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 carbon disulfide. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure - inhalation, oral, and dermal; and then by health effect - death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods - acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest observed- adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify

these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

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

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1989b), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

2.2.1 Inhalation Exposure

Carbon disulfide can exist in air as vapor. Table 2-l and Figure 2-l summarize the available quantitative information on the health effects that have been observed in humans and animals following inhalation exposure to carbon disulfide. All exposure levels are expressed as parts per million (ppm). In many workplace exposures, exposure could be by inhalation and skin exposure, rather than inhalation exposure alone.

2.2.1.1 Death

Several epidemiology studies have reported increased mortality among workers in viscose rayon plants who were occupationally exposed to carbon disulfide as well as other chemicals (Hernberg et al. 1970, 1973; Tolonen et al. 1975, 1979). Deaths have also been reported in a community in India following an accidental release of large amounts of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon plant (Kamat 1994). However, no definitive or consistent conclusions can be drawn from these studies because of concomitant exposure to other chemicals, uncertainty about exposure concentrations, and the likelihood of multiple routes of exposure.

In a 10-year (1975-1985) epidemiological study of 251 workers exposed to carbon disulfide and 124 controls in two viscose rayon factories in Czechoslovakia, increases in total and cardiovascular mortality were noted in spinners exposed to high levels of carbon disulfide (Balcarova and Halik 1991). Although associated levels of exposure were not quantified for this particular group, the study authors estimate that exposures ranged from less than 9.6 to 48 ppm. However, insufficient data were provided to fully support their conclusions. An approximately 15% increase in deaths resulting from circulatory disease was observed among Dutch viscose rayon workers exposed to carbon disulfide concentrations which were described as "at least 7 ppm, and possibly higher" (Swaen et al. 1994). The increased risk of dying from circulatory disease was greatest 20-30 years after the start of the exposure.

The approximate LC_{50} for a 60-minute exposure in male mice was 220 ppm (Gibson and Roberts 1972). Inhalation exposure of pregnant rats to 642 ppm during gestation produced 33% mortality among dams; exposure to 225 ppm produced 35% mortality among pups (Lehotsky et al. 1985). Four of 22 mice died following inhalation exposure to 800 ppm for 6 hours a day, 5 days a week, for 90 days (Toxigenics 1983c). The reliable LC_{50} values for male mice and the LOAEL value for female rats for the acute-duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal and dermal effects in humans or animals after inhalation exposure to carbon disulfide. Information on respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, ocular, body weight, and other systemic effects after inhalation exposure is presented below. The highest NOAEL and all LOAEL values from each reliable study for these systemic effects in each species and each duration category are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. Following an accident involving a railroad car, 27 individuals were exposed via inhalation to an unspecified concentration of carbon disulfide. Subtle and transient changes in pulmonary function were manifested as reduced vital capacity and decreased partial pressure of arterial oxygen (Spyker et al. 1982). Dyspnea was reported in 77 of the 123 persons following an accidental release of large amounts of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon plant in India (Kamat 1994). Exposure concentrations were not stated.

White male Wistar rats exposed to 803 ppm carbon disulfide for 18 hours showed reduced cardiac and respiratory rates and severe narcosis (Tarkowski and Sobczak 1971). However, this study used only six or seven animals and only one dose was tested.

Cardiovascular Effects. In humans, vascular atherosclerotic changes are a primary effect following long-term exposure to carbon disulfide. This is supported by epidemiological studies that have established a relationship between occupational exposure to carbon disulfide and increased mortality due to coronary heart disease (Hernberg et al. 1970, 1971, 1973; MacMahon and Monson 1988; Tiller et al. 1968; Tolonen et al. 1979) and circulatory disease deaths (Swaen et al. 1994). Milder manifestations such as angina have also been documented (Hernberg et al. 1971; Tolonen et al. 1979).

