppm exposure and 5.4 hours for the 50 ppm exposure.

2. HEALTH EFFECTS

As reported by Medlin (1995), Thomas Goldsworthy and other researchers of CIIT are investigating the mechanism by which a number of compounds in unleaded gasoline, including MTBE, cause liver tumors in mice. For MTBE, the hypothesis being tested is that MTBE causes liver tumors in mice through modulation of hormones, specifically estrogen. Abstracts of these studies have been presented at the 35th Annual Meeting of the Society of Toxicology in March, 1996. Borghoff et al. (1996) measured partition coefficients of MTBE in blood, liver, kidney, fat, and muscle of male and female Fischer rats for use in the development of a physiologically based pharmacokinetic model. Moser et al. (1996), of CIIT, found that MTBE exposure of female $B6C3F_1$ mice at 7,988 ppm for 4 months resulted in decreased relative uterine, ovary, and pituitary weights, increased relative liver weight and hepatic microsomal P-P50 pentoxyresorufin O-dealkylase activity, but did not affect DNA synthesis in nonfocal hepatocytes. Furthermore, MTBE did not exhibit tumor-promoting activity in the mice that received an initiating dose of N-nitrosodiethylamine. The authors stated that MTBE produced endocrine modulation suggestive of estrogen antagonism, but did not show tumor-promoting activity. This is unlike the hormonal-modulated mechanism of hepatic tumor promotion of unleaded gasoline (Goldsworthy et al. 1996).

Other abstracts presented at the 35th Annual Meeting of the Society of Toxicology in March 1996, present ongoing studies of MTBE. In male Sprague-Dawley rats gavaged with MTBE in corn oil at 1,000 mg/kg/day for 1 month, serum testosterone was decreased within hours after the first treatment, but not at the time of sacrifice (de Peyster et al. 1996). Total liver cytochrome P-450 was elevated after 1 month, but liver and androgen-dependent organ weights were unchanged. The authors stated that an immediate, transient decrease in serum testosterone is consistent with a direct effect on the hypothalamic-pituitary-gonadal axis, and that long-term changes in steroid metabolism might occur if P-450 induction is sustained.

Scholl et al. (1996) reported that pretreatment of male Fischer rats with phenobarbital or clofibrate, but not beta-naphthoflavone, prolonged the duration of ataxia and narcosis induced by an intraperitoneal dose of MTBE. Phenobarbital and clofibrate also induced hepatic microsomal enzymes that metabolize MTBE to *tert*-butanol, suggesting that the metabolic status is a major determinant of sensitivity to the central nervous system effects of MTBE.