2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of gasoline and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for gasoline based on toxicological studies and epidemiological investigations.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals 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, developmental, reproductive, 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. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear. They should also help to determine 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 tables and figures may differ depending on the user’s perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with “serious” effects. Public

health officials and project managers 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 (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with the carcinogenic effects of gasoline are indicated in Table 2-l and Figure 2- 1.

This chapter discusses the health effects associated with exposure to automotive gasoline. Please see the ATSDR toxicological profiles on jet fuels, Otto fuels II, and fuel oils (ATSDR 1992) for further information on other types of fuels. Furthermore, this chapter will focus on the health effects associated with exposure to the gasoline mixture, and not the individual components of gasoline. For more information on the health effects associated with exposure to specific components of the gasoline mixture, please refer to the ATSDR toxicological profiles on 1,3-butadiene, benzene, 1,2-dibromoethane, ethylbenzene, lead, toluene, and xylene (ATSDR 1989, 1990, 1991). In addition, this profile will not discuss the health effects associated with exposure to automotive gasoline exhaust or combustion products of gasoline because these products contain other substances that are not constituents of gasoline itself.

Gasoline is a complex, highly variable mixture consisting of several hundred hydrocarbons that have boiling points from approximately 40°C to 180°C (Mehlman 1990). The hydrocarbons present in the gasoline mixture include alkanes, or straight-chain C_4 to C_{12} compounds also known as paraffins, isoparaffins, or branched-chain compounds of the same size; alkenes, or olefins, which are unsaturated linear and branched-chain hydrocarbons; and naphthenics, or saturated cyclic hydrocarbons. Also included in the gasoline are aromatic compounds (principally benzene, toluene, ethylbenzene, and xylene). Tetraethyl and tetramethyl lead are being phased out of gasoline (Mehlman 1990). Many of the toxicological effects associated with exposure to gasoline can be attributed to specific components of the mixture (i.e., organic lead compounds, benzene). For the majority of the studies discussed in this chapter, the exact composition of the gasoline mixture used was not specified. For those studies in which the composition of the test mixture was indicated, the percentages of the components are

presented in the text when the study is discussed. Many studies (i.e., those sponsored by the American Petroleum Institute [API]) used a gasoline test mixture formulated by API in 1982 known as API PS-6. Compositional data are available for the mixture formulated in 1982, but the percentages of the formulation are probably atypical because of the large content of paraffinic blending stock: 12.3% n-paraffins, 47.4% isoparaffins, 3.1% cyclopentanes, 14.5% cyclohexanes, 6.4% olefins, 28.7% aromatics (benzene-adjusted 2.0%), 11.5% unclassified hydrocarbons (Anonymous 1989). Another composition (expressed as volume percent) given for API PS-6 is as follows: 11.4% n-paraffins, 46.5% isoparaffins, 4.7% cycloparaffins, 9.0% mono-olefins, 28.4% aromatics (Domask 1984). The API PS-6 used in the chronic study conducted by MacFarland et al. (1984) had the same composition as the one used by Domask (1984), but the benzene content of this mixture was specified as 1.69%. A more recent blend of gasoline, API 91-1, has been reported to contain a greater percentage of aromatics (33.2%) and olefins (12.5%) and a lower percentage of saturated hydrocarbons than the API PS-6 blend (Standeven and Goldsworthy 1993).

Details regarding experimental protocol for most of the studies discussed in this section are presented in Tables 2-1, 2-2, and 2-3, and are generally not reiterated in the text.

2.2.1 Inhalation Exposure

2.2.1.l Death

Several case reports of either accidental or intentional inhalation of gasoline vapors resulting in death have been published (Ainsworth 1960; Boeckx et al. 1977; Poklis 1976; Wang and Irons 1961). Inhalation of ≥5,000 ppm gasoline vapor (20,000 ppm for 5 minutes) has been shown to be lethal (Ainsworth 1960; Wang and Irons 1961). It has been postulated that the cause of death following inhalation of high concentrations of gasoline vapors is either central nervous system depression due to asphyxia leading to respiratory failure, or cardiac sensitization to circulating catecholamines leading to a fatal arrhythmia (Poklis 1976).

Acute median lethal concentrations (LC_{50}s) of gasoline vapor have not been established in experimental animals. Intermediate-duration exposure (90 days) to up to 1,552 ppm unleaded gasoline vapor was not lethal to rats or monkeys (Kuna and Ulrich 1984), and exposure to up to 2,056 ppm unleaded gasoline vapors for 2 years was not lethal to rats or mice (MacFarland et al. 1984).

The highest NOAEL values for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.2 Systemic Effects

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

Respiratory Effects. Adverse respiratory effects were described in one case report of inhalation of gasoline vapors that resulted in death (Ainsworth 1960). In this case, a 3-year-old boy was found with his head lying in a pool of gasoline, and he died shortly thereafter. Autopsy revealed pulmonary congestion, edema, and intrapulmonary hemorrhage. Hyperemia was evident in the tracheal and bronchial mucosa, and there was hemorrhagic fluid in the bronchi. Intraalveolar hemorrhage and alveolar necrosis were seen upon histopathological evaluation. According to the report, these effects were the result of inhalation of gasoline fumes. No gasoline was found in the stomach, and there was no evidence of oral or pharyngeal mucosal damage, thus ruling out the possibility that the lung damage was due to aspiration of ingested gasoline.

Information on the acute respiratory effects of gasoline inhalation in experimental animals is limited to one study in which rats were exposed to 0.1 mL gasoline in a closed container either once or intermittently for 5-7 days. In both exposure scenarios, widespread hemorrhage of the lungs was noted at necropsy (O'Regan and Turgeon 1986). This study is not included in Table 2-l because the unusual exposure conditions precluded quantification of the exposure levels; however, this study does provide qualitative evidence of lung damage resulting from acute-duration exposure to gasoline vapors.

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference/ Chemical form
ACUTE EXPOSURE							
Systemic							
1	Rabbit NS	2 hr	Cardio		70,180 (ECG disturbances, decreased heart rate; decreased myocardial acid phosphatase, sodium, postassium and magnesium; altered myocardial ATPase activity)		Przybylowski 1971 leaded
Neurological							
2	Rabbit NS	2 hr once				70,180 NS (convulsions, restlessness, narcosis)	Przybylowski 1971 leaded
Developmental							
3	Rat CD (SD) BR	10d Gd 6-15 6hr/d		1600			Litton Bionetics 1978 unleaded

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference/ Chemical form
INTERMEDIATE EXPOSURE							
Systemic							
4	Monkey Squirrel	90 d 5d/wk 6hr/d	Resp	1552			Kuna and Ulrich 1984 unleaded
			Cardio	1552			
			Hemato	1552			
			Hepatic	1552			
			Renal	1552			
			Endocr	1552			
			Bd wt	1552			
5	Rat Sprague-Dawley	90 d 6hr/d 5d/wk	Cardio	3866			Halder et al. 1984 unleaded
			Hemato	3866			
			Hepatic	3866			
			Renal	40 M 3866 F	379 M	(renal tubular dilation and necrosis)	
			Endocr	3866			
			Bd wt	3866			

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference/ Chemical form
6	Rat Sprague-Dawley	21 d 5d/wk 6hr/d	Cardio	3316			Halder et al. 1984 unleaded
			Hemato	3316			
			Hepatic	3316			
			Renal	3316 F	29 M (mild tubular degenerative and regenerative changes with hyaline droplets)		
			Endocr	3316			
			Bd wt	3316			
7	Rat Sprague-Dawley	90 d 5d/wk 6hr/d	Resp	1552			Kuna and Ulrich 1984 unleaded
			Cardio	1552			
			Hemato	1552			
			Hepatic	1552			
			Renal	1552 F	384 M (regenerated epithelium; dilated tubules)		
			Endocrine	1552			
			Body wt	1552			
8	Rat Wistar	5-45 d 8hr/d 5d/wk	Resp		100 F (decreased levels of surfactant)		Le Mesurier et al. 1979 leaded
9	Rat NS	12 wk 5d/wk 8hr/d	Resp			100 F (respiratory distress; fibrosis; alveolar collapse)	Lykke et al. 1979 leaded

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference/ Chemical form
10	Rat F344	3 wk 5d/wk 6hr/d	Renal	20 M	200 M (hyperplasia; necrosis of proximal tubular cells)		Short et al. 1987 unleaded
11	Rat Sprague-Dawley	3-50 wk 5d/wk 6hr/d	Renal	300 F	10 M $\alpha 2\mu$-globulin		Short et al. 1989a unleaded
12	Mouse $B6C3F_1$	13 wk 5d/wk 6hr/d	Hepatic	300 F	2039 F (increase liver weight, microsomal enzyme induction, hypertrophy)		Standeven and Goldsworthy 1993 unleaded
13	Mouse $B6C3F_1$	16 wk 5d/wk 6hr/d	Hepatic	292 F	2056 F (increase liver weight, hypertrophy, increase sorbitol dehydrogenase)		Standeven et al. 1994a unleaded
14	Mouse $B6C3F_1$	13 wk 5d/wk 6 hr/d	Hepatic	292F	2056 F (increase liver weight, hepatocyte cell proliferation)		Tilbury et al. 1993 unleaded
	Immuno/Lymphoret						
15	Monkey Squirrel	90 d 5d/wk 6hr/d		1552			Kuna and Ulrich 1984 unleaded
16	Rat Sprague-Dawley	90 d 5d/wk 6hr/d		1552			Kuna and Ulrich 1984 unleaded

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference/ Chemical form
	Neurological						
17	Monkey Squirrel	90 d 5d/wk 6hr/d		1552			Kuna and Ulrich 1984 unleaded
18	Rat Sprague-Dawley	90 d 5d/wk 6hr/d		1552			Kuna and Ulrich 1984 unleaded
	Reproductive						
19	Mouse CD-1	8 wk 5d/wk 6hr/d		1600 M			Litton Bionetics 1980 unleaded
20	Mouse $B6C3F_1$	16 wk 5d/wk 6hr/d		2056 F			Standeven et al. 1994a unleaded
	Cancer						
21	Rat F344	24 wk 5d/wk 6hr/d				300 M (CEL-renal cell adenomas)	Short et al. 1989b unleaded
22	Mouse CrlBr	13 wk 5d/wk 6 hr/d				2039 F (promotion of preneoplastic hepatic foci)	Standeven and Goldsworthy 1993 unleaded

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL			Reference/ Chemical form
					Less serious (ppm)	Serious (ppm)		
23	Mouse $B6C3F_1$	16 wk 5d/wk 6hr/d				2056 F	(promotion of preneoplastic altered hepatic foci)	Standeven et al. 1994a unleaded

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference/ Chemical form
CHRONIC EXPOSURE							
Systemic							
24	Rat F344	107-109 wk 5d/wk 6hr/d	Resp	292 F	2056 F (mild multifocal pulmonary inflammatory response)		MacFarland et al. 1984 unleaded
			Resp	2056 M			
			Cardio	2056			
			Gastro	2056			
			Hemato	2056			
			Musc/skel	2056			
			Hepatic	2056			
			Renal	2056 F	67 M (nephropathy)		
			Dermal	2056			
			Bd wt	292	2056 (decreased body weight gain)		
25	Mouse $B6C3F_1$	103-113 wk 5d/wk 6hr/d	Resp	2056			MacFarland et al. 1984 unleaded
			Cardio	2056			
			Gastro	2056			
			Hemato	2056			
			Musc/skel	2056			
			Hepatic	2056			
			Renal	2056			
			Dermal	2056			
			Body wt	292 M 2056 F	2056 M (decreased body weight)		

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference/ Chemical form
	Immuno/Lymphoret						
26	Rat F344	107-109 wk 5d/wk 6hr/d		2056			MacFarland et al. 1984 unleaded
27	Mouse $B6C3F_1$	103-113 wk 5d/wk 6hr/d		2056			MacFarland et al. 1984 unleaded
	Neurological						
28	Rat F344	107-109 wk 5d/wk 6hr/d		2056			MacFarland et al. 1984 unleaded
29	Mouse $B6C3F_1$	103-113wk 5d/wk 6hr/d		2056			MacFarland et al. 1984 unleaded
	Reproductive						
30	Rat	107-109 wk 5d/wk 6hr/d		2056			MacFarland et al. 1984 unleaded
31	Mouse $B6C3F_1$	103-113wk 5d/wk 6hr/d		2056			MacFarland et al. 1984 unleaded

TABLE 2-1. Levels of Significant Exposure to Automotive Gasoline - Inhalation (continued)

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)		Reference/ Chemical form
32	Mouse B6C3F$_1$	103-113 wk 5d/wk 6hr/d		2056 F				MacGregor et al. 1993 unleaded
	Cancer							
33	Rat F344	107-109 wk 5d/wk 6hr/d				292	(CEL renal adenomas, carcinomas, and sarcomas)	MacFarland et al. 1984 unleaded
34	Mouse B6C3F$_1$	103-113wk 5d/wk 6hr/d				2056 F	(CEL hepatocellular adenomas, and carcinomas)	MacFarland et al. 1984 unleaded

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

Bd wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); ECG = electrocardiogram; Endor = endocrine; F = female(s); Gastro = gastrointestinal; Gd = gestation day; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

Figure 2-1. Levels of Significant Exposure to Automotive Gasoline – Inhalation

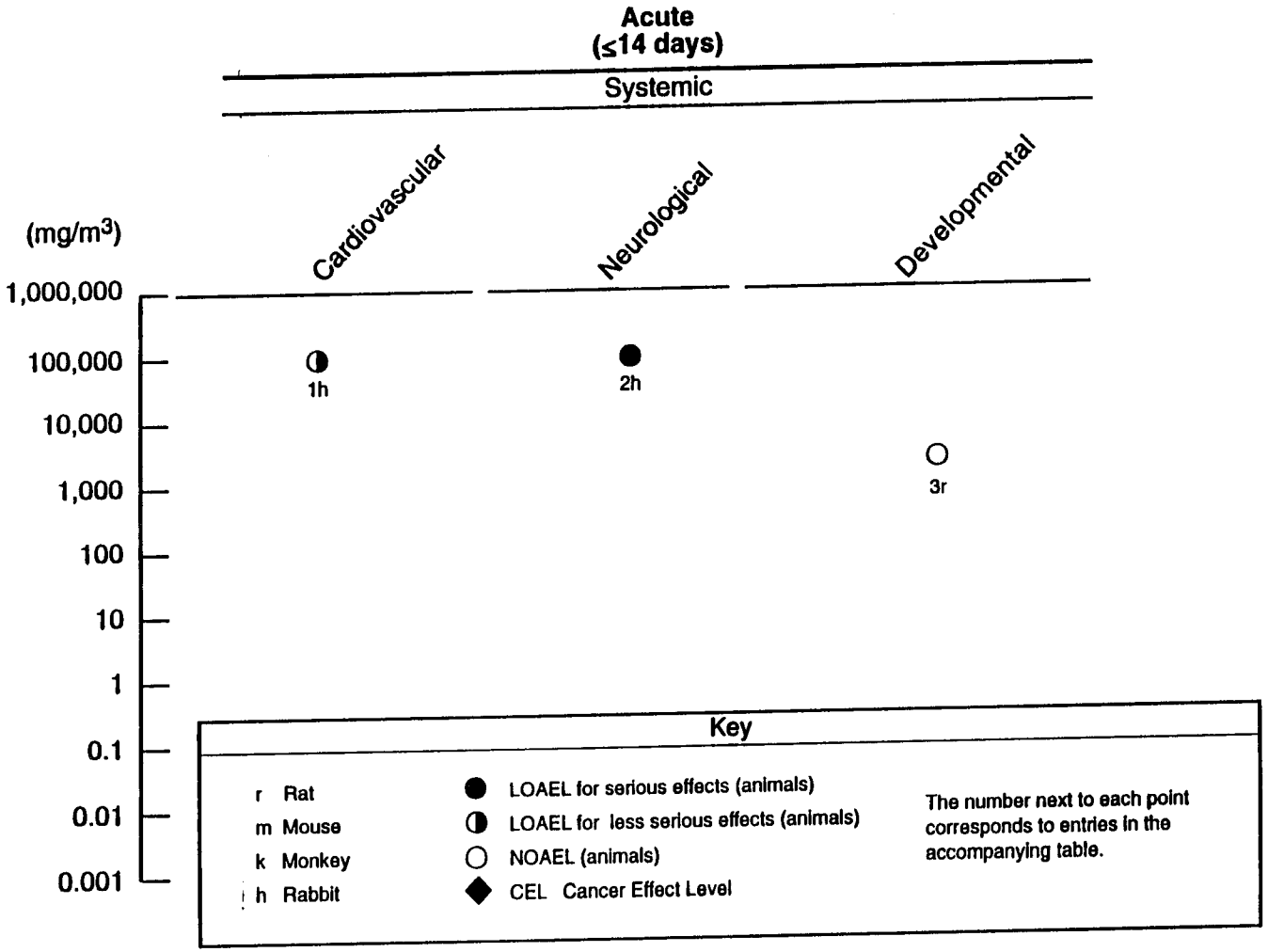


Figure 2-1. Levels of Significant Exposure to Automotive Gasoline – Inhalation (continued)

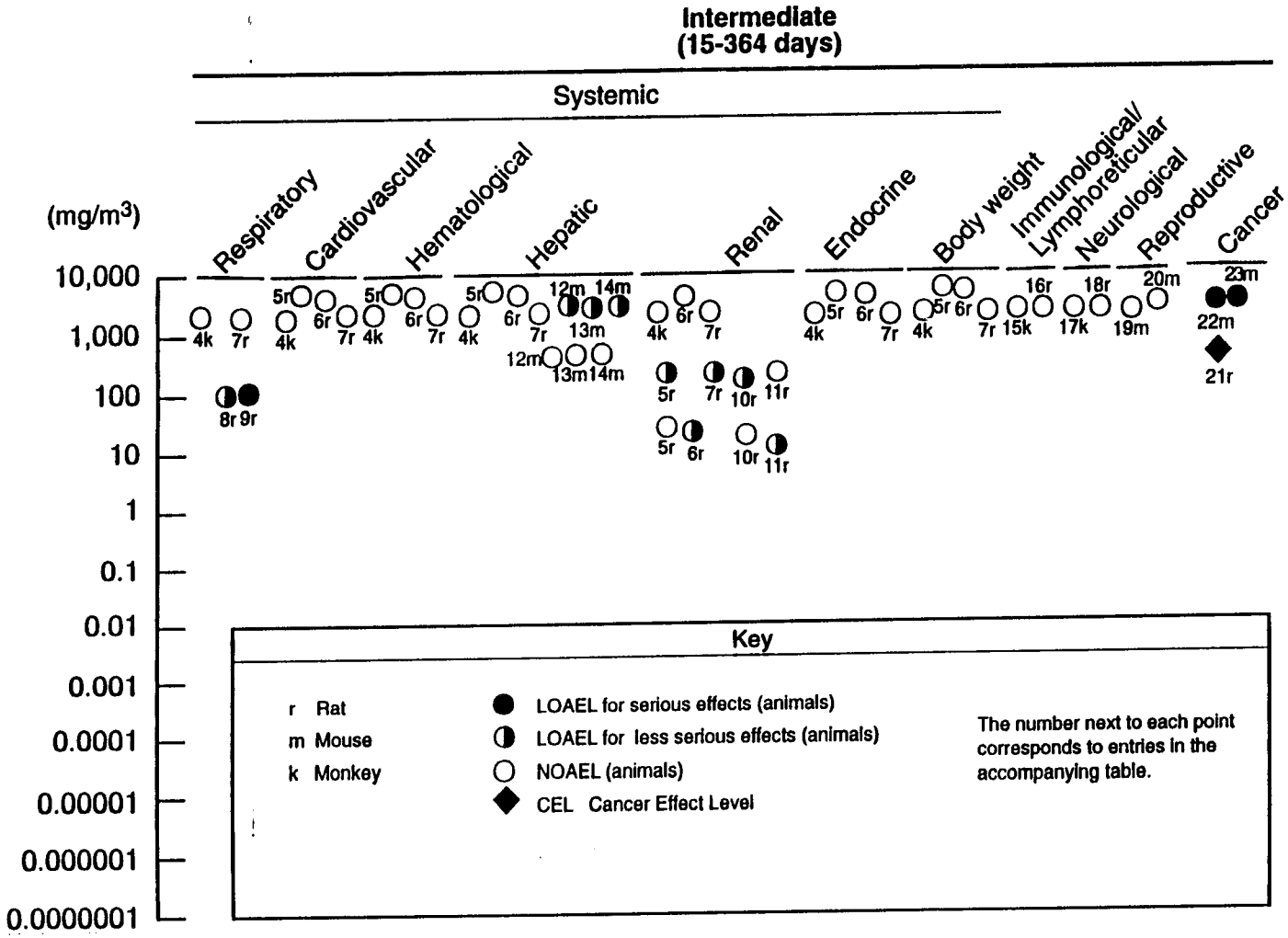
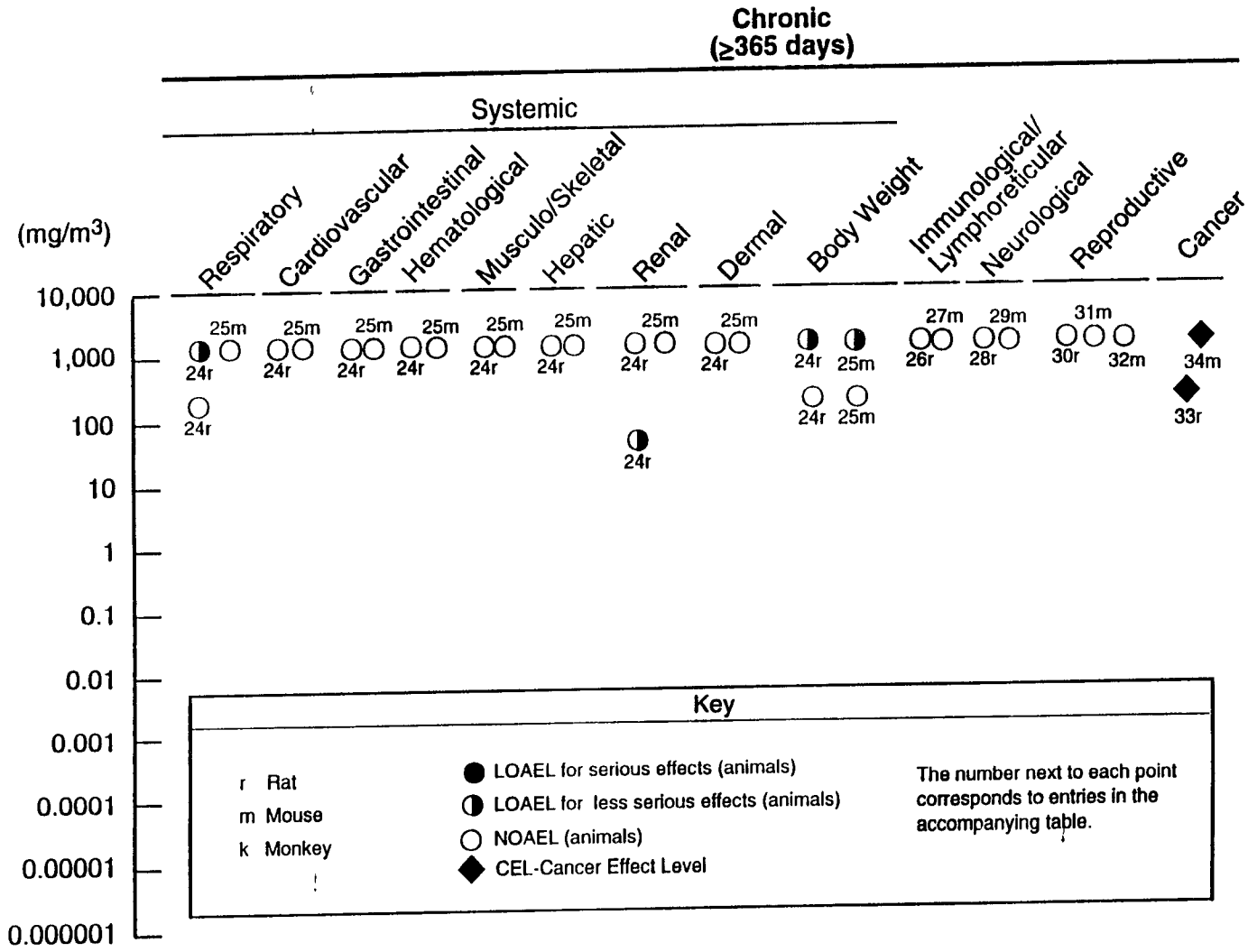