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (CFY)	8 d 6hr/d Gd 7-15				642 F (33% mortality)	Lehotsky et al. 1985
2	Mouse (Swiss-Webster)	60 min				220 M (LC_{50})	Gibson and Roberts 1972
Systemic							
3	Rat (Wistar)	8 hr	Hepatic		20 F (increase in total lipids)		Freundt et al. 1974a
4	Rat (Wistar)	18 hrs	Resp			803 M (decreased respiratory rate)	Tarkowski and Sobczak 1971
			Cardio			803 M (decreased cardiac rate)	
5	Rabbit	12 d 6hr/d	Hemato	1100			Brieger 1949
Neurological							
6	Rat (Wistar)	1 hr			642 M (significant decrease in brain noardrenaline; increased sensitivity to amphetamine)		Magos et al. 1974
7	Rat (Wistar)	18 hr				803 M (severe narcosis, straightening of hindlimbs)	Tarkowski and Sobczak 1971
8	Rat (Wistar)	12 hr			777.1 F (swollen mitochondria in brain; increased ATP)		Tarkowski et al. 1980.

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
	Developmental						
9	Rabbit (New Zealand White)	13 d 6hr/d Gd 6-18		300 F		600 F (increased post-implantation loss)	PAI 1991
	INTERMEDIATE EXPOSURE						
	Death						
10	Mouse (B6C3F1)	90 d 5d/wk 6hr/d				800 (4/22 died)	Toxigenics 1983c
	Systemic						
11	Rat (NS)	1-6 mo 5d/wk 5-8 hr/d	Cardio	3.2 M		16 M (myocardial edema and microhemorrhages)	Antov et al. 1985
12	Rat (Long- Evans)	11 wk 7d/wk 7hr/d	Bd Wt	400 F	800 F (15% decrease body weight)		Rebert and Becker 1986
13	Rat (Sprague-Dawley)	15 d 6hr/d Gd 6-20	Bd Wt	200 F	400 F (19% decreased in maternal body weight gain)		Saillenfait et al. 1989
14	Rat (Wistar)	1-14 mo 5hr/d	Bd Wt		482 (decreased body weight)		Szendzikowski et al. 1974
15	Rat (Long- Evans)	10 wk 5d/wk 5hr/wk	Bd Wt	600 M			Tepe and Zenick 1984

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
16	Rat (Wistar)	8 mo 6d/wk 5hr/d	Hepatic		74 (increased serum lipids)		Wronska-Nofer 1973
					161 (increased liver cholesterol synthesis)		
			Bd Wt	321		546 (26% decrease in body weight)	
17	Mouse (B6C3F1)	90 d 5d/wk 6hr/d	Hemato	300	800 (decreased RBC count, total hemoglobin, and hematocrit)		Toxigenics 1983c
			Hepatic	800			
			Renal	300		800 (nephropathy)	
			Ocular	800			
			Bd Wt	300	800 (decreased body weight 10-11%)		
	Neurological						
18	Monkey (Macaque)	5-13 wk 5d/wk 6hr/d				256 F (severely reduced visual acuity and contrast sensitivity due to effect on optic nerve; retinal ganglion cell degeneration)	Merigan et al. 1988
19	Rat (Long- Evans)	5 or 12 wk 5d/wk 6h/d			500 M (decrease in auditory startle reflex amplitude)		Clerici and Fechter 1991
20	Rat (Sprague-Dawley males, F344 females)	90 d 6h/d 5d/wk		50		300 (occasional swelling of axons in lumbar spinal cord)	Gottfried et al 1985

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
21	Rat (Wistar)	1-14 mo 5hr/d				482 (axonal swelling and distended mitochondria)	Szendzikowski et al 1974
22	Rat (Wistar)	10 mo 6d/wk 5hr/d				578 M (loss of motor equilibrium, muscular weakness, hindlimb paresis)	Tarkowski and Sobczak 1971
23	Rat (Wistar)	10 mo 5hr/d 5d/wk			257 F (uncoupling of oxidative phosphorylation)		Tarkowski et al. 1980
24	Rat (Fischer- 344)	6 wk 5d/wk 4hr/d			642.2 M (decreased hindlimb extensor responses and motor coordination)		Tilson et al. 1979
25	Rat	8 mo 6d/wk 5hr/d		321		546 (paralysis of hindlimbs)	Wronska-Nofer 1973
26	Mouse	90 d 5d/wk 6hr/d		300		800 (degeneration of peripheral nerves)	Toxigenics 1983c
	Reproductive						
27	Rat (Long- Evans)	10 wk 5d/wk 6 hr/d		350 M		600 M (reduced plasma testosterone; slightly lower sperm counts)	Tepe and Zenick 1984
	Developmental						
28	Rat (Wistar, Sprague-Dawley)	19 d 6-7hr/d Gd 1-19		40 F			Hardin et al. 1981