Figure 2-1. Levels of Significant Exposure to Automotive Gasoline – Inhalation (continued)



Intermediate-duration exposure of rats to approximately 453 ppm gasoline vapors resulted in an increase in the relative weight of the lungs in animals sacrificed after 30 days of exposure. This effect was no longer apparent in animals sacrificed after 60 days of exposure (Vyskocil et al. 1988). No biochemical, functional, or histopathological evidence of adverse respiratory effects was reported in this study, so the toxicological significance of the change in organ weight is not known. Rats and monkeys failed to exhibit any consistent adverse respiratory effects following intermediate-duration exposure to 1,552 ppm gasoline vapors (Kuna and Ulrich 1984). Although various parameters of pulmonary function were significantly altered in monkeys exposed to 1,552 ppm gasoline vapors as compared to the controls in this study, there was a lack of consistency with respect to the effects observed between the sexes, and there was a large degree of variability in the responses. Therefore, the evidence is insufficient to conclude that these changes in pulmonary function were treatment related. When the lungs of rats exposed to 100 ppm of gasoline vapors for 12 weeks were examined by electron microscopy, a progression of lesions characteristic of fibrosing alveolitis (interstitial fibrosis and alveolar collapse) was observed (Lykke et al. 1979). These lesions were not apparent at the light microscopic level. The animals began to exhibit signs of respiratory distress in this study after about 7 weeks of exposure, which is consistent with the alveolar collapse seen. Also, consistent with these findings was the observation that surfactant levels in the lung were markedly decreased in animals exposed to 100 ppm gasoline vapors for 5-15 days (Le Mesurier et al. 1979). This observation led the authors to suggest that the sutfactant deficiency was probably involved in the pathogenesis of the fibrosing alveolitis observed in the Lykke et al. (1979) study. The results of these two studies indicate that gasoline-induced pulmonary changes may occur at levels previously thought to cause no effect because the tissues were not examined ultrastructurally and/or other sensitive parameters of pulmonary function were not measured.

Chronic exposure of female rats to 2,056 ppm gasoline vapors for 2 years resulted in an increase in the incidence of mild multifocal pulmonary inflammatory response (compared to respective controls) that was thought to be due to the irritant effect of gasoline (MacFarland et al. 1984). Although the incidence of the pulmonary response was slightly increased in male rats exposed to 292 or 2,056 ppm, the increase was not dose-related, and the incidence was comparable to those of female control rats. This effect was not observed in mice similarly exposed (MacFarland et al. 1984), suggesting that rats are more susceptible to the pulmonary irritating effects of gasoline.

Cardiovascular Effects. Cardiac sensitization to circulating catecholamines leading to a fatal arrhythmia has been postulated as one possible cause of death in humans following inhalation of high concentrations of gasoline vapors (Poklis 1976). Abnormal electrocardiograms (ECGs) of a nonspecific nature have been recorded in individuals with a history of chronic leaded gasoline sniffing (Seshia et al. 1978).

A single 2-hour exposure to 70,180 ppm leaded gasoline vapors was reported to induce ECG changes and disturbances in myocardial enzyme activities and electrolyte levels in rabbits (Przybylowski 1971). The ECG readings were taken from the animals prior to exposure while they were anesthetized with evipan and again "immediately after intoxication" (elapsed time not specified). Exposure to leaded gasoline vapor resulted in a slowing of heart rate in all exposed animals and evidence of disturbed ventricular repolarization such as flattening of the T-wave (10/20), inverted T-wave (7/20), biphasic T-wave (3/20), ST depression (10/20), prolongation of the QT interval (16/20), and prolongation of the QRS complex (7/20). Decreased myocardial acid phosphatase, decreased myocardial sodium, potassium, and magnesium levels, and altered acid phosphatase and ATPase (adenosinetriphosphatase) activity in the myocardium were also observed. A decrease seen in myocardial alkaline phosphatase was not statistically significant. The author concluded that the decreased heart rate was a centrally mediated effect. He also concluded that the prolongation of the QRS complex, which is indicative of disturbed intracellular conduction, was a result of a direct effect on the myocardial electrical conduction system, and that the disturbed ventricular repolarization suggested by the ECG changes may have resulted in myocardial electrolyte disturbances (i.e., the changes in sodium, potassium, and magnesium observed). Furthermore, various enzyme activities also appear to be affected by exposure to gasoline vapor. While these conclusions appear valid, there are a number of limitations associated with this study. Only one exposure level was tested, precluding the determination of a dose-response relationship. The effects observed may have been due to oxygen deprivation because the gasoline vapor concentration was so high (approximately 7%). Therefore, a control group exposed to comparably low levels of oxygen would have been appropriate. The baseline ECGs were done on anesthetized animals, and the postexposure ECGs were done on animals that were apparently in a "narcotic sleep." It is difficult to compare the ECGs obtained under these different conditions. Furthermore, although the authors state that the ECGs were done "immediately after exposure," the

time between exposure and the ECGs was not quantified, and the animals could have been in various stages of recovery from any effects that occurred during exposure. It is possible that the cardiovascular effects may have been due to lead in the gasoline mixture since no cardiovascular effects have been observed in animals exposed to unleaded gasoline vapor (discussed in next paragraph).

No changes in heart weight or in the microscopic anatomy of the heart were observed in rats or monkeys exposed to up to 1,552 ppm unleaded gasoline vapors for 90 days (Kuna and Ulrich 1984). Similarly, rats and mice exposed to 2,056 ppm unleaded gasoline vapors for 2 years exhibited no exposure-related cardiovascular effects (MacFarland et al. 1984). A decrease in relative heart weight was observed in the rats from this chronic study, but in the absence of any biochemical, functional, or histopathological evidence of cardiovascular toxicity, the toxicological significance of a change in heart weight is not known.

Gastrointestinal Effects. No effects on the gastrointestinal system were observed in humans after inhalation exposure to gasoline (Ainsworth 1960; Carlson 1981).

One study in experimental animals was located in which the gastrointestinal tract was examined after inhalation exposure to gasoline vapors. No evidence of adverse effects was found upon histopathological evaluation of the gastrointestinal tract of rats and mice exposed to 2,056 ppm unleaded gasoline vapors for 2 years (MacFarland et al. 1984).

Hematological Effects. Several human case studies have been reported that describe the occurrence of hematological effects in individuals with known long-term exposure to gasoline vapors. However, in all of these cases, the hematological effects reported were most likely due to a constituent of gasoline rather than the gasoline mixture itself. For example, basophilic stippling, increased erythrocyte protoporphyrin, and increased δ-aminolevulinic acid dehydratase (ALAD) activity have been noted in individuals exposed to leaded gasoline vapor (Boeckx et al. 1977; Chessare and Wodarcyk 1988; Young et al. 1977). These effects are also known to occur with exposure to lead (see ATSDR toxicological profile for lead [ATSDR 1991]), and so may be due to the presence of organic

lead compounds in the gasoline. An increased incidence of various blood dyscrasias (anemia, hypochromia, thrombocytopenia, and neutropenia) has been observed in Nigerian males with known exposure to gasoline in their occupations as motor mechanics and road-side vendors of heavy motor oil and/or gasoline as compared to controls with no known exposure to gasoline (Niazi et al. 1989). The authors attributed the increased incidence of these disorders to benzene which is present in gasoline (see ATSDR toxicological profile for benzene [ATSDR 1991]).

No exposure-related adverse effects on any hematological parameters measured or on the bone marrow have been noted in rats or monkeys exposed to 1,552 ppm unleaded gasoline vapors for 90 days (Kuna and Ulrich 1984) or in rats and mice exposed to 2,056 ppm unleaded gasoline vapors for 2 years, despite the presence of benzene (MacFarland et al. 1984).

Musculoskeletal Effects. An 18-year-old male with a history of sniffing leaded gasoline vapors was admitted to the hospital on two occasions complaining of muscle weakness and pain (Kovanen et al. 1983). He claimed to sniff l-l.5 L at a time irregularly over the past year. Neurological examinations were normal on both hospital admissions, but his serum creatinine kinase was markedly elevated, and his urine was positive for myoglobin. Furthermore, his blood and urine lead levels were also elevated. The authors concluded that the boy suffered from acute severe myopathy associated with leaded gasoline sniffing. The mechanism by which gasoline could have induced this myopathy is not known. The authors speculated that the myopathy may have been due to individual susceptibility or that the patient may have had a subclinical, possibly metabolic, myopathy that was exacerbated by gasoline sniffing. The myopathy may also have been the result of lead toxicity (see ATSDR toxicological profile for lead [ATSDR 1991]).

No exposure-related effects were noted upon histopathologic examination of the bones in rats or mice exposed to 2,056 ppm unleaded gasoline vapors for 2 years (MacFarland et al. 1984).

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

No adverse hepatic effects were noted in rats or monkeys exposed to 1,552 ppm unleaded gasoline vapors for 90 days (Kuna and Ulrich 1984) or in rats exposed to 2,056 ppm unleaded gasoline vapors for 2 years (MacFarland et al. 1984). Hepatic hypertrophy and increased hepatic cytochrome P-450 content were observed in mice exposed to 2,039 ppm unleaded gasoline vapor for 13 weeks; however, there was no evidence of hepatic necrosis (Standeven and Goldsworthy 1993). Hepatic hypertrophy without accompanying necrosis was also noted in mice exposed to 2,056 ppm for 13 or 16 weeks (Standeven et al. 1994a; Tilbury et al. 1993). Mice chronically exposed to unleaded gasoline vapors exhibited necrosis and hemorrhage associated with liver tumors (see Section 2.2.1.8), but no other exposure-related hepatic effects were seen (MacFarland et al. 1984).

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

Unleaded gasoline is one of a diverse group of hydrocarbons that have been shown to induce a unique syndrome of nephropathy in male rats following subchronic or chronic inhalation exposure (Halder et al. 1984; Kuna and Ulrich 1984; MacFarland et al. 1984; Short et al. 1987, 1989a, 1989b). The components of gasoline determined to be largely responsible for the hydrocarbon-induced nephropathy in the male rat were identified as branched alkane compounds with six or more carbons. The gasoline used in the studies conducted by Halder et al. had the following composition: 45% alkanes, 12% alkenes, 43% aromatics. The mixture used by MacFarland et al. (1984) and in the studies conducted by Short et al. (1987, 1989a, 1989b) was the API PS-6 specified in the introduction to this chapter. Kuna and Ulrich (1984) used two mixtures of gasoline: “Fuel A - Unleaded EPA Reference Fuel” and “Fuel B - Leaded Commercial.” Fuel A contained 30.1% aromatics, 8.2% olefins, and 61.7% saturates. Fuel A was further described as containing 0.2% benzene, 16.7% toluene, 1.0% n-butane, 5.4% isopentane, and 4.8% n-pentane. Fuel B contained 27.4% aromatics, 7.8% olefins, and 64.4% saturates with 0.4% benzene, 11.4% toluene, 0.4% n-butane, 5.5% isopentane, and 4.0% n-pentane. The lead content of Fuel B was not specified, but the concentrations of lead in the two

exposure levels of Fuel B used in the study were 0.19 μg lead/L at the 384-ppm level and 0.72 μg lead/L at the 1,552-ppm level.

This syndrome of nephropathy is characterized by excessive accumulation of hyaline droplets containing $\propto_{2u}$-globulin in the P2 segment of the proximal tubule region. The accumulation of these droplets may lead to single-cell necrosis and exfoliation of P2 tubular epithelial cells, followed by tubular epithelial cellular proliferation which is often associated with tubular dilation and tubular epithelial necrosis. In addition to inducing this syndrome in young male rats, gasoline has been shown to exacerbate the rat nephropathy commonly seen in aging male rats (MacFarland et al. 1984; Short et al. 1989a). $\propto_{2u}$ -Globulin is produced in large amounts by male rats, accounting for 26% of their total urinary protein. There is no evidence that humans produce $\propto_{2u}$ -globulin. In addition, human urine contains relatively little protein: only 1% of the total concentration measured in male rats. Of this amount, only trace quantities are within the same protein family as $\propto_{2u}$ -globulin (Olson et al. 1990). This suggests that humans are probably not at risk for the type of nephropathy induced by gasoline in male rats. In addition, $\propto_{2u}$,-globulin-induced nephropathy cannot be induced in female rats (Halder et al. 1984; Kuna and Ulrich 1984; Short et al. 1989a), mice of either sex (MacFarland et al. 1984), or monkeys of either sex (Kuna and Ulrich 1984). Thus, it appears to be unique to male rats. Therefore, even though these nephrotoxic effects were seen at exposure levels that are lower than those required to induce other toxic effects, this end point was not used for the derivation of an acute, intermediate, or chronic inhalation MRL for gasoline.

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

A decrease in relative adrenal weight was noted in rats following intermediate-duration exposure to _ . -. gasoline vapors, but no exposure-related histopathological effects were observed in the adrenal gland (Kuna and Ulrich 1984). Therefore, the toxicological significance of this change in adrenal weight is not known.

Dermal Effects. No studies were located regarding dermal effects in humans after inhalation exposure to gasoline.

No exposure-related effects on the skin were observed in rats or mice exposed to 2,056 ppm unleaded gasoline for 2 years (MacFarland et al. 1984).

Ocular Effects. Gasoline vapor at concentrations of about 200, 500, or 1,000 ppm caused eye irritation in volunteers during a 30-minute exposure period (Davis et al. 1960). Ocular irritation was also noted in subjects exposed to 500 ppm for 1 hour (Drinker et al. 1943). Both the Drinker et al. 1943 and the Davis et al. 1960 studies are limited in that the subjects were exposed to atomized gasoline vapors, which has the same composition as liquid gasoline and is not the same as the gasoline vapors that humans would be exposed to in ambient conditions.

No studies were located regarding ocular effects in animals after inhalation exposure to gasoline.

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

Intermediate- and chronic-duration exposures to gasoline vapors have been reported to cause significant decreases in body weight gain in rats (MacFarland et al. 1984; Vyskocil et al. 1988) and mice (MacFarland et al. 1984).

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humansafter inhalation exposure to gasoline.

In animals, exposure to hydrocarbons has been associated with Goodpasture’s syndrome (glometulonephritis and pulmonary hemorrhage caused by the binding of circulating autoantibodies to basement membrane antigens on the glomerular and alveolar basement membranes, respectively)

(O'Regan and Turgeon 1986; Yamamoto and Wilson 1987). To determine whether gasoline exposure causes pulmonary alveolar damage thereby allowing autoantibodies to pass into the alveoli and bind to lung basement membrane, rats were exposed in a closed container to unleaded gasoline once or 5-7 times daily at half-hour intervals for 5-8 days (O'Regan and Turgeon 1986). The animals were then injected with sera containing anti-glomerular basement membrane (anti-GBM) antibodies, sacrificed, and the lungs were taken for light and immunofluorescence microscopy. The anti-GBM failed to bind to alveolar basement membrane (ABM) in the gasoline-exposed animals as determined by immunofluorescence. Based on these results, it does not appear that exposure to gasoline in this manner damages the alveolar endothelium to allow passage of anti-GBM to cause the pulmonary hemorrhage associated with Goodpasture's syndrome.

Rats and monkeys exposed to up to 1,552 ppm gasoline for 90 days were evaluated for the presence and deposition of IgG, a class of immunoglobulin, in the renal glomeruli and lungs (Kuna and Ulrich 1984). There was no evidence of IgG deposition in either the renal glomeruli or the lungs in the exposed animals based on immunofluorescence studies.

No evidence of adverse histopathological effects was noted in the thymus or the bone marrow of rats or mice exposed to 2,056 ppm gasoline vapors for 2 years (MacFarland et al. 1984).
The highest NOAEL values and all reliable LOAEL values for immunological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.4 Neurological Effects

Acute exposure of humans to high levels of gasoline vapors is characterized by a spectrum of neurological effects that progress in severity with increasing dose and duration and can include dizziness, headaches, giddiness, euphoria, vertigo, blurred vision, nausea, numbness, drowsiness, anesthesia, and coma (Poklis and Burkett 1977). Chronic exposure to gasoline (i.e., in those individuals who habitually sniff gasoline for its euphoric/hallucinogenic effects and in those occupationally exposed to gasoline) is associated with neurological effects as well.

In a 2-year retrospective study, hospital records on 40 male and 10 female patients of Native American or Native Canadian origin who sniffed gasoline indicated that exposed individuals exhibited signs of jaw jerk, postural tremor, ataxia, abnormal gait, deep tendon reflexes, and affected speech (Seshia et al. 1978). A study of 51 gasoline station workers reported complaints of headaches, fatigue, sleep problems, memory loss, and general weakness (Pandya et al. 1975). The benzene content of the gasoline was high; values ranged from 10-17%. Therefore, it is difficult to determine the contribution of benzene exposure to the reported symptoms. Gasoline odors were detected periodically by office workers for several years but increased during a 9-month period in which headaches and nausea were reported by 18 individuals (Kullman and Hill 1990). Measurements (unspecified method) that were made on a day when gasoline odors were present indicated total hydrocarbon concentrations of approximately 3-22 ppm. These studies lacked adequate details on the exposure duration and concentrations. Furthermore, the investigators did not assess the contribution of other constituents of gasoline (e.g., lead, benzene) as a potential source of the neurotoxic effects.

The majority of the data on the neurological effects of gasoline have come from case reports describing patients, usually adolescents, who were chronic gasoline sniffers (Owens et al. 1985). In most instances, the exposure concentrations could not be determined and the lead content in the gasoline was not specified. Cerebellar dysfunction (e.g., ataxia, poor coordination, decreased muscle tone, broad-based gait, myoclonic movements), generalized convulsions, and hallucinations, as well as elevated blood lead levels, are typical symptoms observed in gasoline sniffers (Carroll and Abel 1973; Coulehan et al. 1983; Goldings and Stewart 1982; Goodheart and Dunne 1994; Kaelan et al. 1986; Moss and Cooper 1986; Rischbieth et al. 1987; Young et al. 1977). Some exposed individuals were also found to have abnormal electroencephalograms (EEG) and/or slowing of nerve conduction velocity (Goldings and Stewart 1982; Hall et al. 1986; Hansen and Sharp 1978; Rischbieth et al. 1987; Robinson 1978; Seshia et al. 1978). A 14 year-old male who inhaled gasoline 10-20 times a day complained of a loss of strength and paresthesia in the limbs. Motor nerve conduction velocity was slowed on his right side, and Wallerian degeneration and segmental demyelination were reported (Gallassi et al. 1980). Neuropathological changes reported in eight patients who were diagnosed as “chronic gasoline sniffers” included neuronal loss and gliosis in the cerebral cortex, cerebellum, and brainstem, including the reticular formation (Goodheart and Dunne 1994). It is difficult to discern if these effects were due to the lead in the mixture or to the long-term exposure to hydrocarbon mixtures.

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A male patient was hospitalized four times during ages 17-21 years for acute lead encephalopathy due to gasoline sniffing (Valpey et al. 1978). He experienced insomnia, anorexia, agitation, irritability, poor memory, nystagmus, and slurred speech, as well as characteristic cerebellar effects (e.g., ataxia, involuntary movements). He gradually developed permanent dementia and dysmetria and died after his fourth admission. An autopsy revealed mild congestion and atrophy of the brain, as well as a patchy loss of Purkinje cells, neuronal loss, gliosis, and early hemorrhagic pneumonia. Necrosis and demyelination of nerve cells and edema of the brain were noted in a 14-year-old boy who died having repeatedly inhaled gasoline for over 4 years (Robinson 1978). A computerized tomographic scan of a 25-year-old man exposed to gasoline for 5 years indicated cerebellar atrophy (Kaelan et al. 1986). After his death, an autopsy showed decreased brain weight, increased brain lead content, and severe atrophy in the cerebellum as well as the loss of nearly all of the Purkinje cells, some neuronal loss, and severe gliosis.

Many of the neurological changes that were observed in these subjects are related to organic and inorganic lead encephalopathy (Robinson 1978; Valpey et al. 1978). Tetraethyl lead can produce symptoms of nausea, vomiting, diarrhea, irritability, restlessness, and anxiety, progressing to the appearance of tremors, weakness, and confusion, followed by the onset of mania and convulsions (Goldings and Stewart 1982; Robinson 1978). Tetraethyl lead itself is not toxic but is converted to triethyl lead which is water soluble and becomes concentrated in the brain where it induces these neurological changes. Triethyl lead is probably degraded to inorganic lead which explains the slowed nerve conduction velocity (Robinson 1978). Chelation therapy, which increases the excretion of inorganic lead, is commonly given to reduce effects due to the lead exposure (Robinson 1978). In another case report, authors concluded that n-hexane, a component of gasoline, was the probable cause of the motor neuropathy exhibited in a 4-year-old boy who was found comatose beside a can of gasoline (Hall et al. 1986). On admission, he had elevated blood lead level and decreased nerve conduction velocity; he had reportedly demonstrated abnormal gait and imbalance 3 months earlier.