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
29	Rat (Sprague-Dawley)	Pregestation 3 wk 5d/wk 7hr/d, Gd 6-18 or 0-18		40 F			NIOSH 1980
30	Rat (Sprague-Dawley)	15 d 6hr/d Gd 6-20		200	400 (reduced fetal weight)		Saillenfait et al. 1989
31	Rabbit (New Zealand White)	24 d 6-7hr/d Gd 1-24		40 F			Hardin et al 1981
32	Rabbit (New Zealand White)	Pregestation 3 wk 5d/wk Gd 0-21 or Gd 7-21 7 hr/d		40 F			NIOSH 1980
CHRONIC EXPOSURE							
Systemic							
33	Human	3-12 yr	Cardio	9.6			Cirla and Graziano 1981
			Hemato	9.6			
Neurological							
34	Human	3-12 yr occup		9.6			Cirla and Graziano 1981

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
35	Human	12.1 yr occup			7.6[b] M (decreased peroneal nerve MCV and sural nerve SVC)		Johnson et al. 1983

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

[b]Used to derive a chronic inhalation Minimal Risk Level (MRL) of 0.3 ppm for carbon disulfide; the LOAEL of 7.6 ppm was divided by an uncertainty factor of 30 (10 for human variability and 3 for use of a minimal LOAEL).

ATP.= adenosine triphosphate; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); F = female; Gd = gestational day; Hemato = hematological; hr = hour(s); LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M= male; MCV = motor nerve conduction velocity; min = minute(s); mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; RBC = red blood cell; Resp = respiratory; SVC = sensory conduction velocity; wk = week(s); yr = year(s)

Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation

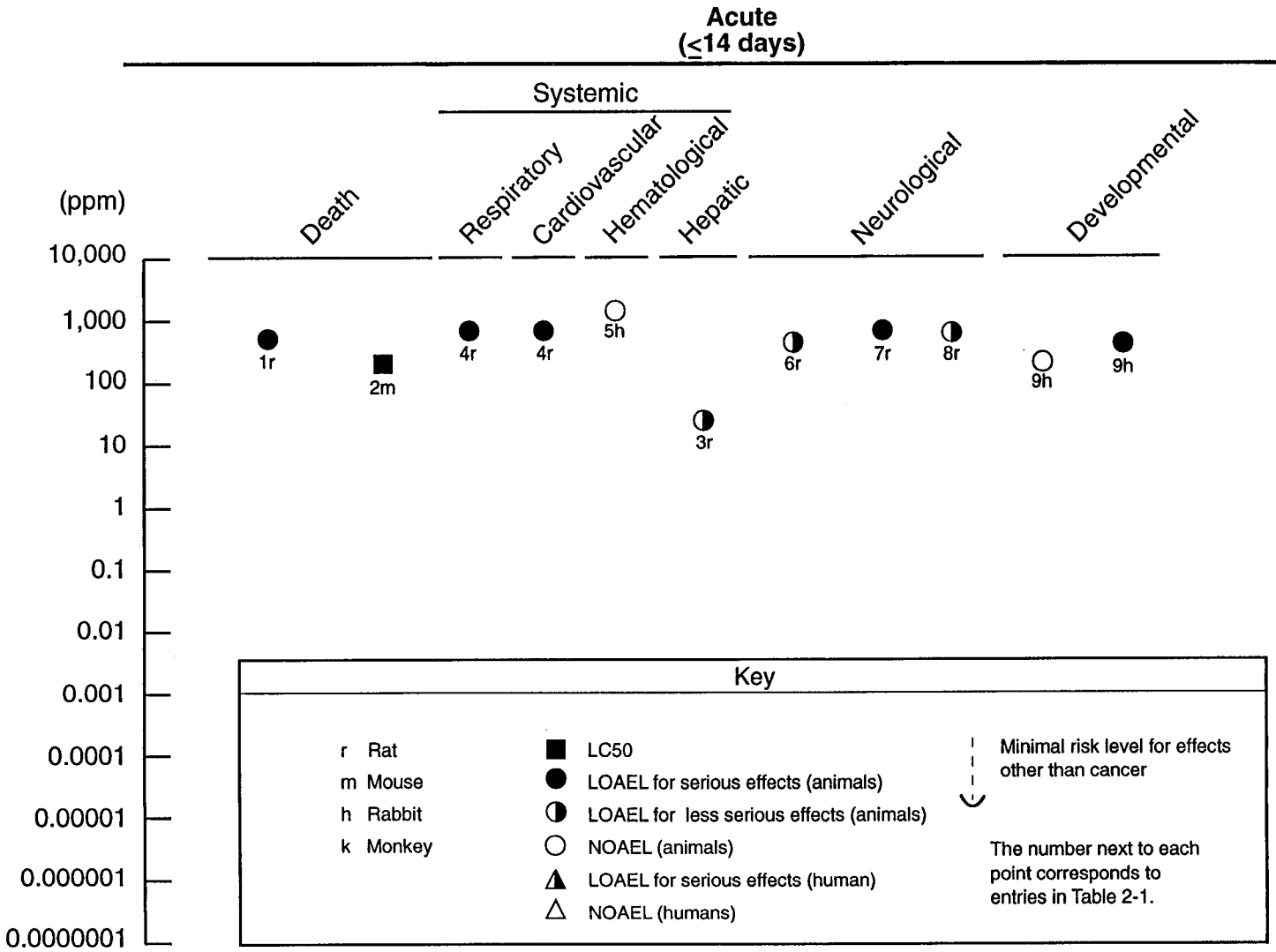


Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation (continued)