Behavioral and intellectual changes have been observed in humans exposed to gasoline (Carroll and Abel 1973; Kumar et al. 1988; Robinson 1978). Ninety exposed gasoline-pump workers had significantly affected immediate and delayed visual memory and perception ($p<0.0l$), psychomotor

disturbances ($p<0.05$), and visuomotor learning ability ($p<0.05$) compared to a control group consisting of 64 subjects matched for age, economic status, education, and location (Kumar et al. 1988). The workers exposed for more than a year had a greater decrease in visual memory and intellectual capacity compared to those workers exposed to gasoline for a shorter duration, while psychomotor disturbances were similar in all workers. Psychological testing was conducted on a 13-year-old boy who sniffed gasoline daily for 6 years (Carroll and Abel 1973). The Wechsler-Bellevue Intelligence Scale indicated an intelligence quotient (IQ) of 29 (severe mental retardation), and the Bender-Gestalt test revealed severe motor incoordination. A follow-up exam 10 weeks after discharge demonstrated an IQ of 44 (moderate retardation). It was noted that his fraternal twin brother, who was not a gasoline sniffer, had an IQ of 62. Therefore, the low IQ in this case may well have preceded the gasoline abuse. Following a 6-month period of gasoline sniffing, a 15 year-old girl had an IQ of 64 with severely impaired perceptual motor skills and an abnormal EEG (Robinson 1978). It is not possible to determine if the psychological and motor impairment seen in this case was a direct result of gasoline sniffing because no information was available on these parameters before the period of gasoline sniffing. No follow-up testing of intellectual functioning was conducted after the girl stopped sniffing gasoline.

Rabbits (15-20/group) of unspecified strain and sex were exposed to 0 or 70,180 ppm leaded gasoline for 2 hours (Przybylowski 1971). All the exposed animals exhibited periods of restlessness, equilibrium disturbances, convulsions, and narcosis after 35 minutes. However, no histopathology of the brain tissue was conducted. These effects may have been partially due to the presumably low oxygen concentration in the exposure atmosphere, as discussed in Section 2.2.1.2, Cardiovascular Effects.

Rats and monkeys exposed to 1,552 ppm gasoline for 90 days exhibited no evidence of neurotoxicity, as assessed by neuropathological examination, either during exposure or at necropsy (Kuna and Ulrich 1984).

In a chronic inhalation study, Fischer-344 rats (three/sex/group) were exposed to 2,056 ppm unleaded gasoline for 7, 12, or 18 months (API 1982). The control groups for each exposure period consisted of only one male and one female rat. Pigmentation of the neuronal cytoplasm was observed in an

unspecified number of exposed animals, and Wallerian degeneration was reported in two rats at the 7-month sacrifice. Age-related axonal degeneration and dystrophy in the distal gracile tract of the spinal cord were exhibited in the control and exposed groups with increasing severity at each exposure period. The effects were more prominent in the exposed rats compared to the controls at 18 months. No statistical analyses were conducted, and the number of animals per group was inadequate for evaluating changes.

Fischer-344 rats and $B6C3F_1$, mice inhaled 2,056 ppm unleaded gasoline for 107-109 weeks with interim sacrifices performed at 3, 6, 12, and 18 months (MacFarland et al. 1984). There were no treatment-related changes in the brain weight or the histopathology of the brain, spinal cord, and peripheral nerves. No other details were reported.

The highest NOAELs and all reliable LOAELs for neurological effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2-l.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to gasoline. A reproductive toxicity study in animals reported no evidence of dominant lethal effect on sperm. Inhalation exposure of male CD-l mice to either 400 or 1,600 ppm unleaded gasoline vapor, 6 hours/day, 5 days/week, for 8 weeks, produced no significant increase in pre- or postimplantation loss of embryos in treated animals compared to controls (Litton Bionetics 1980). The composition of the gasoline used in this study was reported to be 47% paraffins, 4% olefins, 10% naphthenes, and 39% aromatics, which is similar to the mixtures used in other studies sponsored by API. The NOAEL for reproductive effects in male mice was 1,600 ppm; the LOAEL was not established.

No exposure-related histopathological effects were noted in the reproductive organs of rats or mice that were exposed to 2,056 ppm unleaded gasoline vapors for 2 years (MacFarland et al. 1984). A recent reexamination of histological sections from the McFarland et al. 1984 cancer bioassay revealed a marked decrease in the severity of uterine cystic endometrial hyperplasia (a common spontaneous

condition) in female mice exposed to 2,056 ppm unleaded gasoline (MacGregor et al. 1993). In addition, there was an increase in the incidence and severity (primarily graded trace to mild) of uterine atrophy in aged female mice exposed to 2,056 ppm unleaded gasoline. However, the majority of the lesions were observed only at the end of the study. At terminal sacrifice, 14 of 40 female mice exhibited uterine atrophy. Uterine atrophy was not present at terminal sacrifice in control animals or in those exposed to 67 or 292 ppm. A decrease (40%) in uterine weight was observed in female mice following exposure to 2,056 ppm unleaded gasoline for 16 weeks (Standeven et al. 1994a). However, there were no significant histological changes in reproductive organs or significant effects on serum 17β-estradiol levels, uterine peroxidase activity, or uterine cytosolic estrogen receptor levels.
The highest NOAEL values for reproductive effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.6 Developmental Effects

Anecdotal data have suggested a possible link between chronic gasoline vapor exposure of pregnant mothers and congenital central nervous system defects in their children. A gasoline abuse case study reported profound growth retardation and initial hypotonia of muscles progressing to hypertonia, scaphocephaly, a prominent occiput, poor postnatal head growth, and minor anomalies in two children from a small Native American community. The mothers of both children had inhaled leaded gasoline during pregnancy (Hunter et al. 1979). The results of the study are difficult to assess because of the small sample size, possibility of concomitant exposure to alcohol, lack of quantification of exposure levels, and presence of lead which may have contributed to the developmental defects in the children.

A teratogenicity study in animals exposed to gasoline vapors failed to reveal significant developmental effects. In this study, groups of 25 pregnant rats were exposed to atomized unleaded gasoline vapors at 0, 400, or 1,600 ppm for 6 hours/day from day 6 through day 15 of gestation. No adverse effects were noted in maternal animals, and there was no evidence of variation in sex ratio, embryotoxicity, reduced fetal growth, or teratogenic effects in fetuses. The results provide no evidence of developmental toxicity in rats associated with exposure to gasoline vapor at concentrations as high as

1,600 ppm. Thus, the NOAEL for developmental effects was 1,600 ppm; the LOAEL was not established (Litton Bionetics 1978).

The highest NOAEL value for developmental effects in rats after acute inhalation exposure to gasoline is recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.7 Genotoxic Effects

One cytogenetic monitoring study of workers occupationally exposed to gasoline in Sweden was found. Peripheral lymphocytes from 15 male gasoline pump mechanics were analyzed for micronuclei induction; the results were compared to those obtained from 15 male construction workers (Högstedt et al. 1991). Seven of the exposed workers and 8 of the controls were smokers. Ages ranged from 20 to 56 and from 19 to 56 years for the exposed and control groups, respectively. Exposure concentrations, durations, and gasoline type (i.e., leaded versus unleaded) were not reported; however, the authors indicated that gasoline in Sweden may contain up to 5% benzene. Based on the findings from recent studies cited by the authors, parallel lymphocyte cultures from gasoline-exposed and control donor groups were incubated in the presence of pokeweed mitogen (PWM), which stimulates both B- and T-lymphocytes, and phytohemagglutinin (PHA), which primarily activates T-cells. The analysis showed a significant ($p<0.02$) increase in the frequency of micronuclei in the exposure group lymphocytes stimulated by PWM but not by PHA. However, the presence of up to 5% benzene in the gasoline mixture, which is approximately 2-10 times more than the level found in typical American automotive fuels (based on a typical benzene concentration of 0.5-2.5% in American gasoline; see Chapter 3), confounds the interpretation of the results.

In a dominant lethal experiment, groups of 12 male CD-l mice were exposed to 400 or 1,600 ppm unleaded gasoline for 6 hours per day, 5 days per week, for 8 weeks (Litton Bionetics 1980). The composition of the gasoline used in this study is discussed in Section 2.2.1.6. Two days after the final treatment, each male was housed with two untreated virgin females for 5 days. The females were replaced with two new females, and the mating sequence was continued for 2 weeks. One male in the high-dose group and two males in the low-dose group died prior to mating, thereby reducing the sample size of mated females. Fourteen days following the midweek of mating, the uteri of all

females were examined for the numbers of live, dead, and total implants. When these results were statistically compared to the controls, no significant increases in pre- or postimplantation embryo loss were observed. Although the pregnancy rates of the treated groups were comparable to those of the untreated groups, the sample size of pregnant females was less than 20 for the majority of treatment and control groups. The results suggest that exposure of male mice to unleaded gasoline did not induce a clastogenic effect in germinal cells sampled over the spermatogenesis cycle. Nevertheless, the lack of an adequate sample size of pregnant females renders the study insufficient to support a negative conclusion.

In an unscheduled DNA synthesis (UDS) assay, Fischer-344 rats, three males and three females, were exposed to 2,000 ppm of unleaded gasoline, 6 hours per day for up to 18 consecutive days (Loury et al. 1987). Following treatment, kidneys were perfused *in situ* with a buffered salt solution containing collagenase, excised, and dispersed to release individual cells. Cultures of kidney cells were treated with a medium containing tritiated thymidine; gasoline was added directly into the cultures. Autoradiographs were prepared and the incidences of scheduled DNA synthesis (SDS) and unscheduled DNA synthesis (UDS) were determined. SDS is replicative DNA synthesis that occurs during periods of normal or tumorigenic tissue growth or replacement. UDS is a repair process that occurs when DNA has been damaged. There was no increase in UDS following 4 or 18 days of treatment. However, a marked increase in SDS was seen in the kidney cells harvested from male rats 18 days after dosing; no nephrotoxic effects were seen in females. The overall results indicate that acute- and intermediate-duration inhalation exposure to 2,000 ppm unleaded gasoline produced no genotoxic effects in the kidneys of male or female rats. Refer to Table 2-4 for a further summary of these results.

Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

A large number of epidemiological studies have been conducted on workers occupationally exposed to petroleum products and hydrocarbons. These studies all have several inherent limitations that preclude

their use as evidence for an association between gasoline exposure and cancer. These include lack of information on levels of exposure to gasoline vapor; concurrent exposure to other potentially carcinogenic substances (i.e., service station attendants are also exposed to motor oils, diesel fuel oils, and solvents as well as automobile and truck engine exhausts); no adjustment for potential confounding factors (e.g., smoking); and no latency analyses. EPA (1987a) reviewed 55 relevant studies of unleaded gasoline-exposed populations and concluded that the evidence for drawing causal inferences between unleaded gasoline and cancer was inadequate. A few of these and other more recent studies are summarized below.

A case-control study was performed regarding exposure to petroleum-based liquids and the risk of developing cancer (Siemiatycki et al. 1987). Approximately 12.4% of the cases were exposed to gasoline as well as other chemicals. The only significant odds ratio found with regard to gasoline exposure was for stomach cancer. The odds ratio is defined as the ratio of the percentage of exposed individuals with a disease to the percentage of individuals from a control group with the same disease. When data were analyzed by exposure and job category, it was found that persons with long-term low exposures as well as persons with long-term high exposures had an increased risk of stomach cancer. The power to detect risks was only moderate for most of the associations analyzed. Using other cancer patients as controls may have decreased some of the risk estimates. No definition was provided for short, long, or substantial exposures. Auto mechanics tended to have high exposures to gasoline because they used it as a degreasing agent which may account for the excess risk seen in this group. The results of this study are based on a small number of cases in each of these groups.

A case-control study was performed regarding occupational exposure to hydrocarbons and the risk of developing renal cell carcinoma (Kadamani et al. 1989). Most of the workers exposed to hydrocarbons were either operatives or craft workers. An exposure risk index was calculated and was divided into low, medium, and high exposure categories. More cases of renal cell carcinoma occurred in males that had exposure to gasoline than in controls. The highest risk of renal cell carcinoma was among male workers exposed to moderate levels of hydrocarbons. Males <60 years of age exposed to moderate levels of hydrocarbons had the highest risk of renal cell carcinoma in comparison to the control group. Males exposed before 1930 had an increased risk at higher exposures, but males exposed after 1930 had an increased risk when exposed to moderate levels of hydrocarbons. This may

be due to better personal protective equipment being used in the high exposure jobs. There are several limitations to this study. The exposure scores were based only on occupation. No attempt was made to determine the type of solvent exposure. The exposure index did not have sensitivity for multiple exposures and duration of exposure. The study had low power to detect differences in small groups. This would account for the inability of the study to detect an increase in females or, at certain levels of hydrocarbon exposure, in males.

A case-control study of 313 exposed males and 428 male controls who worked in petroleum refineries showed no significant risk of renal cell cancer. Women were not included in the analysis since few women work in the petroleum industry. No significant trend was found regarding increased risk of renal cancer and duration of employment (McLaughlin et al. 1985). A cohort study of 48,417 male and 12,916 female Gulf Oil Company employees revealed no significant excess deaths from kidney cancer (Wen et al. 1984). No information was provided on length or duration of employment. A cohort study of a total of 16,880 employees of the Gulf Oil Company Port Arthur refinery found no excess deaths from kidney cancer as compared to the normal population; employees were followed from January 1937 to January 1978 (Wen et al. 1984). The study included all workers who had worked at least 1 day in the plant. No trend was found for increasing risk with years of employment since duration of employment did not correlate with exposure to gasoline.

A proportionate mortality analysis (PMR) revealed an increase of leukemia deaths in gasoline station attendants and mechanics. In addition, there were four deaths due to brain cancer (Schwartz 1987). No information is provided on length or employment or exposure. The PMR does not reflect the mortality of the population because it is so easily influenced by over- and under-representation of deaths. The automobile mechanics and gasoline station attendants who died of leukemia each had different job titles. Therefore, they may not have had similar exposures. Also, several types of leukemia were found: acute myelogenous leukemia (N=2), chronic myelogenous leukemia (N=2), erythroleukemia (N=l), chronic lymphocytic leukemia (N=2), acute leukemia unspecified (N=l), and chronic leukemia undifferentiated (N=l). It is very difficult to draw any definitive conclusions from this type of analysis because several different types of leukemia were reported, and quantification of exposure is not possible in a retrospective study of this type.

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A meta-analysis (an analysis in which data from several studies are combined to obtain a single result) was performed of several cohort studies in the petroleum industry (Wong and Raabe 1989). A total of 24 studies were evaluated which encompassed a study population of 352,661; 6,405 deaths were observed in the entire cohort. The standard mortality ratio (SMR) for all cancers was reduced when the SMRs from the individual studies were combined. Most of the larger studies demonstrated a deficit for all cancers. The all cancer meta-SMR on 6,405 deaths was 85 (p<.0000l). A British industry-wide study of refinery employees found an excess of deaths from melanoma. However, no explanation of excess was offered. The meta-SMR (SMR obtained from combing SMRs from individual studies) was not significant for skin cancer. For urinary tract cancer in Japanese refinery workers, the SMR was 205 (p=0.05). British drivers had an SMR of 171 (p=0.05) for kidney cancer; a significant excess was found in men aged 55-64 years (SMR=l89, p<0.05). A few studies showed a modest increase in lymphatic and hematopoietic cancers. A significant excess of lymphatic and hematopoietic cancers was found in Mobil Oil Company (Beaumont, Texas) refinery employees. Several studies indicated increased mortality from these cancers with increasing length of service. The meta-SMR was 103 (p=0.58). An unpublished update of the study at the Gulf Oil Company Port Arthur refinery found a significant excess of leukemia among employees with 20 or more years of service. The study of Mobil Oil Company workers found an excess of leukemia in their cohort. Analysis by length of employment detected an SMR of 235 for those with 30 or more years of service. In addition, elevated leukemia mortality was found at the Shell Oil Company refineries at Wood River and Deer Park. Most of the leukemia cases in the petroleum industry were not found among those who worked on benzene units directly. Except for the Shell Oil Company refineries, most refineries reported a variety of cell types. Most of the studies did not provide a dose-response relationship. Few industrial hygiene data existed in the industry prior to the mid-1970s, whereas relevant exposure occurred earlier. This review supports the conclusion that some subgroups of the petroleum industry had an increased risk of cancer, particularly leukemia.

Results of several recent epidemiological studies have examined the possible association between gasoline exposure and increased leukemia and kidney cancer risks. In a recent follow-up study of the cohort of 34,569 British petroleum refinery and 23,306 distribution workers, an SMR of 121 for kidney cancer was reported in distribution workers (Rushton 1993). In particular, an increase in kidney cancer risk (SMR=141) was noted in tank truck drivers. For refinery workers, the SMR was

101. In addition, excesses in leukemia mortality were found in distribution workers (SMR=121), but not refinery workers (SRM=73). For tank truck drivers, the SMR was 155. No estimate of exposure to hydrocarbons was provided in the study.

In a recent retrospective mortality study among 6,672 petroleum marketing and distribution workers from 226 locations throughout Canada, work histories were obtained, and hydrocarbon exposure frequency scores for several jobs were assigned (Schnatter et al. 1993). An increased mortality (SMR=135) for kidney cancer was found for all petroleum marketing distribution workers. The SMR was 158 for employees with "hydrocarbon" exposure in the marketing/distribution segment. When results were examined with respect to exposure frequency, the SMR for kidney cancer was 208 in employees with daily exposure. The kidney cancer SMRs for employees classified as "nonexposed" or "less than daily exposed" were 91 and 99, respectively. A kidney cancer SMR of 210 was noted for tank truck drivers employed more than 1 year. When data were evaluated on the basis of a 20-year latent period after first exposure, the SMR for kidney cancer was 181. Following the application of a Poisson regression model, a relative risk of 3.86 was calculated for employees exposed daily versus those exposed less than daily (relative risk = 0.85). The study authors concluded that although the patterns of kidney cancer risk are consistent with a possible risk due to hydrocarbon exposure, the limited number of observed and expected deaths (9 vs. 6.6) are only suggestive and do not allow a concise interpretation. A significant increase in mortality due to leukemia was noted in tank truck drivers (SMR=335).

A recent case-control study in a cohort of about 100,000 male refinery workers from five petroleum companies revealed no excess in kidney cancer in refinery workers (Poole et al. 1993) The relative risk for any exposure above refinery background levels was estimated to be 1.0. In contrast, workers involved in the distribution, transport, and movement of petroleum products (job category described as receipt, storage, and movements) showed a possible increased kidney cancer risk (relative risk = 2.49). There was no evidence for an increased kidney cancer risk in a cohort of 15,135 distribution workers with potential exposure to gasoline for at least 1 year at land-based terminals or on marine vessels between 1946 and 1985 (Wong et al. 1993). An SMR of 65.4 for kidney cancer was reported in landbased distribution workers. For marine distribution workers, the SMR was 83.7. The SMR for kidney cancer in tank truck drivers was 61. An SMR of 150 for acute myelogenous leukemia was reported

for land-based distribution workers. However, no trend was apparent when data were analyzed by various gasoline exposure indices. The SMR for acute myelogenous leukemia was 74.2 for marine employees. In a follow-up study, the cohort mortality data were further analyzed by using a nested design (Wong et al. 1993b). This study limited analysis to the land-based workers since quantitative exposure data were available for this group. Leukemia (all cell types), acute myeloid leukemia, kidney cancer, and multiple myeloma were selected for additional analyses. Also, a more specific and homogenous job classification was developed in this nested study. Additional gasoline exposure indices consisted of length of exposure, cumulative exposure (ppm-years in terms of total hydrocarbons), and frequency of peak exposure. A time period of first exposure to gasoline (≤1948 vs. ≥1949) was also included as an exposure index. The results of the nested case-control study, along with the findings of the original study, indicated that there was no association between exposure to gasoline and leukemia (all cell types), acute myeloid leukemia, kidney cancer, or multiple myeloma. A parallel study of exposure assessment among the cohort members of the original Wong et al. 1993 study has been reported (Smith et al. 1993). In this exposure assessment, tasks and job exposures during 1975-1985 were evaluated (task-time-weight-average exposure model), and truck and marine exposures before 1975 were extrapolated on the basis of methodology to estimate historic marketing and marine distribution worker exposures to gasoline. Task exposures were highest during tank filling in trucks and marine vessels. Measured average annual, full-shift exposures during 1975-1985 ranged from 9-14 ppm of total hydrocarbon vapor for truck drivers and 2-3 ppm for marine workers on inland waterways. Extrapolated past average exposures in truck operations are highest for truck drivers before 1965 (140-220 ppm).

A recent case-control study was performed to assess the risk of renal cell cancer from occupational exposure to gasoline (Partanen et al. 1991). From a total of 672 cases, 338 eligible cases were selected and matched with 484 controls. The controls were matched to cases based on age, gender, and survival time. The odds ratio was significant when both men and women were considered together, however, this rate was unadjusted. When men were considered alone, even though the number of cases (39) remained the same, the odds ratio was not significantly increased once the rate was adjusted for obesity, smoking, and coffee consumption. The gasoline exposure was considered high for 11 cases and 2 controls, and low for 28 cases and the remaining controls. The persons exposed to gasoline had worked in various occupations such as taxis drivers and service station

attendants. Because of possible confounding of gasoline with other fuel exposures, the odds-ratio for gasoline exposure only was calculated and found to be significantly elevated. Conditional logistic regression showed a significant risk of renal cell cancer for exposure to gasoline of 1.0-2.0 ppm, cumulative exposure of 14-102 ppm-years, and a latency of 27-33 years; duration of exposure was not significant. A ppm-year is a measure of cumulative exposure. It is the product of the mean level of exposure and the duration of exposure. The majority of the cases were deceased. Therefore, next-of-kin were contacted to fill out the questionnaire. (Next-of-kin information is unreliable because relatives may not know what other confounding factors may have been involved, such as smoking.) Subjects that were alive filled out their own questionnaires. Interviews were also conducted with personnel familiar with the workplace conditions and job exposures so that exposures could be classified with little misclassification bias. Based on the results of this study, it can be concluded that exposure to gasoline was associated with the risk of kidney cancer.

Male and female Fischer-344 rats were exposed to unleaded gasoline vapors in an initiation/promotion assay in an attempt to determine the mechanism for unleaded gasoline-induced renal tumors in male rats (Short et al. 1989b). The incidences of chronic progressive nephrosis (CPN), atypical cell foci (ACF) (believed to be the precursors to tumors), and renal cell tumors (RCT) were evaluated by light microscopy. The rats were exposed to unleaded gasoline vapors for 24 weeks or for 59-61 weeks after a 2-week exposure to the initiator, N-ethyl-N-hydroxyethylnitrosamine (EHEN) and a 4-week control period (initiation-promotion group) or a 6-week control period. There was a statistically significant linear trend for an increase in the incidence of RCT in the males initiated with EHEN but not in the rats who had not been initiated. These results show that unleaded gasoline has a promoting effect on the incidence of RCT in male, but not female, rats initiated with EHEN.

In an initiation-promotion protocol, l2-day-old female B6C3F_1 mice were administered N-nitrosodiethylamine (DEN) by the intraperitoneal route prior to exposure to gasoline (Standeven et al. 1994). At 5-7 weeks of age, mice from the DEN-initiated and control groups were exposed to 0, 292, or 2,056 ppm of wholly vaporized PS-6 blend unleaded gasoline. Exposure conditions were chosen to simulate those utilized in the previous cancer bioassay reported by MacFarland et al. (1984) (discussed below). Treatment with 2,056 ppm unleaded gasoline (but not 292 ppm) increased the size

and number of altered hepatic foci (primarily basophilic) in DEN-initiated mice. Treatment with gasoline failed to induce altered hepatic foci in the absence of prior DEN treatment. These results show that unleaded gasoline is a liver tumor promotor in female mice. Similar results were obtained in female $B6C3F_1$ mice exposed to 2,039 ppm unleaded gasoline (PS-6 blend) for 13 weeks (Standeven and Goldsworthy 1993).