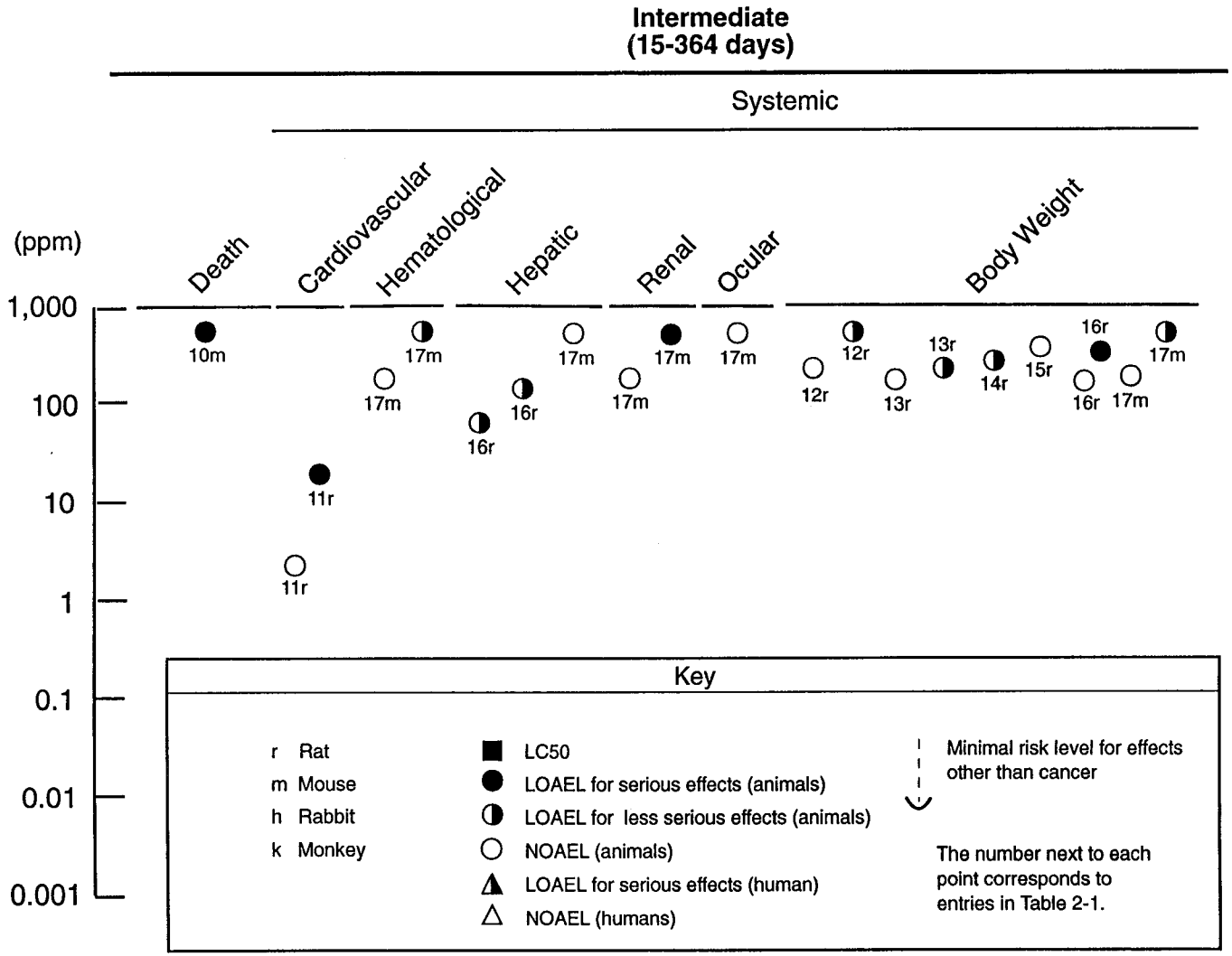


Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation (continued)