One chronic-duration animal study investigated the potential carcinogenicity of inhaled gasoline vapors in experimental animals (MacFarland et al. 1984). Groups of 100 Fischer-344 rats and $B6C3F_1$, mice per sex were intermittently exposed to gasoline vapors for 2 years with interim sacrifices at 3, 6, 12, and 18 months. A decrease in the average body weight gain at the highest exposure level (2,056 ppm) provided evidence that the maximum tolerated concentration had been achieved. The test atmosphere consisted of completely volatilized gasoline. A statistically significant concentration-related increased incidence of primary renal neoplasms (adenoma, carcinoma, and sarcoma) was observed in the male rats that died after 18 months or at terminal sacrifice (one tumor was seen in a female rat). A statistically significant increase in the incidence of hepatocellular tumors was observed in the female mice at 18 months and at study termination. Some of these tumors had metastasized to the lungs. A recent reevaluation of the liver tumor data in mice, as well as a reexamination of the slides prepared from hepatic tissue, supported the conclusion that hepatocellular tumors were increased in female mice following treatment with 2,056 ppm unleaded gasoline (Magaw et al. 1993). However, the revised incidence rates for the hepatocellular tumors in mice were approximately 20-25% lower than those reported by MacFarland et al. 1984. The MacFarland study is limited with respect to its relevance to human health risk for the following reasons. First, the animals were exposed to wholly vaporized gasoline, which has the same composition as liquid gasoline and is not the same as the gasoline vapors that humans would be exposed to in ambient conditions. Gasoline emissions normally found in the environment contain lower concentrations of hydrocarbons with very low vapor pressures (i.e., the branched-chain hydrocarbons, such as 2,2,4-trimethyl-pentane, that have been shown to induce nephrotoxicity) than those found in liquid gasoline. Secondly, the relevance of male rat kidney tumors believed to have arisen from $\propto_{2u}$ -globulin accumulation to human cancer risk is questionable (see Section 2.4).

Oral Exposure

2.2.2.1 Death

Accidental or intentional ingestion of large quantities of gasoline can cause death in humans (Camevale et al. 1983). The lethal ingested dose of gasoline has been estimated to be 12 ounces (350 g, or 5 g/kg for a 70-kg individual) (Anonymous 1989). The cause of death following ingestion of gasoline is either severe chemical pneumonitis resulting from the aspiration of gasoline that leads to asphyxiation, central nervous system depression leading to respiratory failure, or cardiac sensitization to circulating catecholamines resulting in the occurrence of fatal arrhythmias (EPA 1987a).

The acute oral LD_{50} in rats for gasoline has been reported to be 14,063 mg/kg (Beck et al. 1983; Vemot et al. 1990). No treatment-related deaths were reported in a 4-week study in rats administered up to 2,000 mg/kg/day by gavage (Halder et al. 1985), but a few treatment-related deaths were seen in another study in which rats were administered 500 mg/kg/day API PS-6 by gavage (Borriston Labs 1985). The basis for this discrepancy between studies is not known. No information is available on death in experimental animals following chronic oral exposure to gasoline.

The highest NOAEL values and all reliable LOAEL and LD,, values for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal, dermal, or ocular effects in humans or animals after oral exposure to gasoline.

The highest NOAEL values and all reliable LOAEL values for systemic effects for 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 Automotive Gasoline - Oral

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical form
	ACUTE EXPOSURE						
	Death						
1	Rat	once				14,063 (LD_{50})	Beck et al. 1983; Vernot et al. 1990
	Sprague-Dawley	(G)					NS
	Systemic						
2	Human	once	Resp			6429 M (pulmonary edema)	Janssen et al. 1988
							NS
			Gastro			6429 M (severe esophagitis and gastritis)	
			Hemato			6429 M (hemolysis; disseminated intravascular coagulation)	
			Hepatic		6429M (serum enzyme changes)		
			Renal			6429 M (tubular necrosis)	
3	Rat F344	2 wk 5d/wk 1x/d (G)	Renal		375M (minimal to slight regenerative tubular epithelial lesions)		Gerin et al. 1988 unleaded
4	Rat F344	9 d 1x/d (GO)	Renal	3 M	30M ($\alpha 2\mu$-globulin accumulation and exfoliation of renal tubular cells)		Olson et al 1987 unleaded

TABLE 2-2. Levels of Significant Exposure to Automotive Gasoline - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ duration/ frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference/ Chemical form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
5	Mouse B6C3F$_1$	3 days (GO)	Hepatic	1800			Standeven and Goldsworthy 1993 unleaded
	Neurological						
6	Human	1 x				6429 M (seizures)	Janssen et al. 1988 NS
	Reproductive						
7	Mouse B6C3F$_1$	3 days (GO)		3000			Standeven et al. 1994b unleaded

TABLE 2-2. Levels of Significant Exposure to Automotive Gasoline - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ duration/ frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference/ Chemical form
	INTERMEDIATE EXPOSURE						
	Death						
8	Rat F344	28 d 1x/d 7d/wk (G)				500 M (4/73 died) 500 F (2/72 died)	Borriston Labs 1985 unleaded
	Systemic						
9	Rat F344	28 d 1x/d 7d/wk (G)	Renal		500M (hyaline droplets; tubular regenerative epithelium; intratubular cast formation)		Borriston Labs 1985 unleaded
			Body wt		2000 (decreased body weight)		
10	Rat F344	4 wk 1x/d 5d/wk (G)	Gastro		2000M (erythema; erosion of gastric mucosa; ulceration of gastric epithelium)		Halder et al. 1985 unleaded
			Renal		500M (hyaline droplet accumulation; tubular regenerative epithelium; intratubular cast formation)		
			Body wt		2000M (18% reduction in body weight gain)		

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

Bd wyt = body weight; d = day(s); F = female(s); (G) = gavage; Gastro = gastrointestinal; (GO) = gavage in oil; Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; M = male(s); LD_{50} = lethal dose 50% kill; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

Figure 2-2. Levels of Significant Exposure to Automotive Gasoline – Oral

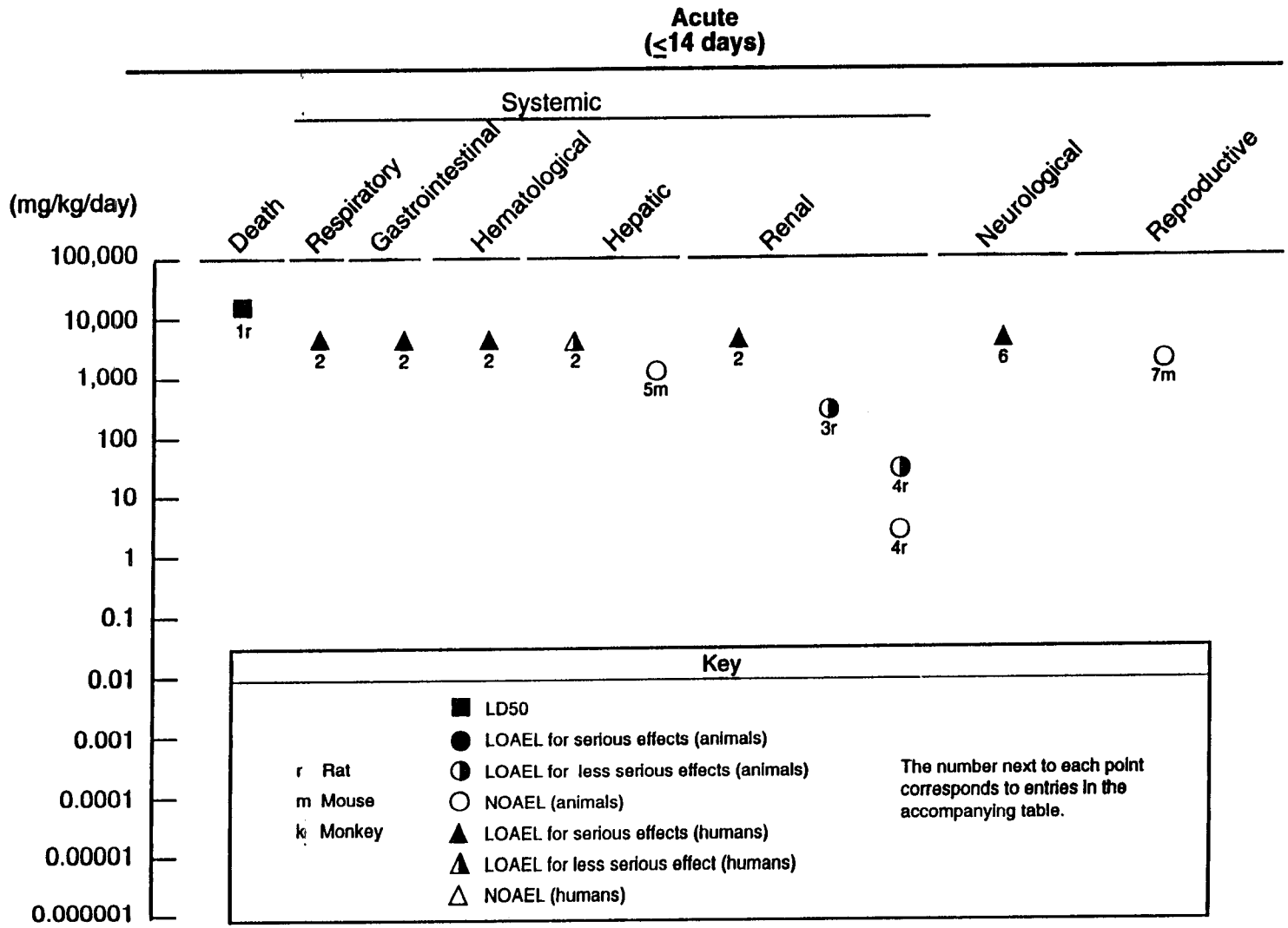
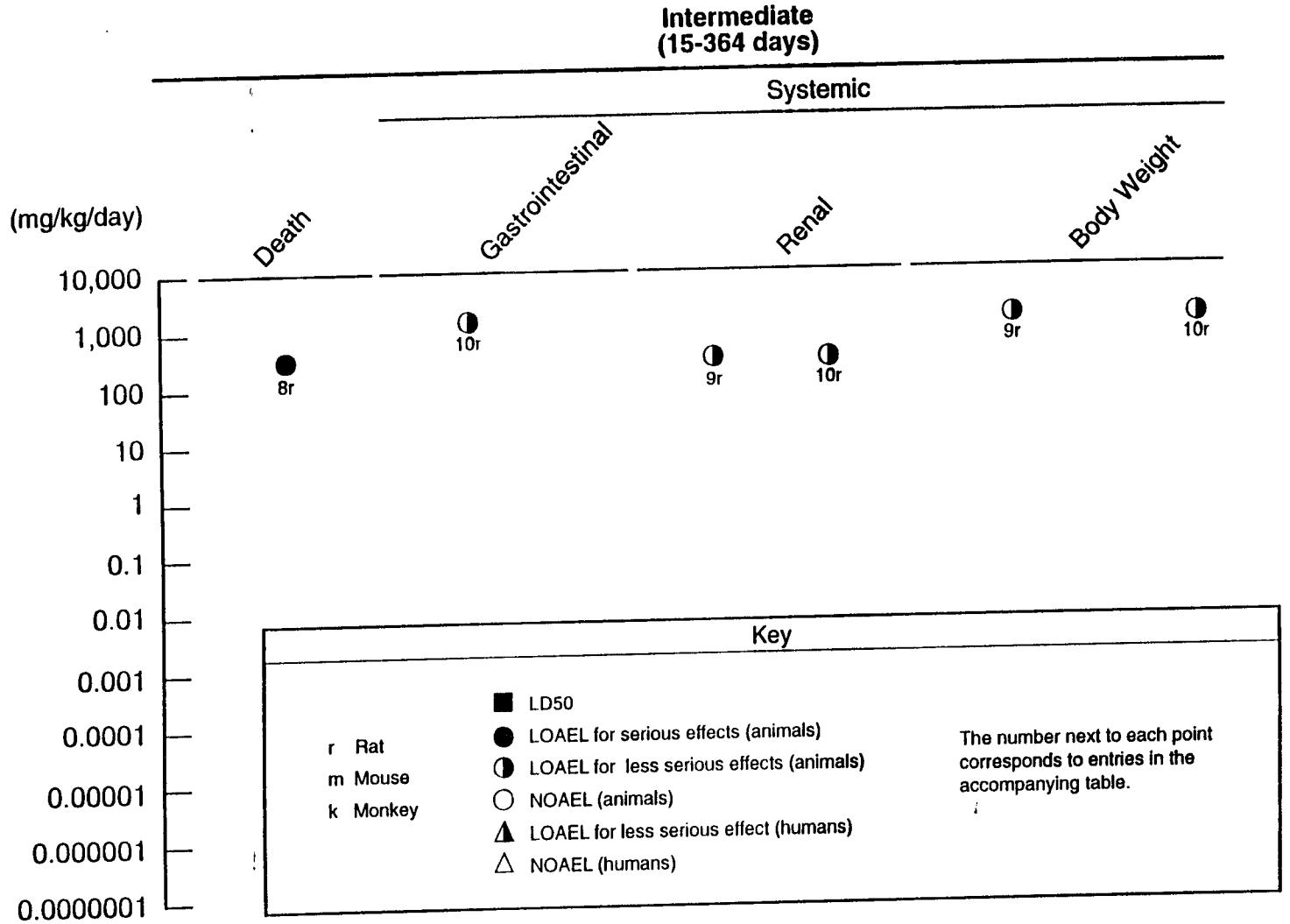


Figure 2-2. Levels of Significant Exposure to Automotive Gasoline – Oral (continued)



Respiratory Effects. Intentional or accidental ingestion of gasoline often results in aspiration of the gasoline into the lungs because of its high volatility and low surface tension. Therefore, the most common effect associated with acute gasoline ingestion in humans is aspiration pneumonia which is often accompanied by respiratory distress, pulmonary edema, emphysema, and focal alveolar hemorrhage (Banner and Walson 1983; Beamon et al. 1976; Carnevale et al. 1983; Grufferman and Walker 1982; Janssen et al. 1988). Death from asphyxia is often the result in cases of gasoline ingestion when the aspiration pneumonia becomes severe.

No studies were located regarding respiratory effects in experimental animals after oral exposure to gasoline.

Cardiovascular Effects. The only study located regarding the cardiovascular effects of ingested gasoline in humans was reported by Banner and Walson (1983). A 15-month-old male ingested approximately 1 pint (≈5,000 mg/kg) of gasoline and was found to be hypotensive upon hospital admission. However, because the child exhibited multi-organ system toxicity (see discussions of Respiratory, Hematological, and Renal Effects in this Section 2.2.2.2) it is not possible to ascertain whether the hypotension was a direct effect of the ingested gasoline or a consequence of other adverse effects.

No studies were located regarding cardiovascular effects in experimental animals after oral exposure to gasoline.

Gastrointestinal Effects. Damage to the digestive tract (severe esophagitis, gastritis, congestive failure, degeneration of the epithelium, and mucositis of the oral cavity) has been observed in individuals who accidentally or intentionally ingested gasoline (Camevale et al. 1983; Hoffman et al. 1980; Janssen et al. 1988).

Rats that were administered 2,000 mg/kg/day unleaded gasoline by gavage for 4 weeks were found to have gastric erythema, erosion of the gastric mucosa, and ulceration of the epithelium (Halder et al.

1985). The combined findings in humans and animals demonstrate the irritating effect of gasoline on mucosal tissue.

Hematological Effects. Hemolysis, as evidenced by a decrease in hematocrit and an increase in free urine hemoglobin, and disseminated intravascular coagulation have been observed in cases of accidental or intentional ingestion of gasoline (Banner and Walson 1983; Janssen et al. 1988). In addition, evidence of intravascular consumption of clotting factors (coagulopathy, hypofibrinogenima, and elevated prothrombin and partial thromboplastin times) was seen in a 15-month-old male who ingested approximately 1 pint (≈5,000 mg/kg) of gasoline (Banner and Walson 1983).

No studies were located regarding the hematological effects of gasoline after oral exposure in animals.

Hepatic Effects. A transient increase in serum enzymes indicative of liver function (y-glutamyl transferase, serum glutamic-oxaloacetic transaminase [SGOT], and serum glutamic pyruvic transaminase [SGPT]) has been noted in individuals who accidentally or intentionally ingested gasoline (Janssen et al. 1988). Mild centrilobular congestion was observed at autopsy in a death following gasoline ingestion (Carnevale et al. 1983). However, all of the visceral organs were congested in this individual, so it is not likely that gasoline had a direct toxic effect on the liver.

Mild hepatic centrilobular hypertrophy and increases in pentoxyresorufin-o-dealkylase activity, which quantitates cytochrome P-450 2B, and in the hepatocyte labeling index were observed in female $B6C3F_1$ mice following the administration of 1,800 mg/kg/day unleaded gasoline by gavage for 3 consecutive days (Standeven and Goldsworthy 1993). However, there was no effect on the activity of serum sorbitol dehydrogenase (SDH), nor was there evidence of hepatic necrosis.

Renal Effects. Renal injury has been reported in a number of case reports of individuals who accidentally or intentionally ingested gasoline (Banner and Walson 1983; Kuehnel and Fisher 1986; Janssen et al. 1988). A 23-year-old male developed oliguria requiring hemodialysis for 3.5 weeks after ingesting approximately 6,400 mg/kg of gasoline (Janssen et al. 1988). A biopsy taken 20 days after ingestion revealed tubular necrosis and interstitial edema. It is not known whether any

preexisting kidney insufficiency was present in this individual prior to the gasoline ingestion. Radiologic tests (intravenous pyelography [IVP] and a computerized tomography [CT]) revealed an altered appearance in the upper poles of both kidneys of a 26-year-old man who accidentally ingested gasoline and experienced flank pain, cramps, nausea, weakness, and red-brown urine (Kuehnel and Fisher 1986). Urinalysis revealed hematuria and reduced creatinine clearance and his serum creatinine was elevated. By the 19th day after ingestion, he was asymptomatic, his urinalysis was normal, his serum creatinine decreased, and his CT scan was normal. The authors concluded that accidental acute ingestion of gasoline resulted in an acute reversible toxicity, particularly to the kidneys. Evidence of renal failure (increased urine specific gravity with elevated protein, glucose, and hemoglobin, oliguria, and elevated blood urea nitrogen [BUN]) was seen 24 hours after a 15-month-old boy ingested gasoline (Banner and Walson 1983). Serum creatinine was elevated by the 3rd post-ingestion day. Diuretic therapy did not resolve the renal failure, and peritoneal dialysis was initiated and continued for 13 days until urine output increased. The authors speculated that the renal failure was either the result of the hypotension or hemoglobinuria observed or a direct effect of gasoline.

Acute and intermediate gasoline ingestion produces the same syndrome of lesions (i.e., hyaline droplet accumulation and the deposition of g,-globulin in the kidney) in male rats as is seen after inhalation exposure (Borriston Labs 1985; Garg et al. 1988, 1989; Gerin et al. 1988; Halder et al. 1985; Murty et al. 1988; Olson et al. 1987a, 1988) (see Section 2.2.1.2, Renal Effects). The composition of the gasoline used in the studies conducted by Murty et al. and Olson et al. was reported to be (on a percent weight basis) 16.4% n-paraffins, 34.3% isoparaffins, 5.4% naphthenes, 10.0% olefins, 26.4% aromatics, and 7.5% not specified. Biochemical parameters of renal tubular function have also been observed to be affected by gasoline administration in male rats. Urinary lactate dehydrogenase (LDH) and β-N-acetyl-D-glucosaminidase (NAG) activities (indicators of tubular function) were significantly increased in male rats administered unleaded gasoline for 2 weeks, whereas BUN values. (an indicator of glomerular function) were not different from controls (Gerin et al. 1988).

As with inhalation exposure, these renal effects were not seen in female rats administered API PS-6 (Borriston Labs 1985). In addition, this syndrome could not be induced in male NCI-Black-Reiter rats administered API PS-6 (Dietrich and Swenberg 1991). The NCI-Black-Reiter rat is an inbred strain of

rats that does not synthesize α_{2u}-globulin, which is believed to be associated with the etiology of hydrocarbon-induced nephropathy (see Section 2.4).

The effects of acute-duration oral gasoline administration on the accumulation of α_{2u}-globulin and associated nephropathy in the proximal convoluted tubules were compared in young (35-month) versus old (26-month) male rats (Murty et al. 1988). α_{2u} Globulin content was assessed by electrophoresis and radioimmunoassay (RIA). Old rats had a markedly reduced α_{2u}-globulin content in their kidneys prior to gasoline treatment as compared to the young rats, and they failed to exhibited an increase in renal α_{2u}-globulin content after administration. The young rats exhibited a 173% increase in renal α_{2u}-globulin content, and their phagolysosomes exhibited altered morphology. These results led the authors to conclude that the failure of gasoline to alter hyaline droplet accumulation and α_{2u}-globulin content in the kidneys of aged rats suggests that only young adult male rats are susceptible to hydrocarbon-induced α_{2u}-globulin-mediated nephropathy. Because α_{2u}-globulin-induced male rat nephropathy may not be relevant to humans (see Section 2.2.1.2, Renal Effects), this end point was not chosen as the basis for an acute or intermediate MRL for gasoline.

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

A significant decrease in body weight gain was exhibited by rats administered unleaded gasoline (API PS-6) by gavage for 28 days as compared to the controls (Borriston Labs 1985).

2.2.2.3 Immunological and Lymphoreticular Effects

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

2.2.2.4 Neurological Effects

There were no quantitative data on the neurological effects of gasoline following oral exposure; however, accounts of neurological effects after short-term exposure, usually by accidental ingestion, were available in case reports. A retrospective study on the hospital records of 24 children who accidentally ingested gasoline indicated central nervous system complications such as convulsions, coma, and lethargy (Beamon et al. 1976). There were no other details provided. A case study described a man who drank approximately 6,000 mg/kg of gasoline and developed seizures after gastric lavage was performed (Janssen et al. 1988). These effects were attributed to hypoxia that is secondary to chemical pneumonitis. The autopsy of a man who accidentally ingested gasoline revealed edema in the brain (Carnevale et al. 1983).

No studies were located regarding neurological effects in animals after oral exposure to gasoline.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to gasoline. Although oral administration of 3,000 mg/kg/day unleaded gasoline to female mice for 3 days resulted in a three-fold increase in estrogen metabolism in isolated hepatocytes, there were no functional antiestrogenic effects as assessed by uterotrophic assays (Standeven et al. 1994b).

2.2.2.6 Developmental Effects

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

2.2.2.7 Genotoxic Effects

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

The oral administration of 500, 750, or 1,000 mg/kg/day unleaded gasoline (API PS-6) to groups of five male Sprague-Dawley rats for 5 consecutive days did not cause an increase in structural chromosome aberrations in bone marrow cells harvested 6 hours following the final treatment (Dooley et al. 1988). In general, animals orally exposed to gasoline did not exhibit unscheduled DNA synthesis (UDS) induction. The ability to induce UDS is a strong indication that a compound is genotoxic. One exception was a UDS assay conducted with male and female $B6C3F_1$ mice. The mice received 2,000 mg/kg unleaded gasoline (API PS-6) by gavage; at 2, 12, and 24 hours post-treatment, harvested hepatocytes were analyzed for UDS (Loury et al. 1986). The researchers observed slight but significant ($p<0.01$) elevations in UDS in both sexes 12 hours after gasoline administration. No evidence of a genotoxic response was observed in the hepatocytes analyzed 2 hours after exposure. However, a marked increase in scheduled DNA synthesis (SDS) was apparent in male mice 24 hours after treatment; no hepatotoxic effects were seen in the females.

Fischer-344 rats were also gavaged with unleaded gasoline, and both liver and kidney cells were analyzed for UDS activity. To assess potential genotoxicity, hepatocytes from male rats were analyzed for UDS activity 2, 12, 24, or 48 hours after treatment with 2,000 mg/kg and 2 or 24 hours after treatment with 100 or 5,000 mg/kg. Significant UDS activity was not observed at any concentration or at any harvest time. In agreement with other in viva rodent UDS assays, SDS was increased 24 hours and 48 hours following treatment with 2,000 mg/kg. UDS activity was not increased in kidney cells harvested from male rats 2 and 24 hours after administration of 2,000 or 5,000 mg/kg unleaded gasoline (API PS-6) (Loury et al. 1987). Similarly, increasing the exposure time of the high dose to 4 days failed to induce genotoxicity. Elevations in SDS activity were observed in both sexes following the administration of 2,000 mg/kg/day or 135 mg/kg/day unleaded gasoline for 4 or 18 days, respectively. Refer to Table 2-4 for a further summary of these results.

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to gasoline.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans following dermal exposure to gasoline.
In an acute-duration study, dermal application of up to 8.0 mL/kg (approximately 6,000 mg/kg) gasoline to rabbits resulted in no deaths (Beck et al. 1983).

2.2.3.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, or musculoskeletal effects in humans or animals after dermal exposure to gasoline.

The highest NOAEL values and all reliable LOAEL values for systemic effects for each species and duration category are recorded in Table 2-3.

Respiratory Effects. The only information located regarding the respiratory effects of gasoline following dermal exposure comes from a case report in which a 34-year-old man suffered from atelectasis, laryngeal edema, and upper airway obstruction following immersion in a pool of unleaded gasoline for approximately 8 hours after an automobile accident (Simpson and Cruse 1981). Exposure by other routes was possible.

No studies were located regarding respiratory effects in animals after dermal exposure to gasoline.

TABLE 2-3. Levels of Significant Exposure to Automotive Gasoline - Dermal

Species/ (strain)	Exposure/ duration/ frequency/	System	NOAEL	LOAEL		Reference/ Chemical form
				Less Serious	Serious	
ACUTE EXPOSURE						
Systemic						Beck et al. 1983
Rabbit New Zealand	12 d 5d/wk 24hr/d	Hemato	8.0 mL/kg			unleaded
		Hepatic		8.0 mL/kg	(pale congested liver)	
		Renal		8.0 mL/kg	(pale congested kidney)	
		Dermal		8.0 ml/kg	(severe dermal irritation)	
Rabbit New Zealand	24 hr once	Dermal		0.5mL	(slight dermal irritant)	Vernot et al. 1990 unleaded
Rabbit New Zealand	once	Ocular	0.1mL			Vernot et al. 1990 unleaded
Immuno/Lymphoret						Vernot et al. 1990
Gn pig Hartley	3 wk 3d/wk 6hr/d		0.5mL M 50% solu-tion			unleaded

d = day(s); Gn pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immuological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified; wk = week(s); x = time(s)

Hematological Effects. No studies were located regarding hematological effects in humans after dermal exposure to gasoline.

No adverse hematological effects were noted in rabbits that had 8.0 mL/kg applied to their shaved skin for 12 days (Beck et al. 1983).

Hepatic Effects. A transient increase in serum enzymes indicative of liver function (creatine phosphokinase [CPK], SGOT, and SGPT) was noted in a 34-year-old male who was immersed in a pool of unleaded gasoline for approximately 8 hours (Simpson and Cruse 1981). Exposure by other routes was possible.

Pale, congested livers were observed in rabbits who had 8.0 mL/kg unleaded gasoline applied to their shaved skin for 12 days (Beck et al. 1983). No other details were provided regarding the extent of the liver toxicity, if any, in this study.

Renal Effects. Renal function tests were performed in two individuals who had been immersed in gasoline for several hours (Hansbrough et al. 1985; Simpson and Cruse 1981). There was no evidence of abnormal renal function in either case.

Pale, congested kidneys were observed in rabbits that had 8.0 mL/kg unleaded gasoline applied to their shaved skin for 12 days (Beck et al. 1983). No details regarding pathological findings were reported.

Dermal Effects. A number of case reports of individuals who were immersed in gasoline for several hours described the occurrence of either partial or full skin-thickness chemical bums in the area of contact with the gasoline (Ainsworth 1960; Hansbrough et al. 1985; Simpson and Cruse 1981).

Studies in experimental animals show that gasoline is irritating to the skin. Application of a single dose of 0.5 mL undiluted gasoline to the skin of rabbits resulted in slight dermal irritation (Vemot et al. 1990), whereas daily application of 8.0 mL/kg of undiluted gasoline to the skin of rabbits for

12 days produced severe dermal irritation (Beck et al. 1983). Gasoline was shown not to be a dermal sensitizer in guinea pigs (Vemot et al. 1990).

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to gasoline.

Gasoline instilled into the eyes of rabbits did not cause ocular irritation (Vemot et al. 1990).

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after dermal exposure to gasoline.

Gasoline was shown not to be a dermal sensitizer in guinea pigs (Vemot et al. 1990). The NOAEL from this study is recorded in Table 2-3.

No studies were located regarding the following effects in humans or animals after dermal exposure to gasoline:

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to gasoline. However, in many of the epidemiological studies discussed in Section 2.2.1.8, exposure probably occurred by the dermal route as well as by inhalation.

2 3 TOXICOKINETICS

There are limited data on the toxicokinetics of gasoline in humans and animals. Information on the toxicokinetics of several components of gasoline is available (see the ATSDR toxicological profiles for benzene, toluene, and xylene [ATSDR 1991, 1989, 1990]); it should be noted, however, that the interaction of these compounds may influence their individual absorption, distribution, metabolism, and elimination characteristics.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Although there are no data on the absorption rate of gasoline, indirect evidence from case reports of gasoline sniffers indicates that it can be absorbed following inhalation exposure. The increases in blood and urinary lead levels, as well as the characteristic neurological signs, are indicators of exposure (Goldings and Stewart 1982; Robinson 1978). Because gasoline is a mixture, the pattern of absorption following inhalation varies for the individual components (NESCAUM 1989). The compounds with higher blood/gas coefficients (e.g., xylene, benzene, toluene) have a higher rate of absorption than the compounds with lower coefficients (e.g., cyclohexane, ethane, ethylene) (NESCAUM 1989).

2.3.1.2 Oral Exposure

There is no quantitative information on the absorption of gasoline following oral exposure in humans and animals. IIowever, the absorption is believed to be relatively complete because ofthe high lipophilicity of the hydrocarbon compounds, the large surface area of the gastrointestinal tract, and the long resident time in the tract (NESCAUM 1989).

2.3.1.3 Dermal Exposure

Although no studies on the dermal absorption of gasoline in humans and animals are available, the dermal absorption of hydrocarbon solvents is known to be low relative to the oral route (NESCAUM 1989). The aromatic hydrocarbons, such as benzene, are expected to have higher skin penetration than the aliphatic hydrocarbons (NESCAUM 1989).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

Autopsies of humans who were apparently exposed to gasoline indicated elevated blood levels of hydrocarbons such as benzene, toluene, pentane, and hexane (Brugnone et al. 1986; Ikebuchi et al. 1986; Matsubara et al. 1988). An initial concentration of 247 μg/mL gasoline was estimated from blood samples of an adult male who was found unconscious in a gasoline-vapor-filled car (Matsumoto et al. 1992). However, the patient was also exposed dermally to gasoline. The estimated half-life of the gasoline was 16.9 hours. The lead concentrations were slightly elevated in the blood of exposed gasoline station workers (Moore et al. 1976). It has been reported that triethyl lead and inorganic lead, metabolites of tetraethyl lead, may accumulate in the brain and produce encephalopathy and slowed nerve conduction (Kaelan et al. 1986; Robinson 1978). However, quantitative data on the lead content in the brain tissue were not presented in these studies.

Benzene, toluene, and xylenes were detected in the blood samples collected from Wistar rats immediately after exposure to 5,000 ppm gasoline vapor for 30 minutes (Kimura et al. 1988).

2.3.2.2 Oral Exposure

There are limited data on the distribution pattern of gasoline in humans and animals. The distribution of gasoline (gasoline concentration measured as the ratio of the concentrations of [2-methylpentane/2,2-dimethylbutane] in sample/[2-methylpentane/2,2-dimethylbutane] in standard) was determined in a male who died following accidental ingestion of gasoline (Camevale et al. 1983).

The liver, gastric wall, and lungs had the highest gasoline concentrations at 663, 324, and 457 ppm, respectively. The brain, bile, and kidney contained 44.2, 59, and 51.5 ppm, respectively, while the concentrations in the blood from the brain, lungs, and heart were 29.4, 132, and 51.5 ppm, respectively. Autopsies of humans who were apparently exposed to gasoline indicated elevated blood levels of hydrocarbons such as benzene, toluene, pentane, and hexane (Brugnone et al. 1986; Ikebuchi et al. 1986; Matsubara et al. 1988).

2.3.2.3 Dermal Exposure

No studies were located regarding the distribution of gasoline in humans or animals after dermal exposure.

2.3.3 Metabolism

The metabolism of gasoline is not known, although it is expected that the interaction of the various components of gasoline may affect the metabolic products that are formed. The interaction of the components of gasoline is likely to influence the metabolizing enzymes such that the elimination rate of a compound may be altered (NESCAUM 1989). The increased metabolism of antipyrine suggested that mixed function oxygenase activity was induced after inhalation of gasoline vapors in humans (43-1,312 mg/m^3) and in rats (5,000 mg/m3) (Dossing et al. 1988; Harman et al. 1981). The composition (expressed as volume percent) of the gasoline used in the Dossing et al. (1988) study was 30-40% aromatic hydrocarbons (including 3-5% benzene).

Organic tetraethyl lead was a component of gasoline (Goldings and Stewart 1982). It is converted in the liver to triethyl lead, a water-soluble metabolite that can accumulate in the brain (Robinson 1978). This compound can be further broken down to inorganic lead.

2.3.4 Excretion

Although there are no specific data on the elimination of gasoline following inhalation, oral, or dermal exposure, the elimination rate of the components of gasoline probably varies because of the

metabolism of the gasoline components by the hepatic enzymes. Metabolites of benzene, toluene, and xylene are known to be excreted primarily in the urine (NESCAUM 1989).

Chelation therapy in individuals exposed to gasoline indicates that inorganic lead is eliminated in the urine of patients exposed to leaded gasoline (Robinson 1978). Urinary phenol, which is commonly used to indicate benzene exposure, was measured in gasoline pump workers (Pandya et al. 1975). There was an elevated amount of phenol (40 mg/L) in these subjects compared to normal values (<20 mg/L).

2.3.5 Mechanisms of Action

Several mechanisms have been proposed to account for the unique syndrome of nephropathy in male rats following exposure to certain hydrocarbons, including unleaded gasoline. Currently, the most likely mechanism is that a metabolite of gasoline or one of its constituents binds to α_{2u}-globulin; the complex is then reabsorbed in the proximal tubule and phagocytized by lysosomes within the tubule cells. Investigators at the Chemical Industry Institute of Toxicology (CRT) have demonstrated that 2,2,4-trimethylpentane (TMP), a component of gasoline, accumulates in the renal cortex, and that 2,4,4-trimethyl-2-pentanol (TMPOH), a metabolite of TMP, binds to α_{2u}-globulin in these cells (Lock et al. 1987; Swenberg et al. 1989). This protein complex is difficult to catabolize; therefore, the TMPOH-α_{2u}-globulin complexes accumulate in the lysosomes. Eventually, the lysosomes burst, and digestive enzymes contained within the lysosomes induce cytotoxicity and cell death, which in turn leads to the accumulation of casts and the hyperplastic events described above (Swenberg et al. 1989).

The antiestrogenic effects of unleaded gasoline have been proposed as a possible underlying mechanism of female mouse liver tumor induction by this chemical (Standeven et al. 1994a). In a study utilizing an initiation-promotion protocol, female mice exposed to 2,056 ppm of PS-6 blend unleaded gasoline vapor for 6 hours/day, 5 days/week for 16 weeks exhibited increases in relative liver weight, in the number of gross hepatic neoplasms, and in the size and volume of altered hepatic foci in N-nitrosodiethylamine-initiated mice. The PS-6 blend of unleaded gasoline was derived from the same lot used in the cancer bioassay conducted by MacFarland et al. 1984. Cotreatment with ethinyl

estradiol (EE2) (added to the diets at a concentration of 1 ppm) potentiated unleaded gasoline-induced liver tumor promotion. Treatment with 2,056 ppm unleaded gasoline also resulted in antiestrogenic effects, including decreased relative uterine weight and partial reversal of EE2-induced body weight loss, anestrus, and vaginal keratinization. Unleaded gasoline at a concentration of 292 ppm was without effect, either in the presence or absence of EE2. The conclusion that unleaded gasoline may promote liver tumors in female mice secondary to antiestrogenicity was based on findings that estrogens normally act to suppress liver tumor promotion in mice, and unleaded gasoline exhibits potential antiestrogenic properties (Standeven et al. 1994a). While available data provide strong support for an antiestrogenic effect of unleaded gasoline with respect to the pharmacological actions of exogenous estrogen, the support is weak for such an effect on endogenous estrogen (Standeven et al. 1994a). In this regard, only the effect of unleaded gasoline on uterine weight is indicative of a potential antiestrogenic effect (Standeven et al. 1994a). Subchronic exposure of mice to unleaded gasoline vapor did not affect serum 17β-estradiol levels, uterine estrogen receptor levels, or reproductive tract histology (Standeven et al. 1994a). Although acute oral administration of unleaded gasoline to female mice resulted in an increase in estrogen metabolism in isolated hepatocytes, there were no functional antiestrogenic changes associated with the enhanced estrogen metabolism (Standeven et al. 1994b). Therefore, it is unclear whether or not the uterine effects observed in chronic toxicity studies of unleaded gasoline are due to a direct antiestrogenic effect of gasoline. Also, the potential link between possible antiestrogenic effects of unleaded gasoline and the development of hepatocellular tumors in female mice is not clear.

In female $B6C3F_1$, mice, unleaded gasoline vapor has been demonstrated to induce the activity of the hepatic microsomal enzyme pentoxyresorufin-o-dealkylase, an enzyme associated with CYP2B, a cytochrome P-450 isoform commonly induced by rodent liver tumor promotors (Standeven and Goldsworthy 1993). The unleaded gasoline associated with the induction of CYP2B was the PS-6 blend; mice were exposed to 2,039 ppm 6 hours/day, 5 days/week for 13 weeks. Exposure to PS-6 unleaded gasoline also increased cytochrome P-450 content and promoted hepatic preneoplastic lesions. Data have also demonstrated that the API 91-1 blend of unleaded gasoline, a blend containing a high content of saturated hydrocarbons, displays promotional effects similar to the PS-6 blend (Standeven and Goldsworthy 1993).

2.4 RELEVANCE TO PUBLIC HEALTH

Humans living in areas surrounding hazardous waste sites may be exposed to gasoline via inhalation of gasoline vapors or ingestion of and dermal contact with contaminated water. For the majority of the general population (i.e., those not living in the vicinity of hazardous waste sites), the major route of exposure to gasoline is inhalation of gasoline vapors during automobile refueling, refueling of gasoline-powered equipment (e.g., lawn mowers), and through the use of untreated surface water or groundwater that has become contaminated with gasoline from spills or leaking underground storage tanks. For occupationally exposed individuals, the predominant route of exposure is the inhalation of gasoline vapors, but the possibility for dermal contact with gasoline also exists. Occupational exposure to gasoline can occur for workers all along the chain from gasoline production to consumer use. Workers involved in onloading and offloading gasoline at docks, bulk storage terminals, and gas stations, delivering fuel to storage terminals and gas stations, and refueling and automotive repair operations at service stations have a large potential for exposure (Runion 1988). Workers involved in the clean-up and maintenance of underground storage tanks and service station pump equipment are also exposed to higher-than-background levels of gasoline and gasoline vapor (Runion 1988). However, the majority of the general population is not likely to have significant exposures to gasoline.

Gasoline is composed of at least 150 hydrocarbons, several of which have toxic effects of their own (e.g., benzene, toluene, xylene, and ethylbenzene). Gasoline has been shown to be irritating at the portal of entry (i.e., the eyes, the lungs after inhalation, or the gastrointestinal mucosa after ingestion). One target of gasoline-induced toxicity appears to be the nervous system in both humans and animals. A whole spectrum of neurological effects can be seen following acute exposure to high levels of gasoline, either by inhalation or ingestion, that increase in severity with increasing dose. The neurotoxicity observed in humans or animals exposed to leaded gasoline may be partially attributed to the organic lead compounds present in the mixture. Inhalation of very high concentrations of gasoline vapors or ingestion of gasoline can be fatal in both humans and animals. Adverse respiratory effects (e.g., chemical pneumonitis, pneumonia, pulmonary hemorrhage, and edema) are sometimes seen in humans after ingestion of large amounts of gasoline. These effects are due to the aspiration of gasoline from the stomach following vomiting. Blood dyscrasias have been noted in humans acutely and chronically exposed to gasoline vapors, but these effects are most likely due to benzene, and the

incidence of these findings has decreased as the benzene content in gasoline has decreased. No such effects have been observed in experimental animals. Gasoline, along with a diverse group of hydrocarbons, has been shown to induce α_{2u} -globulin-mediated nephropathy and renal tumors in male rats. These nephrotoxic and renal carcinogenic effects are believed to be unique to male rats and are most likely not relevant to humans. There is insufficient epidemiological evidence to link exposure to gasoline with cancer in humans. However, inhalation of gasoline vapors has been shown to induce an increased incidence of hepatocellular tumors in female mice. The relevance of the observed hepatocellular tumors in female mice to humans is not known.

Minimal Risk Levels for Automotive Gasoline

Inhalation

No inhalation MRLs were derived because gasoline contains many components which can vary significantly among the many compositions of gasoline. The toxicity of gasoline would depend on the specific composition.

Oral

No oral MRLs were calculated because of the variability in the composition of gasoline. Also, no quantitative information on adverse effects other than α_{2u} -globulin-mediated nephropathy in male rats was available. As discussed in Section 2.2.1.2, Renal Effects, α_{2u}-globulin-mediated nephrotoxicity is not considered an appropriate end point for the derivation of MRLs for gasoline because it is unique to male rats and, thus, not relevant to human risk assessment.

Death. Inhalation of gasoline vapors or ingestion of gasoline can be fatal to both humans and experimental animals. Several case reports of either accidental or intentional inhalation or ingestion of gasoline resulting in death have been published (Ainsworth 1960; Boeckx et al. 1977; Camevale et al. 1983; Poklis 1976; Wang and Irons 1961). Lethal concentrations of gasoline vapors have been reported to range from >5,000 ppm to 20,000 ppm in humans (Ainsworth 1960; Wang and Irons

1961), whereas the lethal ingested dose of gasoline has been estimated to be 12 ounces (350 g, or 5 g/kg for a 70-kg individual) (Anonymous 1989). The cause of death following inhalation of gasoline vapors or ingestion of gasoline has been postulated to be either central nervous system depression (asphyxia) leading to respiratory failure or cardiac sensitization to circulating catecholamines resulting in the occurrence of a fatal arrhythmia (EPA 1987a; Poklis 1976). In addition, ingested gasoline can be aspirated leading to severe chemical pneumonitis (EPA 1987a).

Acute lethal concentrations (LC_{50}s) of airborne gasoline in experimental animals have not been reported. The acute oral LD_{50} for gasoline in rats has been reported to be 18.8 mL/kg, or approximately 14,063 mg/kg (Beck et al. 1983; Vemot et al. 1990). This is higher than the approximate lethal dose estimated for humans cited above (12 ounces, which is equivalent to approximately 5 mL/kg, or 3,740 mg/kg). The lethal dose of gasoline following dermal exposure has not been determined in animals, but it does exceed 8.0 mL/kg, or 6,000 mg/kg, indicating that it is relatively nontoxic by the dermal route (Beck et al. 1983). Under the exposure conditions expected to be present at hazardous waste sites, it is not expected that lethal air or water concentrations of gasoline will be achieved.

Systemic Effects

Respiratory Effects. Intentional or accidental ingestion of gasoline often results in aspiration of the gasoline into the lungs because of its high volatility and low surface tension. Therefore, the most common effect associated with acute gasoline ingestion in humans is aspiration pneumonia which is often accompanied by respiratory distress, pulmonary edema, emphysema, and focal alveolar hemorrhage (Banner and Walson 1983; Beamon et al. 1976; Carnevale et al. 1983; Grufferman and Walker 1982; Janssen et al. 1988). Death from asphyxia is often the result in cases of gasoline. ingestion when the aspiration pneumonia becomes severe. Atelectasis, laryngeal edema, and upper airway obstruction were observed in a 34-year-old man who had been immersed in a pool of gasoline for approximately 8 hours, suggesting that adverse respiratory effects may occur following a combination of dermal, inhalation, and oral exposure to gasoline (Simpson and Cruse 1981). No

information is available on adverse respiratory effects in animals exposed to gasoline by oral administration.

While intermediate-duration inhalation exposure to 1,552 ppm unleaded gasoline vapors has been shown to have no effect on the lungs in rats or monkeys when examined at the light microscopic level (Kuna and Ulrich 1984), a progression of lesions indicative of fibrosing alveolitis was observed in rats exposed to 100 ppm gasoline vapors for 12 weeks (Lykke et al. 1979). In addition, a decrease in surfactant levels was reported in rats similarly exposed (Le Mesurier et al. 1979). This surfactant deficiency is probably involved in the pathogenesis of the fibrosing alveolitis observed at similar exposure levels. The results of these two studies indicate that gasoline-induced pulmonary changes may occur at levels previously thought to have no effect because the tissues were not examined ultrastructurally and/or other sensitive parameters of pulmonary function were not measured.

Gasoline vapors have been shown to be irritating to the lungs of rats, but not in mice that were similarly exposed (MacFarland et al. 1984). A mild multifocal pulmonary inflammatory response was seen in rats exposed to 2,056 ppm gasoline for 2 years, but not in mice, suggesting that rats are more susceptible to the pulmonary irritating effects of gasoline.

Cardiovascular Effects. No direct cardiovascular effects have been reported in humans after exposure to gasoline. One study in animals described ECG changes and disturbances in myocardial enzyme activities and electrolyte levels in rabbits after inhalation exposure to high levels of gasoline (Przybylowski 1971). However, limitations associated with this study preclude its use in assessing the potential cardiovascular risk to humans from exposure to gasoline. No adverse cardiovascular effects have been observed in animals exposed to levels of up to 2,056 ppm unleaded gasoline vapors for up to 2 years. The. air. and water concentrations of gasoline expected to be present at hazardous waste sites are unlikely to cause adverse cardiovascular effects.

Gastrointestinal Effects. Reports of human and animal ingestion of gasoline indicate that gasoline has a direct irritating effect on the gastrointestinal mucosal tissue. Damage to the digestive tract (severe esophagitis, gastritis, congestive failure, degeneration of the gastric epithelium, and mucositis of the

oral cavity) has been observed in individuals who accidentally or intentionally ingested gasoline (Camevale et al. 1983; Hoffman et al. 1980; Janssen et al. 1988). Similarly, rats that were administered unleaded gasoline for 4 weeks by gavage were found to have gastric erythema, erosion of the gastric mucosa, and ulceration of the gastric epithelium (Halder et al. 1985). This appears to be a direct portal-of-entry effect as inhalation exposure to gasoline does not result in any adverse gastrointestinal effects.

Hematological Effects. Several human case studies have been reported that describe the occurrence of hematological effects in individuals with known long-term exposure to gasoline vapors. However, in most of these cases, the hematological effects reported were most likely due to a constituent of gasoline. For example, basophilic stippling, increased erythrocyte protoporphyrin, and increased ALAD activity have been noted in individuals exposed to leaded gasoline vapor (Boeckx et al. 1977; Chessare and Wodarcyk 1988; Young et al. 1977). These effects are also known to occur with exposure to lead (see ATSDR toxicological profile for lead [ATSDR 1991) and so may have been due to the presence of organic lead compounds in the gasoline. An increased incidence of various blood dyscrasias (anemia, hypochromia, thrombocytopenia, and neutropenia) has been observed in Nigerian males with known exposure to gasoline in their occupations as motor mechanics and road-side vendors of heavy motor oil and/or gasoline as compared to controls with no known exposure to gasoline (Niazi et al. 1989). The authors attributed the increased incidence of these disorders to benzene that is present in gasoline (see ATSDR toxicological profile for benzene [ATSDR 1991).

Hemolysis and disseminated intravascular coagulation have been observed in cases of accidental or intentional acute ingestion of gasoline (Banner and Walson 1983; Janssen et al. 1988). The mechanism for these effects is not known, but they resolved within a few days after ingestion.

No adverse hematological effects have been noted in animals after inhalation exposure to up to 2,056 ppm unleaded gasoline vapors for up to 2 years (Kuna and Ulrich 1984; MacFarland et al. 1984). No information is available, however, on the hematological effects of gasoline following oral exposure in animals.

Based on the findings reported above in humans, it appears that adverse hematological effects can occur following short-term, high-level exposure and longer-term, lower-level exposure to gasoline. These effects are most likely the result of exposure to benzene or lead, constituents of gasoline. The effects seen after acute exposure are most likely reversible, whereas the effects seen after long-term exposure may not be reversible.

Musculoskeletul Effects. One case of acute severe myopathy was reported in an l8 year-old male with a history of sniffing leaded gasoline (Kovanen et al. 1983). The mechanism by which gasoline could have induced this myopathy is not known. The authors speculated that the myopathy may have been due to individual susceptibility or that the patient may have had a subclinical, possibly metabolic, myopathy that was exacerbated by gasoline sniffing. No other reports of adverse muscular or skeletal effects in humans or animals following exposure to gasoline were found. The relevance of this one case of myopathy to humans exposed to gasoline at hazardous waste sites is not known.

Hepatic Effects. A transient increase in serum enzymes indicative of liver function (γ-glutamyl transferase, SGOT, SGPT, and CPK) has been noted in individuals who accidentally or intentionally ingested gasoline (Janssen et al. 1988) or who were immersed in gasoline (Simpson and Cruse 1981). While these enzyme changes suggest that acute exposure to gasoline caused liver toxicity, no histopathological evaluations were performed to ascertain the presence and extent of this toxicity. Given that no adverse noncancer hepatic effects have been noted in animals exposed to gasoline for 90 days or 2 years (Kuna and Ulrich 1984; MacFarland et al. 1984; Standeven and Goldsworthy 1993; Standeven et al. 1994a; Tilbury et al. 1993), the relevance of these transient enzyme changes in humans following acute exposures with regard to long-term, low-level exposure near hazardous waste sites is not known.

Renal Effects. Reversible renal injury (oliguria, tubular necrosis, interstitial edema, hematuria, and reduced creatinine clearance) has been reported in a number of case reports of individuals who accidentally or intentionally ingested gasoline (Banner and Walson 1983; Kuehnel and Fisher 1986; Janssen et al. 1988). Unleaded gasoline is one of a diverse group of hydrocarbons that have been shown to induce a unique syndrome of nephropatby in male rats that is associated with the

accumulation of the protein, α_{2u} -globulin, following acute, subchronic, or chronic inhalation and oral exposure (Borriston Labs 1985; Garg et al. 1988, 1989; G&in et al. 1988; Halder et al. 1984, 1985; Kuna and Ulrich 1984; MacFarland et al. 1984; Murty et al. 1988; Olson et al. 1987a, 1988; Short et al. 1987, 1989a, 1989b) (see Section 2.2.1.2, Renal Effects).

The available data indicate that the nephrotoxic syndrome described above that is induced by unleaded gasoline and several other hydrocarbons is unique to male rats and not relevant to humans. The hepatic synthesis of α_{2u} -globulin is under androgenic control, and the protein is found at 100-300 times higher concentrations in male rat urine than in female rat urine (Shapiro and Sachchidananda 1982; Van Doren et al. 1983). There is no evidence that humans produce α_{2u}-globulin. Only trace quantities of proteins within the same protein family as α_{2u}-globulin have been identified in human urine (Olson et al. 1990). α_{2u}-Globulin and hyaline droplet accumulation, and the associated constellation of nephrotoxic effects that are observed in male rats, have not been observed in female rats or mice or monkeys of either sex exposed to unleaded gasoline (Kuna and Ulrich 1984; MacFarland et al. 1984). Estradiol treatment in male rats after the administration of 2 mL/kg of unleaded gasoline by gavage for 3 days reduced the content of α_{2u}-globulin in the renal cortex to 25%, 41%, and 52% on post-exposure days 3, 6, and 9, respectively, as compared to rats administered gasoline but given no hormone treatment (Garge et al. 1988). Furthermore, the removal of hyaline droplets was increased in the rats receiving estradiol treatment as compared to those receiving no hormones. In addition, this syndrome could not be induced in male NCI-Black-Reiter rats administered API PS-6 (Dietrich and Swenberg 1991). The NCI-Black-Reiter rat is an inbred strain of rats that does not synthesize α_{2u}-globulin. In their document entitled, “Alpha-2u-Globulin: Association with Chemically-Induced Renal Toxicity and Neoplasia in the Male Rat” (EPA 1991), EPA’s Risk Assessment Forum concluded that in light of this evidence,

> “If a compound induces α_{2u}-globulin accumulation in hyaline droplets, the associated nephropathy in male rats is not an appropriate end point to determine noncancer (systemic) effects potentially occurring in humans. Likewise, quantitative estimates of noncancer risk (e.g., reference doses and margin-of-exposure determinations) are based on other end points.

Further,

> "...if the sequence of lesions characteristic of the α_{2u}-globulin syndrome are present, the associated nephropathy in the male rat does not contribute to determinations of noncarcinogenic hazard or risk."

Thus, it does not appear that the nephrotoxicity attributable to the α_{2u}-globulin syndrome observed in male rats after exposure to gasoline is relevant to humans exposed to gasoline at hazardous waste sites.

Endocrine Effects. No studies were located regarding endocrine effects in humans after exposure to gasoline. In rats, a decrease in relative adrenal weight was noted following intermediate-duration exposure to gasoline vapors (Kuna and Ulrich 1984). However, the toxicological significance of this change in adrenal weight is unknown since there no accompanying treatment-related histological effects.

Dermal Effects. Gasoline is irritating to the skin of both humans and animals. Partial or full skin thickness bums have been noted in individuals who were immersed in gasoline for several hours (Hansbrough et al. 1985; Simpson and Cruse 1981), and single as well as repeated applications of neat gasoline to the skin of rabbits cause slight to severe irritation (Beck et al. 1983). Gasoline is not a dermal sensitizer in guinea pigs (Vemot et al. 1990).

Ocular Effects. Ocular irritation was noted in human subjects exposed acutely to 200, 500, or 1,000 ppm atomized gasoline vapor (Davis et al. 1960; Drinker et al. 1943).

No ocular irritation was seen following the instillation of undiluted gasoline into the eyes of rabbits (Vemot et al. 1990).

Body Weight Effects. No studies were located regarding body weight effects in humans after exposure to gasoline. Intermediate- and chronic-duration exposures to gasoline vapors have been reported to cause significant decreases in body weight gain in rats and mice (MacFarland et al. 1984).

Immunological and Lymphoreticular Effects. No studies were located regarding immunological and lymphoreticular effects in humans after exposure to gasoline.

No apparent immunological effects (as measured by IgG deposits in the kidney and lungs) were noted in rats or monkeys exposed to gasoline by inhalation for 90 days (Kuna and Ulrich 1984). Gasoline is not a dermal sensitizer in guinea pigs (Vemot et al. 1990).

Exposure to hydrocarbons has been loosely associated with Goodpasture's syndrome in humans. This syndrome is characterized by glomerulonephritis and pulmonary hemorrhage caused by the binding of circulating antibodies to basement membrane antigens on the glomerular and alveolar basement membranes, respectively (O'Regan and Turgeon 1986; Yamamoto and Wilson 1987). To determine whether gasoline exposure causes pulmonary alveolar damage, thereby allowing the passage of antibodies into the alveoli where they bind to lung basement membrane, rats were exposed by inhalation to unleaded gasoline (O'Regan and Turgeon 1986). The animals were then injected with sera containing anti-GBM, sacrificed, and the lungs were removed for light and immunofluorescence microscopy. The anti-GBM failed to bind to alveolar basement membrane in the gasoline-exposed animals as determined by immunofluorescence. Based on these results, it does not appear that exposure to gasoline in this manner damages the alveolar endothelium which would allow passage of anti-GBM and cause the pulmonary hemorrhage associated with Goodpasture's syndrome. However, in another study in which unleaded gasoline was administered to rabbits by intratracheal administration, horse anti-GBM/ABM antibodies were found bound to the ABM in a linear, but focal, fashion (Yamamoto and Wilson 1987). No deposits were found in the lungs of rabbits administered saline instead of gasoline. In addition, there was a significant increase in the uptakes of radiolabeled anti-GBM/ABM in the lungs of animals administered gasoline as compared to the animals administered saline. These results suggest that gasoline does damage the alveolar endothelium allowing the passage of antibodies into the alveoli, in contrast to the findings of O'Regan and Turgeon (1986). The reason for the discrepancy between the studies is not clear. One possible explanation is that intratracheal administration delivered a higher dose of gasoline to the lung than inhalation of gasoline vapors. The relevance of these findings to human exposure to gasoline at hazardous waste sites is not known.

2. HEALTH EFFECTS

Neurological Effects. Acute exposure to gasoline vapors is characterized by a spectrum of effects that progress in severity and can include dizziness, headaches, giddiness, euphoria, vertigo, blurred vision, nausea, numbness, drowsiness, anesthesia, and coma (Poklis and Burkett 1977). Acute ingestion of large amounts of gasoline has also been reported to induce adverse neurological effects such as lethargy, convulsions, and coma (Beamon et al. 1976). Chronic exposure to gasoline (i.e., in individuals who habitually sniff gasoline for its euphoric/hallucinogenic properties) is associated with neurological effects, such as cerebellar effects including postural tremor, ataxia, abnormal gait, affected speech, fatigue, headaches, memory loss, and sleep problems (Carroll and Abel 1973; Coulehan et al. 1983; Goldings and Stewart 1982; Kaelan et al. 1986; Kullman and Hill 1990; Moss and Cooper 1986; Pandya et al. 1975; Rischbieth et al. 1987; Young et al. 1977). Behavioral and intellectual changes (effects on visual memory and perception, psychomotor disturbances, visuomotor learning ability) have been observed in individuals chronically exposed to gasoline (Carroll and Abel 1973; Kumar et al. 1988; Robinson 1978). Many of the neurological changes associated with exposure to leaded gasoline can be attributed to organic and inorganic lead encephalopathy (Robinson 1978; Valpey et al. 1978). However, the exact mechanism by which unleaded gasoline induces neurological effects is not known.

Animals acutely exposed to high levels of gasoline also exhibit neurotoxic effects, including restlessness, equilibrium disturbances, convulsions, and narcosis (Przybylowski 1971). Evidence of neuropathological effects (pigmentation of the neuronal cytoplasm, Wallerian degeneration, axonal degeneration, and axonal dystrophy) was found in rats chronically exposed to unleaded gasoline vapors (API PS-6) (API 1982). However, because of the small number of animals studied and the lack of statistical analyses, these results are of questionable value. Other chronic studies failed to note any exposure-related effects in rats or mice with respect to functional and behavioral indices or the histopathology of the brain, spinal cord, or peripheral nerves.

Based on information obtained in humans and animals, it is reasonable to expect that humans acutely exposed by inhalation to high levels of gasoline vapors or chronically exposed to low levels such as those found near hazardous waste sites will experience adverse neurological effects.

Reproductive Effects. No studies were located regarding reproductive effects in humans after inhalation, oral, or dermal exposure to gasoline.

Only one study was available in animals in which male mice intermittently exposed to either 400 or 1,600 ppm unleaded gasoline vapors for 8 weeks showed no evidence of dominant effect on the sperm cells (Litton Bionetics 1980). The relevance of this finding with regard to adverse effects in humans is not known.

Although no exposure-related histopathological effects were noted in the reproductive organs of rats or mice exposed to 2,056 ppm unleaded gasoline vapors for 2 years (MacFarland et al. 1984), a recent reevaluation of slides from this bioassay revealed a decrease in the severity of uterine cystic endometrial hyperplasia in female mice. In addition, an increase in the incidence and severity of uterine atrophy was observed in aged female mice. It has been postulated that the uterine effects may be due to possible antiestrogenic effects of gasoline (Standeven et al. 1994a). The available data, however, provide only weak support for the antiestrogenic effect of gasoline on endogenous estrogen (Standeven et al. 1994a, 1994b). The available information is inadequate to assess the potential risk for reproductive effects in humans living in the vicinity of hazardous waste sites containing gasoline.

Developmental Effects. Anecdotal data have suggested a link between chronic gasoline vapor exposure of pregnant women and congenital defects of the central nervous system in their children. *In utero* exposure to leaded gasoline was found to cause retarded development and anomalies of head and muscles in two children (Hunter et al. 1979). Based on the limited available data, the study was considered inadequate to establish a relationship between inhalation exposure to gasoline and human developmental toxicity.

In contrast, no developmental effects were found in rats exposed to unleaded gasoline vapors at concentrations as high as 1,600 ppm during gestation (Litton Bionetics 1978). These data are insufficient to assess the developmental toxicity of gasoline in animals. No data were available for other routes of exposure in animals. Therefore, there are insufficient data to assess whether gasoline could induce developmental effects in humans exposed at hazardous waste sites.

Genotoxic Effects. The potential genotoxicity of gasoline in humans was evaluated by measuring micronuclei induction in the peripheral lymphocytes of male gasoline pump mechanics in Sweden (Högstedt et al. 1991). In this study, the investigators compared the effects of gasoline on both B- and T-lymphocytes. The data showed that significant micronuclei induction was observed in the B-cells of the gasoline-exposed group but not in the T-cells. Since gasoline in Sweden may contain as much as 5% benzene (typical American automotive fuel contains 0.5-2.5% benzene), the study results should be interpreted with caution. This inclusion of benzene was also considered by the authors, who suggested that benzene may have been responsible for the genotoxic effects observed in this study (Högstedt et al. 1991). No other human epidemiology or case/control studies were found concerning the genotoxicity of gasoline. From this study it seems that gasoline, or at least components of gasoline (i.e., benzene), may produce some chromosomal damage in human B-lymphocytes. However, because exposure concentrations and exposure durations were not discussed and because Swedish gasoline contains higher concentrations of benzene than American fuel (see Chapter 3), it is difficult to determine the degree of exposure to American gasoline that would be harmful to humans.

In a UDS test, primary hepatocytes were recovered from three human surgery patients and were exposed to unleaded gasoline doses ranging from 0.01% to 0.1%. The results were difficult to interpret because neither the cytotoxic nor the genotoxic response was uniform. A weakly positive effect occurred in the cells from one of the three subjects and was confined to a single dose (0.01% unleaded gasoline). From these observations, the researchers concluded that human hepatocytes may not be particularly sensitive to gasoline genotoxicity (Butterworth et al. 1989). In a second UDS assay involving primary human hepatocytes, a significant increase in UDS was obtained at one dose (0.01%); exposure to higher levels (0.05%) resulted in severe cytotoxicity (Loury et al. 1986). Of additional interest was the marked increase in the percentage of cells in repair (25% in treated cells as compared to 1% in control cells).

Studies exposing whole animals to gasoline in viva were largely negative for such genotoxic effects as chromosome aberrations in somatic cells, dominant lethal mutations in male germinal cells, and DNA damage (see Table 2-4). The oral exposure of Sprague-Dawley rats to unleaded gasoline (API PS-6) did not produce significant chromosome damage (Dooley et al. 1988). The lack of a clastogenic effect

TABLE 2-4. Genotoxicity of Gasoline *In Vivo*

Species (test system)	End Point	Results	Reference
Invertebrate animal cells:			
Drosophila melanogaster (*sc z w*$^+$)	Gene mutation	+[a]	Nylander et al. 1978
Mammalian cells:			
Mouse (germinal cells)	Dominant lethal mutation	+/–	Litton Bionetics 1980
Mouse (primary hepatocytes)	Unscheduled DNA synthesis	+/–	Loury et al. 1986
Rat (primary hepatocytes)	Unscheduled DNA synthesis	–	Loury et al. 1986
Rat (kidney cells)	Unscheduled DNA synthesis	–	Loury et al. 1987
Rat (bone marrow cells)	Chromosome aberrations	–	Dooley et al. 1988
Rat (bone marrow cells)	Chromosome aberrations	–	Conaway et al. 1984
Human (peripheral lymphocytes)	Micronuclei induction	+/–[b]	Högstedt et al. 1991

[a]Leaded gasoline containing approximately 0.01% 1,2-dichloroethane was used.
[b]Positive result only from pokeweek mitogen induced lymphocytes; ≤ 5% benzene was present in the gasoline.

+ = positive result; +/– = inconclusive result; – = negative result; DNA = deoxyribonucleic acid; *sc z w*$^+$ = transposable genetic element

was supported by the negative findings of an additional in viva cytogenetic assay. These results showed that neither the single intraperitoneal injection of 0.03, 0.1, or 0.3 mL per rat nor the intraperitoneal administration of 0.013, 0.04, or 0.13 ml/rat/day unleaded gasoline that contained 2% benzene and 39% aromatics for 5 days induced a clastogenic response in the bone marrow cells of male and female Sprague-Dawley rats (Conaway et al. 1984). No significant decrease in pregnancy or increase in pre- or postimplantation loss were observed in female CD-1 mice impregnated by males that inhaled unleaded gasoline prior to mating (Litton Bionetics 1980). The findings, however, are not conclusive; the sample size of pregnant females was too small to clearly establish that unleaded gasoline did not induce dominant lethal mutations. UDS was not significantly increased in the kidney cells of Fischer-344 rats exposed to unleaded gasoline (API PS-6) by inhalation or gavage (Lout-y et al. 1987), nor was UDS activity increased tn the hepatocytes of male rats exposed orally to unleaded gasoline (API PS-6) (Loury et al. 1986). However, a significant rise in the number of dividing kidney cells (SDS) was observed in male rats exposed to unleaded gasoline via both oral and inhalation routes. The oral route of exposure produced the greater effect. However, cell turnover was not increased in the females (Loury et al. 1987). A weakly positive response for UDS activity was observed in hepatocytes from $B6C3F_1$ mice orally exposed to unleaded gasoline. In agreement with the findings in male rat kidney cells, male mice exhibited a statistically significant increase in percentage of replicating hepatocytes; no S-phase induction was seen in the females (Loury et al. 1986).

The weight of evidence from in viva animal studies suggests that, as a mixture, unleaded gasoline is not genotoxic to rats and probably not strongly genotoxic to mice. It is possible, however, that unleaded gasoline is toxic to organs such as the kidney and liver in male rodents. The relevance of these results to humans is indeterminable at this time.

Leaded gasoline, containing approximately 0.01% 1,2-dichloroethane, was evaluated for mutagenic effects in *Drosophila melunogaster.* Larvae fed 1.0% and 2.5% gasoline showed significantly increased frequencies of somatic mutations (Nylander et al. 1978). Because 1,2-dichloroethane alone also induced a powerful mutagenic response in this study, the authors concluded that the mutagenic activity observed with gasoline was probably due to the 1,2-dichloroethane component. Although 1,2-

dichloroethane is typically included in American unleaded gasoline as a lead scavenger, the concentration was not specified in this study. Therefore, the relevance of this finding to public health is inconclusive.

Gasoline genotoxicity was evaluated using human cells *in vitro* (see Table 2-5). Human TK6 lymphoblastoid cells were examined for mutations at the $TK^{+/-}$ locus following treatment with 0.6% and 1.2% unleaded gasoline; higher concentrations were cytotoxic. No significant increase in incidence of mutations was observed either with or without metabolic activation (Richardson et al. 1986). The test mixture used in this study was API PS-6. Similarly, exposure to the volatile components of unleaded gasoline both with and without metabolic activation failed to induce a mutagenic effect. The same investigators reported that unleaded gasoline (API PS-6) (0.6% and 1.2% with or without activation) did not induce sister chromatid exchange in the human TK6 lymphoblastoid cell line (Richardson et al. 1986).

In vitro rodent studies produced mixed results (see Table 2-5). Mouse lymphoma L5178Y ($TK^{+/-}$) cells were considered negative for gene mutations because no consistent upward trend in the mutation frequency was observed (Conaway et al. 1984). In another study using mouse lymphoma cells, increases in the mutation frequency to at least twice that of the controls were observed at dose levels of API PS-6 that reduced cell growth to approximately 5% or lower (0.060 and 0.070 μL/mL without metabolic activation, 0.150 and 0.175 μL/mL with activation); there was, however, no dose-related effect (Dooley et al. 1988). Without evidence of a dose response, the increased mutation frequencies at severely cytotoxic levels should not be considered indicative of mutagenesis. Mouse hepatocytes were positive for UDS activity at the lowest assayed concentration (0.01% unleaded gasoline [API PS-61]); higher doses, 0.03% and 0.05%, were cytotoxic (Loury et al. 1986). Exposure of rat hepatocytes to unleaded gasoline (API PS-6) produced inconsistent results for UDS. In .one study, concentrations of 0.05% and 0.1% unleaded gasoline induced reproducible and significant dose-dependent UDS responses (Loury et al. 1986). In the second study, rat hepatocytes exposed to concentrations of API PS-6 ranging from 0.0001% to 0.010% (from 0.1 to 10 μL/mL) showed no significant increase in UDS activity at any level (API 1988). It was noted, however, that cell survival at the highest assayed level (88%) was only marginally affected by treatment. It is conceivable that

TABLE 2-5. Genotoxicity of Gasoline *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, TA100)[a]	Gene mutation	–	–	Conaway et al. 1984
S. typhimurium (TA98, TA100)[b]	Gene mutation	–	+/–	Conaway et al. 1984
S. typhimurium (TA1535, TA1537, TA1538)[b]	Gene mutation	–	–	Conaway et al. 1984
Eukaryotic organisms:				
Mammalian cells:				
Mouse L5178Y lymphoma cells ($TK^{+/-}$ locus)	Gene mutation	–	–	Dooley et al. 1988
Mouse L5178Y lymphoma cells ($TK^{+/-}$ locus)	Gene mutation	–	–	Conaway et al. 1984
Mouse (primary hepatocytes	Unscheduled DNA synthesis	+[c]	NA	Loury et al. 1986
Rat (primary hepatocytes)	Unscheduled DNA synthesis	+	NA	Loury et al. 19986
Rat (kidney cells)	Unscheduled DNA synthesis	No data	–	Loury et al. 1987
Rat (primary hepatocytes)	Unscheduled DNA synthesis		NA	API 1988
Human TK6 lymphoblastoid cells ($TK^{+/-}$ locus)	Gene mutation	–	–	Richardson et al. 1986
Human TK lymphoblastoid cells	Sister chromatid exchange	–	–	Richardson et al. 1986

TABLE 2-5. Genotoxicity of Gasoline *In Vitro* (*continued*)

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Human (primary hepatocytes)	Unscheduled DNA synthesis	–[d]	NA	Butterworth et al. 1989
Human (primary hepatocytes)	Unscheduled DNA synthesis	+[c]	NA	Loury et al. 1986

[a]Plate assay
[b]Suspension assay
[c]Lowest of three doses produced significantly positive result; higher doses were cytotoxic or lethal and prevented an accurate evaluation of unscheduled DNA synthesis activity.
[d]Hepatocytes were analyzed from three surgery patients; the cells from two out of three patients were negative for unscheduled DNA synthesis.

– = negative result; +/– = inconclusive result; + = positive result; DNA = deoxyribonucleic acid; NA = not applicable; TK = thymidine kinase

the concentrations evaluated in this second study were below the range of detection of a UDS response. Rat kidney cells were negative for UDS activity following the *in vitro* exposure to 0.005% and 0.010% unleaded gasoline (API PS-6); higher doses, 0.050% and O.100 %, were cytotoxic (Loury et al. 1986).

Ames *Salmonella* tests were negative for gene mutation both with and without metabolic activation (see Table 2-5). No mutagenic effect was seen in the Ames Salmonellulmicrosome plate incorporation assay conducted with unleaded gasoline containing 2% benzene and 39% aromatics at concentrations ranging from 0.001 to 5 μL/plate (Conaway et al. 1984). When the assay was repeated using the preincubation modification of the Ames test with a dose range of 3.75-30 μL/mL, increased mutant colony counts for strains TA98 and TA100 were observed but were confined to the highest nonactivated dose (30 μL/ mL). At this concentration, there was an approximate 50% reduction in cell survival. In the absence of a dose-related effect, increases in mutant colonies at cytotoxic levels cannot be considered conclusive evidence of mutagenesis.

Cancer. A number of epidemiological studies have been conducted on workers occupationally exposed to petroleum and hydrocarbons, including gasoline. These studies all have several inherent limitations that preclude their use as evidence for an association between gasoline exposure and cancer in humans. These include lack of information on levels of exposure to gasoline vapor; concurrent exposure to other potentially carcinogenic substances (i.e., service station attendants are also exposed to motor oils, diesel fuel oils, and solvents as well as automobile and truck engine exhausts); no adjustment for potential confounding factors (e.g., smoking); and no latency analyses. EPA (1987a) reviewed 55 relevant studies of unleaded gasoline-exposed populations and concluded that the evidence for drawing causal inferences between unleaded gasoline and cancer was inadequate. Some of the recent epidemiological data suggest a possible association between gasoline exposure and kidney cancer and leukemia.

An exposure-related increase in the incidence of renal tubular tumors was observed in male rats exposed to unleaded gasoline vapors for 2 years (MacFarland et al. 1984). Female rats and mice of both sexes similarly exposed in this experiment failed to exhibit renal tumors. The renal tubular

tumors observed in the male rats are considered to be the result of a process involving the accumulation of α_{2u}-globulin that ultimately leads to cytotoxicity, tubular epithelium degeneration, hyperplastic regeneration, and tumors (MacFarland et al. 1984). The relevance of α_{2u}-globulin-mediated male rat nephropathy and carcinogenicity to humans has been questioned (see previous subsection on Renal Effects). EPA (1991) has concluded that.

> "Male rat renal tubule tumors arising as a result of a process involving α_{2u}-globulin accumulation do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk."

An exposure-related increase in the incidence of hepatocellular tumors was observed in female mice exposed to unleaded gasoline vapors for 2 years (MacFarland et al. 1984). Some of these tumors metastasized to the lungs.

The MacFarland et al. (1984) study is limited with respect to its relevance to human health risk because the animals were exposed to wholly vaporized gasoline, which has the same composition as liquid gasoline and is not the same as the gasoline vapors that humans would be exposed to in ambient conditions. Gasoline emissions normally found in the environment contain lower concentrations of hydrocarbons with very low vapor pressures (i.e., the branched-chain hydrocarbons, such as 2,2,4-trimethylpentane, that have been shown to induce nephrotoxicity) than those found in liquid gasoline. Thus, the studies in animals using wholly vaporized gasoline may not adequately reflect the carcinogenic risk to humans.

Based on the weight-of-evidence from the animal data discussed above, EPA (1987a) classified gasoline as a Group B2 (probable) carcinogen.[1]

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[1]This classification has not been verified by EPA's Carcinogenicity Risk Assessment Verification Endeavor (CRAVE) Workgroup, is not on IRIS, and is currently undergoing review by EPA.

Benzene, a component of gasoline, is a known human carcinogen that has been shown to cause an increased incidence of hematopoietic cancers (leukemia) in occupationally exposed workers (see toxicological profile for benzene [ATSDR 19911]). However, as discussed above and in Section 2.2.1.8, the evidence for an association between increased incidence of cancer (including leukemia) and exposure to gasoline in humans is inadequate. Furthermore, while there is sufficient evidence that benzene is carcinogenic in rats, causing an increased incidence of tumors at multiple sites including the oral and nasal cavities, Zymbal gland, and the liver, as well as myelogenous neoplasms (see toxicological profile for benzene [ATSDR 1991]), gasoline has only been shown to cause increased incidences of renal cell tumors in male rats (a finding that is not considered relevant to humans) and liver tumors in female mice. Therefore, there is no conclusive evidence to support or refute the carcinogenic potential of gasoline in humans or animals based on the carcinogenicity of one of its components, benzene.

2.5 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 (NASLNRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself 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 biologic 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 gasoline are discussed in Section 2.5.1.

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 often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by gasoline are discussed in Section 2.5.2.

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, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "Populations That Are Unusually Susceptible."

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Gasoline

Increased blood and urine lead levels were at one time commonly used as indicators of exposure to gasoline. However, the phasing out of leaded gasoline and increased use of unleaded gasoline have decreased the effectiveness of monitoring for blood or urine lead as a biomarker for gasoline exposure. It should also be noted that elevated blood lead and urine levels are not specific for exposure to gasoline and could indicate exposure to any of the lead compounds. Elevated blood and.urine lead levels have been observed in several chronic gasoline sniffers (Goldings and Stewart 1982; Robinson 1978). Gasoline vendors who had been exposed to gasoline in the workplace for anywhere from 4 months to 35 years had mean total blood lead levels of 32.9 μg/100 mL, significantly higher than levels found in the control population (14.3 μg/l00 n-L) (Moore et al. 1976).

Increased urinary thioether output has limited use as a biomarker of exposure to gasoline. Many of the hydrocarbons in gasoline are metabolized by the mixed function oxidase system and the metabolites then undergo conjugation with glutathione, followed by urinary excretion as mercapturates (thioethers). Gasoline pump attendants and garage mechanics excreted higher than normal levels of urinary thioether at the end of the day (Stock and Priestly 1986). The differences were greater in attendant-operated outlets than in self service outlets. These results indicate that occupational exposure to gasoline induces increased urinary thioether output. However, increased urinary thioether output is not specific for exposure to gasoline and could indicate exposure to a number of chemicals. It should be noted that this study was complicated by the fact that several of the workers were smokers. Cigarette smoke is known to induce mixed fiurction oxidase activity and thus may alter the output of metabolites that conjugate with glutathione. The influence of cigarette smoking on the urinary excretion of thioethers has been studied in workers employed in suburban petroleum retail outlets. Workers exposed to gasoline at driveway attended stations excreted a significantly greater amount of thioethers in the urine than those at self service outlets (Edwards and Priestly 1993). Urinary thioethers excretion tended to be higher in smokers than in nonsmokers. The difference between the 2 groups was not statistically significant. In addition, postwork urinary thioether concentrations were positively correlated with cigarette smoking.

Biomarkers of benzene exposure such as elevated urinary phenol levels are often used to detect exposure to gasoline. The average level of urinary phenol excreted by gasoline workers was 40 mg/L which is higher than the normal amount (<20 mg/L) (Pandya et al. 1975). It should be noted that the benzene content of the gasoline used in this study was relatively high, 10-17%, as compared to typical American gasoline which contains 0.5-2.5% benzene (see Chapter 3). For more information on the use of urinary phenol as a biomarker of benzene exposure, see the ATSDR toxicological profile for benzene (ATSDR 1991).

The hydrocarbon components of gasoline such as benzene, toluene, pentane, and hexane have been measured in the blood of gasoline-exposed humans (Brugnone et al. 1986; Kimura et al. 1988; Matsubara et al. 1988). Also, benzene, toluene, and xylenes were detected in the blood samples collected from Wistar rats immediately after exposure to 5,000 ppm gasoline vapor for 30 minutes

(Kimura et al. 1988). Increased hydrocarbon blood levels are not specific for gasoline exposure and are not commonly used as biomarkers of exposure.

2.5.2 Biomarkers Used to Characterize Effects Caused by Gasoline

Potential biomarkers for neurological effects of gasoline are slowed motor nerve conduction velocity and indices of cerebellar dysfunction. Some chronically exposed individuals were found to have abnormal slowing of nerve conduction (Gallassi et al. 1980; Goldings and Stewart 1982; Hall et al. 1986; Hansen and Sharp 1978; Rischbieth et al. 1987; Robinson 1978; Seshia et al. 1978). Cerebellar dysfunction (e.g., ataxia, poor coordination, decreased muscle tone, broad-based gait, myoclonic movements) is a typical symptom observed in gasoline sniffers (Carroll and Abel 1973; Coulehan et al. 1983; Goldings and Stewart 1982; Kaelan et al. 1986; Moss and Cooper 1986; Rischbieth et al. 1987; Young et al. 1977). These neurological effects are not specific for gasoline exposure and could indicate exposure to lead or any other neurotoxic substance. General neurological effects such as nausea, vomiting, irritability, restlessness, and anxiety were observed in people exposed to lead and other components of gasoline. These could possibly be used as biomarkers of exposure to high levels of gasoline but, because of their nonspecificity, would only be useful when gasoline exposure is suspected.

2.6 INTERACTIONS WITH OTHER CHEMICALS

Limited data are available on the interactions between the gasoline mixture and other substances. The metabolic interactions of benzene and gasoline vapor have been investigated in male Fischer-344 rats by utilizing a closed chamber gas-uptake exposure system to study uptake and metabolism. It was demonstrated that benzene metabolism in male rats can be decreased in the presence of gasoline vapor (Travis et al. 1992). The gasoline used in the study contained 170 ppm benzene. A physiologically based pharmacokinetic model of benzene metabolism indicated that the inhibitory effect could not be accounted for by the presence of toluene. Some information is available on the interactions between the individual components of gasoline and other substances. For example, benzene toxicity is affected by compounds that alter its metabolism such as alcohol, drugs, and industrial chemicals (Goldstein 1977; NESCAUM 1989). Also, the toxicity of benzene may be enhanced by other myelotoxic agents

such as radiation, metals, halogenated hydrocarbons, and pesticides (Goldstein 1977; NESCAUM 1989). For more information on the interactions of the individual components of gasoline with other substances, see the appropriate ATSDR toxicological profiles on these compounds (ATSDR 1989, 1990, 1991).

The interactions among the components of gasoline should also be considered. The number of potential interactions within gasoline increases exponentially with the number of components (NESCAUM 1989). Since gasoline may contain as many as 1,000 chemical substances, the number of possible interactions is very large. It is not possible, however, to reliably predict the effects of these complex interactions (NESCAUM 1989). There are data indicating that the interactions among the components may influence their characteristic absorption, distribution, metabolism, and elimination patterns. For more information on toxicokinetics of gasoline, see Section 2.3.

The interaction between unleaded gasoline and exogenous estrogen in liver tumor promotion has been demonstrated in female mice (Standeven et al. 1994). Female mice (12 day old) were administered intraperitoneal injections of N-nitrosodiethylamine (DEN) or vehicle. The mice were exposed to wholly vaporized PS-6 blend unleaded gasoline vapor at concentrations of 0, 292, or 2,056 ppm for 6 hours/day, 5 days/week for 16 weeks. Increases in relative liver weight, the number of gross hepatic neoplasms, and the size and volume of altered hepatic foci were observed in DEN-initiated mice treated with 2,056 ppm unleaded gasoline. These effects were not observed in DEN-initiated mice treated with 292 ppm unleaded gasoline. Additionally, groups of mice were exposed to 1 ppm ethinyl estradiol (EE2) in the diet or to 1 ppm EE2 in the diet as well as to 2,056 ppm unleaded gasoline vapor for 16 weeks. Cotreatment with EE2 potentiated the unleaded gasoline-induced liver tumor promotion. Treatment with 2,056 ppm unleaded gasoline also resulted in antiestrogenic effects, including decreased relative uterine weight and partial reversal of EEZ-induced body weight loss, anestrus, and vaginal keratinization.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to gasoline than will most persons exposed to the same level of gasoline in the environment. Reasons for such a response include genetic make-up, developmental stage, health and nutritional status, and chemical exposure history. These parameters may result in decreased function of the detoxification and excretory processes (mainly hepatic and renal) or may affect the pre-existing compromised function of target organs. 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.”

Limited data are available on populations that are unusually susceptible to the toxic effects of gasoline. There are limited data that gasoline may enhance sister chromatid exchange in circulating lymphocytes of cigarette smokers, possibly as a consequence of increased hepatic metabolism of gasoline to reactive metabolites (Edwards and Priestly 1993). Most of the information available on susceptible populations of gasoline toxicity pertains to the benzene component. In general, benzene exposures are likely to present a concern for those whose immune systems are not functioning optimally, such as the very young and the very old. There is some evidence to suggest that females are more susceptible to benzene toxicity (Goldstein 1977). Furthermore, pregnant women, whose hematopoietic systems are naturally under stress, may be particularly susceptible to benzene toxicity (Calabrese 1978). Benzene toxicity may be potentiated in the presence of thalassemia (abnormal hemoglobins) and malnutrition (Aksoy 1989; Calabrese 1978; Goldstein 1977). The hematotoxic effects of benzene may be enhanced by ethanol (Baarson et al. 1982, Nakajima et al. 1985). This is of particular concern for gasolineexposed workers who consume alcohol. (Note that this is not a comprehensive discussion of populations that are susceptible to benzene toxicity. For more information on populations unusually susceptible to the toxic effects of gasoline’s components, see the appropriate toxicological ATSDR profiles [ATSDR 1989, 1990, 1991).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section describes clinical practice and research concerning methods for reducing toxic effects of exposure to gasoline. 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 gasoline. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

Supportive treatment following ingestion or inhalation of gasoline may include the following: assisted ventilation techniques, treatment for pulmonary edema and seizures, administration of oxygen, administration of antiarrhythmics and anticonvulsants, intravenous administration of dextrose in water, dialysis, and hepatic failure treatment (Bronstein and Currance 1988; Ervin and Manske 1990; Goldfrank et al. 1990; Graham 1990). In cases of chronic gasoline inhalation, it may be necessary to screen for electrolyte imbalance and acid-base disturbances and to check serum lead and free erythrocyte protoporphyrin levels (Ellenhom and Barceloux 1988). For serious cases of aspiration secondary to gasoline ingestion, it may be necessary to follow acid-base status, fluid and electrolyte balance, renal and liver function, and serial arterial blood gases (Ellenhom and Barceloux 1988). The use of epinephrine is not suggested because it may precipitate lethal arrhythmias in the sensitized myocardium (Bronstein and Currance 1988; Ervin and Manske 1990; Goldfrank et al. 1990). Antibiotic and steroid treatment are not usually advocated; however, it may be necessary to administer steroids in the case of “shock lung” (Ellenhom and Barceloux 1988; Ervin and Manske 1990; Goldfrank et al. 1990).

Methods used to increase the elimination of gasoline hydrocarbons from the gastrointestinal tract following ingestion include induction of vomiting, lavage, and the administration of cathartics. Much controversy surrounds the use of emetics as a means for gastrointestinal elimination of gasoline. The traditional view is that vomiting should not be induced because of the risk of aspiration and damage to the lung (Bronstein and Currance 1988; Ervin and Manske 1990). According to another point of view, the risk of aspiration during emesis is not very great; therefore, ipecac-induced emesis is recommended

in the alert patient who has ingested large amounts of gasoline (Ellenhorn and Barceloux 1988). In children, it is recommended that nothing be done to remove the substance from the stomach (Goldfrank et al. 1990). Since emesis causes fewer pulmonary complications than lavage, it is the preferred method for gastrointestinal decontamination (Ellenhorn and Barceloux 1988; Goldfrank et al. 1990; Ng et al. 1974). However, for those patients who are too obtunded to ingest syrup of ipecac, lavage may be necessary. Lavage with the use of an endotracheal tube to protect the airway is suggested (Ellenhorn and Barceloux 1988; Ervin and Manske 1990; Goldfrank et al. 1990). Since aspirated oils can cause lipoid pneumonia and increase absorption of gasoline, the use of oils as cathartics should be avoided (Beamon et al. 1976; Goldfrank et al. 1990). The use of saline cathartics or sorbitol has been suggested (Ervin and Manske 1990). Activated charcoal does not effectively adsorb gasoline (Ellenhorn and Barceloux 1988; Goldfrank et al. 1990). In the case of leaded gasoline ingestion, chelation therapy may be used to lower the inorganic lead produced in the body. There is no antidote for triethyl lead intoxication (Garrettson 1990).

Following dermal exposure to gasoline, the skin should be either washed with copious amounts of soapy water or soaked in water for a prolonged period of time (Goldfrank et al. 1990; Stutz and Janusz 1988). In the case of extensive dermal injuries, early debridement might be considered especially if the gasoline contains lead additives (Hansbrough et al. 1985). If the eyes are exposed, they should be thoroughly flushed with water (Goldfrank et al. 1990; Stutz and Janusz 1988).

2.8.2 Reducing Body Burden

There are no known effective methods for reducing the body burden of gasoline. Very little is known about the toxicokinetics of gasoline. However, the elimination rate of components of gasoline, for example, benzene and toluene, probably varies because of the metabolism of the gasoline components by the hepatic enzymes. Thus it is conceivable that alteration of the activities of hepatic enzymes may affect the elimination of gasoline.

2.8.3 Interfering With the Mechanism of Action for Toxic Effects

Although several mechanisms have been proposed for the development of α_{2u}-globulin nephropathy in the male rate following exposure to gasoline, this syndrome is unique to male rats and is not relevant to humans. The antiestrogenic effects of unleaded gasoline have been proposed as a possible underlying mechanism of the induction of hepatic tumors in female mice; however, this effect may be species and sex specific (Standeven et al. 1994a).

2.9 ADEQUACY OF THE DATABASE

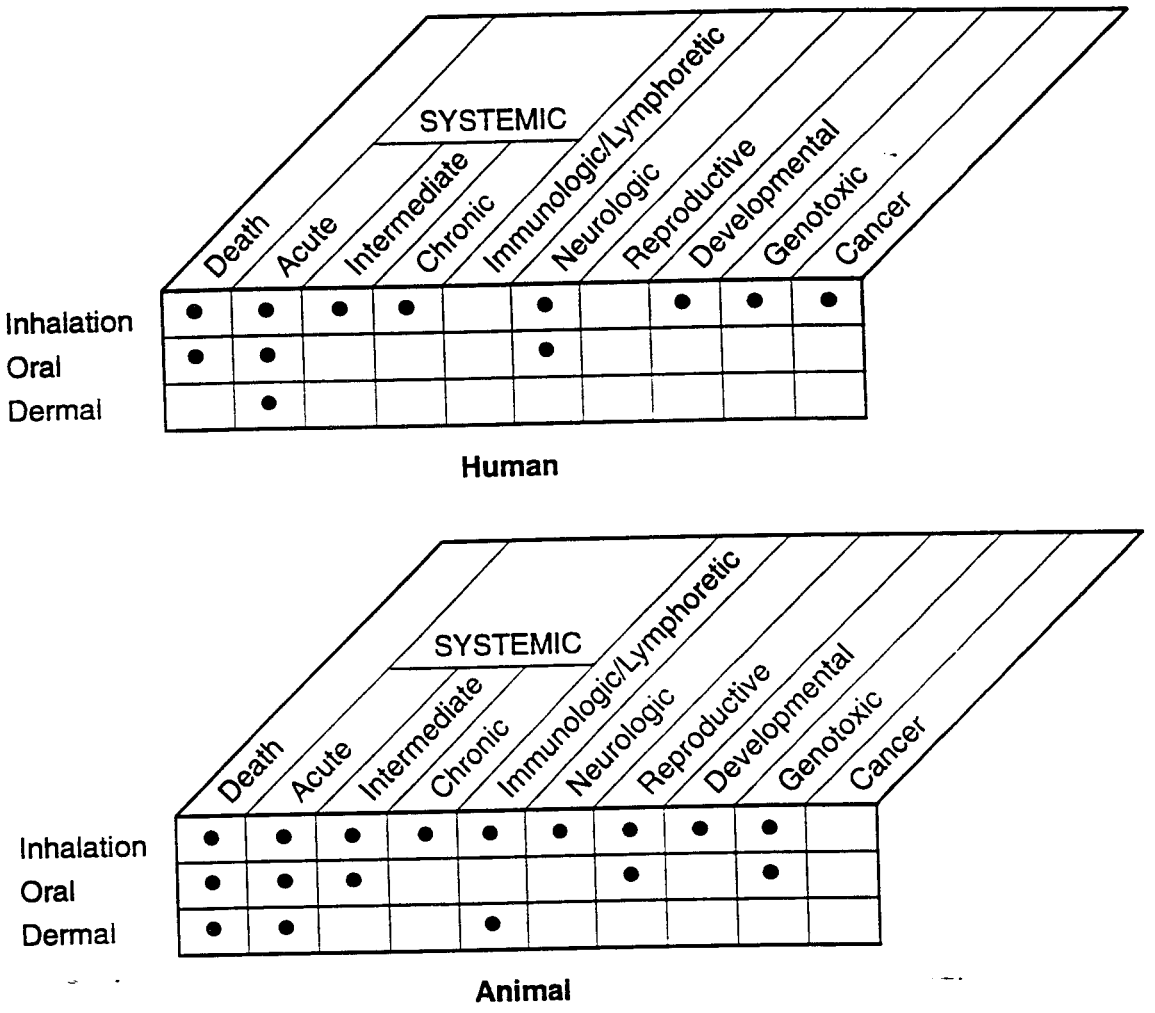
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of gasoline is available. Where adequate information is not available, 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 gasoline.

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 or eliminate 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 may be proposed.

2.9.1 Existing Information on Health Effects of Gasoline

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to gasoline are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of gasoline. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply

FIGURE 2-3. Existing Information on Health Effects of Automotive Gasoline



● Existing Studies

anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information (i.e., data gaps that must necessarily be filled).

Information is available on death and acute, chronic, neurological, developmental, genotoxic, and carcinogenic effects following inhalation exposure to gasoline; death and acute and neurological effects following oral exposure to gasoline; and acute effects following dermal exposure to gasoline in humans. Animal data exist for all end points following inhalation exposure to gasoline; for death and acute, intermediate, and genotoxic effects following oral exposure to gasoline; and death and acute and immunological effects following dermal exposure to gasoline. Therefore, as can be seen in Figure 2-3, the majority of the available information is on the health effects of inhaled gasoline in humans and animals, with very little information on the effects of oral and dermal exposure.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. The central nervous system appears to be a target of gasoline toxicity following acute-duration exposures in both humans and animals. Acute exposure to high levels of gasoline, either by inhalation or ingestion can result in death in both humans and animals (Ainsworth 1960; Beck et al. 1983; Boeckx et al. 1977; Carnevale et al. 1983; Poklis 1976; Vemot et al. 1990; Wang and Irons 1961). Acute inhalation exposure to gasoline is characterized by a spectrum of effects that progress in severity with exposure to increasing levels and can include eye irritation, dizziness, headaches, giddiness, euphoria, vertigo, blurred vision, nausea, numbness, drowsiness, anesthesia, and coma in humans (Poklis and Burkett 1977). Children admitted to the hospital as a result of gasoline ingestion exhibited central nervous system complications such as convulsions, coma, and lethargy (Beamon et al. 1976). Animals acutely exposed by inhalation to high levels of gasoline also exhibit neurotoxic effects, including restlessness, equilibrium disturbances, convulsions, and narcosis, but these effects may have been partially due to oxygen deprivation (Przybylowski 1971). The most common effect associated with acute ingestion of gasoline in humans is aspiration into the lungs resulting in respiratory distress, pulmonary edema, emphysema, pneumonia, and focal alveolar hemorrhage (Banner and Walson 1983; Beamon et al. 1976; Carnevale et al. 1983; Grufferman and Walker 1982; Janssen et al. 1988). Gasoline has been shown to be irritating at the portal of entry (i.e., the lungs after

inhalation or the gastrointestinal mucosa after ingestion) in both humans and animals (Carnvale et al. 1983; Halder et al. 1985; Hoffman et al. 1980; Janssen et al. 1988). α_{2u}-Globulin-mediated nephrotoxicity has been observed in male rats orally administered gasoline (Gerin et al. 1988; Olson et al. 1987a). This lesion is not considered to be relevant to humans, although evidence of reversible renal injury has been reported in a number of cases of individuals who accidentally or intentionally ingested gasoline (Banner and Walson 1983; Janssen et al. 1988; Kuehnel and Fisher 1986). Information on the health effects of dermal exposure to gasoline are lacking, but case reports of individuals who had been immersed in gasoline indicate that toxic effects similar to those seen after inhalation or ingestion of gasoline occur (Ainsworth 1960; Hansbrough et al. 1985; Simpson and Cruse 1981). However, it is possible that these individuals were also exposed to gasoline via the inhalation and oral routes. Acute-duration dermal studies in animals report only slight to severe dermal irritation (Beck et al. 1983; Vernot et al. 1990). There are no toxicokinetic data available by the dermal route of exposure. However, given that the dermal absorption of hydrocarbon solvents is generally low relative to the oral route (NESCAUM 1989), systemic effects resulting from dermal exposure to gasoline are not as likely to occur as they would following oral or inhalation exposure. No acute MRLs have been developed because reliable exposure data are not available for end points of gasoline toxicity other than α_{2u}-globulin-mediated nephrotoxicity and because of the wide variation in the composition of gasoline. More quantitative information on the levels of exposure that elicit various neurological effects in humans and animals following inhalation or oral exposure would be helpful. In addition, there are certain populations that might be exposed to gasoline for brief periods; therefore, this information is important in assessing the risk to these populations.

Intermediate-Duration Exposure. No information is available on the effects of intermediateduration inhalation, oral, or dermal exposure to gasoline in humans. Intermediate-duration inhalation exposure to gasoline vapors has been reported to induce ultrastructural pulmonary changes in rats (fibrosing alveolitis) (Lykke et al. 1979). Ultrastructural pulmonary changes have been observed in rats exposed by inhalation to leaded gasoline (Lykke et al. 1979). These changes occur in conjunction with a reduction in the level of surfactant in the lungs of rats similarly exposed (Le Mesurier et al. 1979). This end point was not chosen as the basis for an inhalation MRL because it is considered a serious effect and because of the variability in the composition of gasoline. However, no changes of

biological significance in pulmonary function were observed in monkeys following exposure to 1,552 ppm gasoline vapor for 90 days (Kuna and Ulrich 1984). α_{2u}-Globulin-mediated nephrotoxicity has been observed in male rats inhaling gasoline vapors or that have been orally administered gasoline (Borriston Labs 1985; Halder et al. 1984, 1985; Kuna and Ulrich 1984; Short et al. 1987, 1989a, 1989b). This lesion is unique to male rats and therefore, not considered to be relevant to humans. There is no information available on the health effects of gasoline following dermal exposure in either humans or animals. Intermediate oral MRLs have not been developed because quantitative information on target organs of gasoline other than male rat nephropathy has not been reported for intermediate inhalation and oral exposure. In addition, gasoline contains many components that can vary significantly among the many compositions of gasoline. The toxicity of gasoline would depend on the specific composition. More quantitative information from animal studies on the effects of intermediate-duration inhalation and oral exposure to gasoline would be useful to identify target organs other than the kidney in male rats, to verify the ultrastructural effects observed in the lungs of rats, and to establish threshold levels for any effects that may be seen following intermediate-duration exposure.

Chronic-Duration Exposure and Cancer. Chronic inhalation exposure to gasoline (i.e., in those individuals who habitually sniff gasoline for its euphoric/hallucinogenic properties) is associated with neurological effects, such as cerebellar effects including postural tremor, ataxia, abnormal gait, affected speech, fatigue, headaches, memory loss, and sleep problems (Carroll and Abel 1973; Coulehan et al. 1983; Goldings and Stewart 1982; Kaelan et al. 1986; Kullman and Hill 1990; Moss and Cooper 1986; Pandya et al. 1975; Rischbieth et al. 1987; Young et al. 1977). Behavioral and intellectual changes (effects on visual memory and perception, psychomotor disturbances, or changes in visuomotor learning ability) have been observed in individuals chronically exposed to gasoline vapors (Carroll and Abel 1973; Kumar et al. 1988; Robinson 1978). However, based on pathological examinations, chronic inhalation of gasoline vapors has not been shown to induce adverse neurological effects in animals (MacFarland et al. 1984). Chronic inhalation of gasoline vapors has been shown to be mildly irritating to the lungs of female rats, but this effect was not observed in mice in the same study and has not been reported in humans (MacFarland et al. 1984). Several human case studies have reported the occurrence of various hematological effects in individuals with known long-term exposure to gasoline vapors (Boeckx et al. 1977; Chessare and Wodarcyk 1988; Niazi et al. 1989; Young et al.

1977). In most of these cases, the hematological effects reported were most likely due to a constituent of gasoline (i.e., lead, benzene). α_{2u}-Globulin-mediated nephrotoxicity has been observed in male rats inhaling gasoline vapors (MacFarland et al. 1984; Short et al. 1989a, 1989b). Again, because this syndrome is unique to male rats, it is not considered to be relevant to humans. No information is available on the effects of chronic oral or dermal exposure to gasoline in either humans or animals. No chronic oral MRLs have been developed because quantitative exposure data are not available for end point of chronic gasoline toxicity other than α_{2u},-globulin-mediated nephrotoxicity and because of the variability in the composition of gasoline. More quantitative information on the levels of exposure that elicit various neurological effects in humans and animals would be helpful, as would information on what the thresholds are for these effects following both inhalation and oral exposure. Information regarding the thresholds for adverse effects are important since certain populations might be exposed to gasoline for chronic durations.

An exposure-related increased incidence of renal tubular tumors was observed in male rats that were exposed to unleaded gasoline vapors for 2 years (MacFarland et al. 1984). The renal tubular tumors observed in the male rats are believed to have been the result of a process involving the accumulation of α_{2u}-globulin. The relevance of α_{2u}-globulin-mediated male rat nephropathy and carcinogenicity to humans has been questioned (EPA 1991). An exposure-related increase in the incidence of hepatocellular tumors was observed in female mice exposed to unleaded gasoline vapors for 2 years (MacFarland et al. 1984). Some of these tumors metastasized to the lungs. The MacFarland et al. (1984) study is limited with respect to its relevance to human health risk because the animals were exposed to wholly vaporized gasoline, which has the same composition as liquid gasoline and is not the same as the gasoline vapors that humans would be exposed to under ambient conditions. Gasoline emissions normally found in the environment contain lower concentrations of those hydrocarbons that have lower vapor pressures (i.e., the branched-chain hydrocarbons, such as 2,2,4-trimethylpentane, that have been shown to induce nephrotoxicity) than is found in liquid gasoline. Thus, the studies in animals using wholly vaporized gasoline may overestimate the carcinogenic risk in humans. No information is available in the literature on the carcinogenicity of gasoline in animals following chronic oral exposure. Additional inhalation bioassays using gasoline vapors of a composition similar to that which is normally found in the ambient environment and also bioassays employing the oral

route of exposure would be helpful to more fully assess the potential carcinogenic risk of gasoline for humans exposed via inhalation or ingestion of contaminated groundwater.

Genotoxicity. Gasoline as a mixture does not appear to be strongly genotoxic to either humans or animals (see Tables 2-4 and 2-5). Although an increased incidence of micronuclei was observed in the B-lymphocytes of workers occupationally exposed to gasoline, it is probable that the lower concentration of benzene in American fuel would reduce this potential hazard (Högstedt et al. 1991). However, cytogenetic studies, specifically in human B-lymphocytes, are needed to determine the validity of this assumption. There is sufficient evidence to conclude that gasoline is not mutagenic in bacteria or mammalian cells in cultures (Conaway et al. 1984; Dooley et al. 1984; Richardson et al. 1986). The relevance of the positive findings with Drosophila melunogaster (Nylander et al. 1978), which appears to be associated with the 1,2-dichloroethane component, cannot be determined without further information on the concentration of 1,2-dichloroethane in American fuel. Well-conducted in viva rodent cytogenetic studies showed that gasoline is not clastogenic in somatic cells (Conaway et al. 1984; Dooley et al. 1988); no conclusions can be reached for germinal cells at this time (Litton Bionetics 1980). The findings from *in vivo* and *in vitro* UDS assays with mouse, rat, and human cells produced conflicting results for genotoxicity (API 1988; Butterworth et al. 1989; Lout-y et al. 1986, 1987). The only consistent finding from the *in vivo* rodent assays was the elevation of SDS in male rat kidney cells (Loury et al. 1987) and male mouse and rat liver cells (Loury et al. 1986,. 1987). Increases in SDS in female rat or mouse liver and/or kidney cells were either modest or negligible. There was, however, no correlation between SDS activity and tumor initiation in rats and mice of both sexes chronically exposed to unleaded gasoline. Overall, the findings from the *in vivo* UDS assays tend to suggest a possible nephrotoxic and/or hepatotoxic role for unleaded gasoline rather than an ability to initiate tumorigenesis (Loury et al. 1986, 1987). Therefore, it is doubtful that the performance of additional UDS/SDS studies would provide additional meaningful information.

Reproductive Toxicity. No information is available on the reproductive effects of gasoline in humans following inhalation, oral, or dermal. Only one study (a dominant lethal study that investigated toxic effects on sperm cells) was found on the reproductive effects of gasoline in animals, and the results were negative (Litton Bionetics 1980). In addition, no adverse effects on the

reproductive organs were reported in chronic-duration inhalation studies in rats or mice (MacFarland et al. 1984). No reproductive organ toxicity data are available in the literature following oral exposure to gasoline. Pathological examination of the reproductive organs in 90-day oral studies would be useful to establish whether gasoline has the potential to induce adverse reproductive effects by this route of exposure, since chronic-duration inhalation studies failed to demonstrate any such effects.

Developmental Toxicity. Anecdotal data are available to suggest an association between chronic exposure of pregnant women to leaded gasoline vapor and congenital defects of the central nervous system in children (Hunter et al. 1979). However, these data are inadequate to assess the risk of developmental toxicity following exposure to gasoline in humans because of the small sample size, possibility of concomitant exposure to alcohol, genetic background, lack of quantification of exposure levels, and presence of lead which may have contributed to the developmental defects in the children. No developmental effects were observed in pregnant rats exposed to gasoline vapors at concentrations as high as 1,600 ppm (Litton Bionetics 1978). No information is available on the developmental toxicity of gasoline following oral or dermal exposure. Additional information on the developmental toxicity of gasoline in rats following maternal inhalation exposure would be useful to confirm the negative results obtained in the Litton Bionetics (1978) study.

Immunotoxicity. No information is available in the current literature on the immunological effects of gasoline following inhalation, oral, or dermal exposure in humans, or following oral exposure in animals. No apparent immunological effects (as measured by IgG deposits in the kidney and lungs) were noted in rats and monkeys exposed to gasoline by inhalation for 90 days (Kuna and Ulrich 1984), and no other effects were noted on lymphoid tissue or blood components in animals exposed to gasoline vapors for intermediate or chronic durations (Kuna and Ulrich 1984; MacFarland et al. 1984). Studies conducted.in rats to investigate the possible contribution of gasoline to the manifestation of pulmonary hemorrhage in Goodpasture's syndrome yielded mixed results (O'Regan and Turgeon 1986; Yamamoto and Wilson 1987). The discrepancy in results between these two studies may be due to differences in route of administration and dose. Since the relationship between gasoline exposure and

Goodpasture's syndrome is unclear and hence the possible involvement of the immune system uncertain, additional information would be useful in resolving the issue.

Neurotoxicity. The central nervous system appears to be a target of gasoline toxicity following acute-duration exposures in both humans and animals. In humans, acute inhalation exposure to gasoline is characterized by a spectrum of effects that progress in severity with increasing dose and duration and can include eye irritation, dizziness, headaches, giddiness, euphoria, vertigo, blurred vision, nausea, numbness, drowsiness, anesthesia, and coma (Poklis and Burkett 1977). Children admitted to the hospital as a result of gasoline ingestion exhibited central nervous system complications such as convulsions, coma, and lethargy (Beamon et al. 1976). Chronic intermittent exposure to high levels of gasoline (i.e., in those individuals who habitually sniff gasoline for its euphoric/hallucinogenic properties) is associated with neurological effects, such as cerebellar effects including postural tremor, ataxia, abnormal gait; affected speech; fatigue; headaches; memory loss; and sleep problems (Carroll and Abel 1973; Coulehan et al. 1983; Goldings and Stewart 1982; Kaelan et al. 1986; Kullman and Hill 1990; Moss and Cooper 1986; Pandya et al. 1975; Rischbieth et al. 1987; Young et al. 1977). Behavioral and intellectual changes (effects on visual memory and perception, psychomotor disturbances, or changes in visuomotor learning ability) have been observed in individuals chronically exposed to gasoline vapors (Carroll and Abel 1973; Kumar et al. 1988; Robinson 1978). Animals acutely exposed by inhalation to high levels of gasoline also exhibit neurotoxic effects, including restlessness, equilibrium disturbances, convulsions, and narcosis, but these effects may have been partially due to oxygen deprivation (Przybylowski 1971). However, chronic inhalation of gasoline vapors has not been shown to induce adverse neurological effects in animals as indicated by pathological examinations (MacFarland et al. 1984). More quantitative information on the levels of exposure that elicit various neurological effects, including changes in learning and behavior, as well as additional studies that examine sensitive neuropathological end points via inhalation and oral routes would be useful.

Epidemiological and Human Dosimetry Studies. A number of epidemiological studies have been conducted on workers occupationally exposed to petroleum products and hydrocarbons (e.g., Kadamani et al. 1989; McLaughlin et al. 1985; Partanen et al. 1991; Poole et al. 1993; Schnatter et al. 1993; Schwartz 1987; Siemiatycki et al. 1987; Wong et al. 1993). These studies all have several inherent limitations that preclude their use as evidence for an association between gasoline exposure and cancer in humans. These limitations include lack of information on levels of exposure to gasoline hydrocarbons; concurrent exposure to other potentially carcinogenic substances (i.e., service station attendants are also exposed to used motor oils, diesel fuel, and solvents as well as automobile and truck engine exhausts); no adjustment for potential confounding factors (e.g., smoking); and no latency analyses. EPA (1987a) reviewed 55 relevant studies of unleaded gasoline exposed populations and concluded that the evidence for drawing causal inferences between unleaded gasoline and cancer was inadequate. Since occupational exposure to gasoline invariably occurs in workers who are also exposed to a number of other toxic and potentially carcinogenic substances, additional epidemiological studies in occupationally exposed populations that identify these variables would be difficult to conduct and are thus not likely to yield any more useful data with respect to adverse health effects specific to gasoline. Exposure to low levels of gasoline is extremely common in the general population, and it would be difficult to conduct meaningful epidemiological studies in any subset of the population. If populations exposed primarily to automotive gasoline can be identified, monitoring gasoline exposure and gathering information regarding neurological, developmental, and cancer effects would be useful for establishing cause/effect relationships.

Biomarkers of Exposure and Effect. Increased blood and urine lead levels, urinary thioether, urinary phenol, and blood benzene, toluene, pentane, and hexane levels can all be used as biomarkers of exposure to short-term exposure to gasoline (Brugnone et al. 1986; Goldings and Stewart 1982; Kimura et al. 1988; Matsubara et al. 1988; Pandya et al. 1975; Robinson 1978; Stock and Priestly 1986). However, none of these biomarkers are specific for gasoline, and there do not appear to be any biomarkers of effect that would be useful for monitoring either intermediate or long-term exposure to gasoline. Development of additional, more sensitive biomarkers that are specific for gasoline exposure would be useful in monitoring populations at risk.

Potential biomarkers of effect for intermediate and long-term gasoline exposure include slowed motor nerve conduction velocity and indices of cerebellar dysfunction (Carroll and Abel 1973; Coulehan et al. 1983; Gallassi et al. 1980; Goldings and Stewart 1982; Hall et al. 1986; Hansen and Sharp 1978; Kaelen et al. 1986; Moss and Cooper 1986; Rischbieth et al. 1987; Robinson 1978; Seshia et al. 1978; Young et al. 1977). However, these effects are not specific for gasoline exposure. Biomarkers of effect for short-term exposure to gasoline include nausea, vomiting, diarrhea, irritability, restlessness, and anxiety. These are also nonspecific for gasoline. Development of additional, more sensitive biomarkers that are specific for gasoline effects would be useful in monitoring populations at high risk. However, there are no adverse health effects known at this time that are specific for gasoline.

Absorption, Distribution, Metabolism, and Excretion. There are no quantitative data available on the rates and extent of absorption, distribution, metabolism, or excretion of gasoline in humans or animals following inhalation, oral, or dermal exposure. Although data are available on these parameters for many of the individual components of gasoline (i.e., benzene, toluene, xylene) that may be used to predict the toxicokinetics of gasoline, it is possible that interactions between these components may influence the toxicokinetics of the mixture as a whole. Quantitative data on the toxicokinetics of gasoline following inhalation, oral, and dermal exposure would be useful to predict the behavior of this mixture in the body.

Comparative Toxicokinetics. There is relatively little quantitative information on the toxicokinetics of gasoline in any species, including humans. However, the toxicity data available on gasoline indicate that, with the exception of α_{2u} -globulin-mediated nephrotoxicity in male rats, the target organs/systems are similar in humans and animals (Beamon et al. 19761; Poklis and Burkett 1977; Przybylowski 1971).

Methods for Reducing Toxic Effects. All of the treatment modalities currently available for use in cases of gasoline ingestion, inhalation, or skin contact are supportive in nature and/or involve hastening the elimination of gasoline from the body. The mechanisms of gasoline toxicity are not known, so there are currently no methods geared towards mitigating the effects of gasoline by

interfering with its mechanism of action. Development of further methods to mitigate the effects of gasoline would rely on characterizing its mechanism of action.

2.9.3 On-going Studies

W.L. Backes at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, is studying the toxicological significance of the metabolism in rats and rabbits of alkylbenzenes (including toluene, xylenes, ethylbenzene, and n-propylbenzene), which are major constituents of gasoline. Specifically, the identification of changes in the metabolic fate of the alkylbenzenes due to their prior administration, the effects of age, sex, and strain differences, the effects of exposure time and exposure to hydrocarbon mixtures, and the effect of hydrocarbon induction of cytochrome P-450 on the association of the alkylbenzenes with the enzyme will be studied. The objective of these studies is to provide information that will aid in the identification of conditions under which individuals might be susceptible to alkylbenzene-induced toxicity.

R.W. Wood at the National Institutes of Health, National Institute on Drug Abuse in Rockville, Maryland, is studying drug self-administration by inhalation of several compounds including alkylbenzenes in the primate. In conjunction with models of learned and unlearned behavior, self administration models will be developed that will provide a method to assess abuse potential, assist in setting workplace exposure limit values to prevent substance abuse and performance impairment, determine whether inhalants maintain “drug-seeking” behavior as strongly as other abused drugs, measure the severity of dependence, withdrawal, and toxicity syndromes, assess irritancy and its consequences, and characterize direct behavioral effects of solvents.