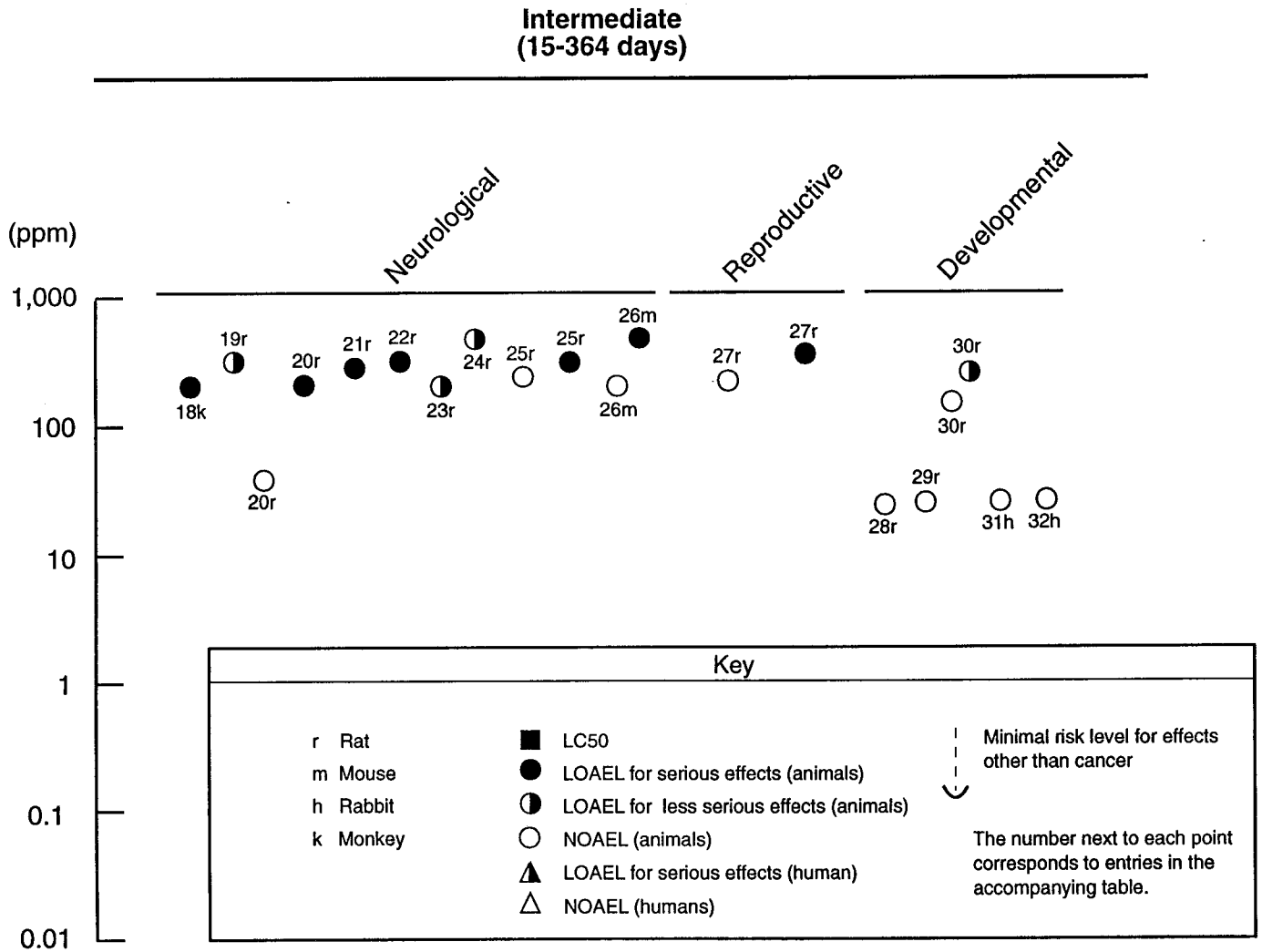
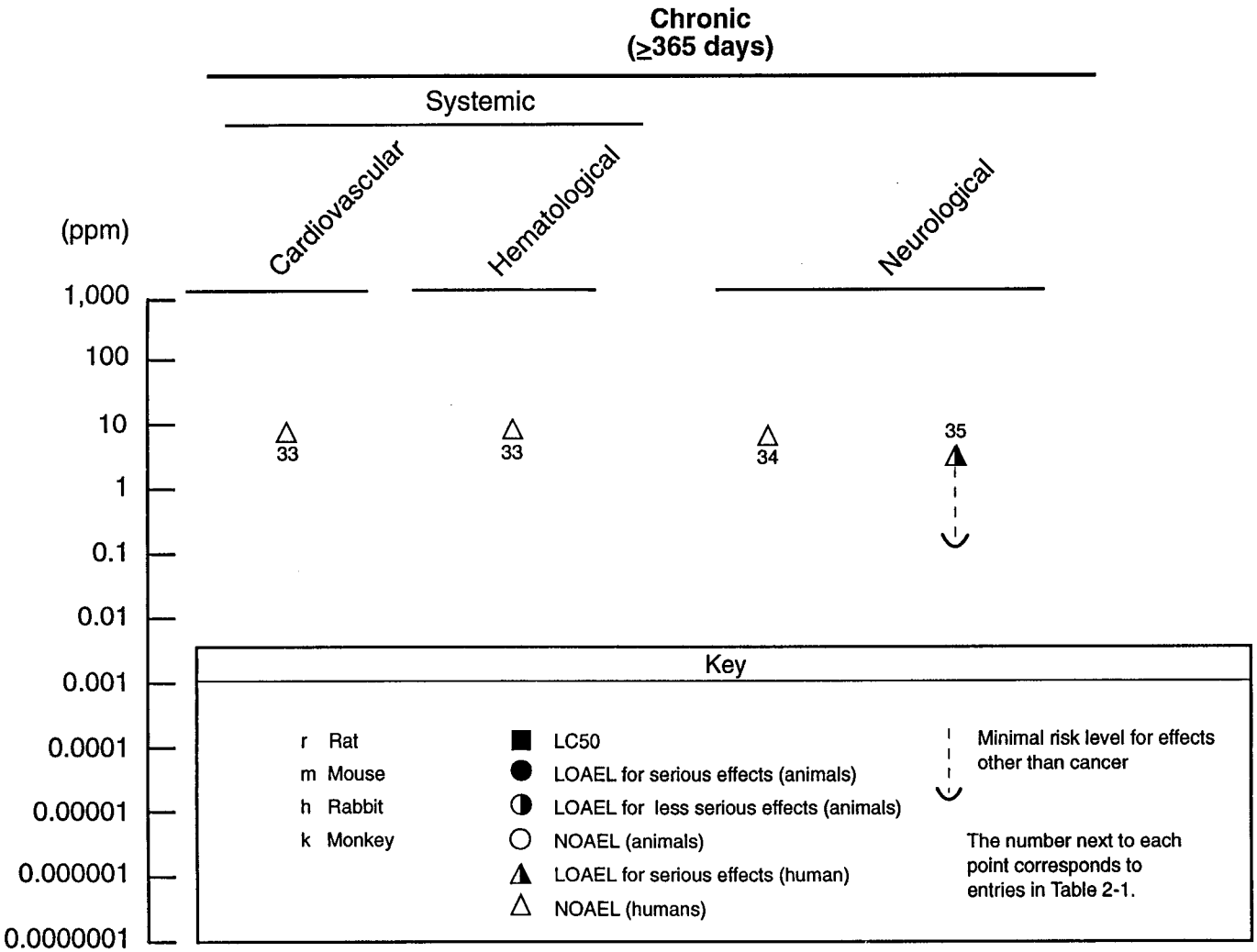


Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation (continued)



However, since reliable data on exposure levels were not available, it is impossible to establish a dose-response relationship or a NOAEL. In addition, coronary heart disease has a multicausal origin that is in part related to the saturated fat intake of the population and is also influenced by a large number of other risk factors such as smoking, other dietary habits, diabetes, and physical inactivity. A combination of two or more risk factors greatly increases the incidence of coronary heart disease, and therefore carbon disulfide may be a cofactor in the presence of other risk factors (WHO 1979). Another limitation with occupational studies reported from the viscose rayon industry is concurrent exposure to other chemicals such as hydrogen sulfide (Hernberg et al. 1970; Rubin and Arieff 1945; Swaen et al. 1994; Tolonen et al. 1979).

A retrospective mortality study revealed that 223 viscose rayon process workers employed for more than 10 years and exposed to carbon disulfide at concentrations in excess of 20 ppm had a statistically significant increase (2.5-fold) in deaths due to coronary heart disease from 1933 to 1962, compared to 174 nonprocess workers from the same factory (Tiller et al. 1968). Over the 30-year study period, 42% of all deaths in rayon process workers were attributed to coronary heart disease; the proportion was 24% for other rayon workers and 17% for other local males used as controls. The excess mortality was more pronounced in the 1940s and declined towards 1960, indicating a strong dependence on the intensity of exposure, which had decreased during this interval. The same study demonstrated that the death rate from coronary heart disease was proportionally higher among workers engaged in the viscose spinning process than in other workers. However, nonexposed workers also had a significantly higher death rate than expected for coronary heart disease, as did controls not employed in the viscose industry. These factors limit the value of this study. Other limitations include an inappropriately selected control group, failure to control for other coronary heart disease risk factors such as smoking, dietary habits, physical inactivity, and obesity, and failure to monitor blood pressure and blood lipid levels. In addition, there may have been concomitant exposures to other chemicals in these industrial environments.

A prospective mortality study at a Finnish plant during the period of 1967-1977 revealed a similar excess of deaths (2.5-fold) due to coronary heart disease (Tolonen et al. 1979). Two cohorts were followed over a 10-year period, 1967-1977; 343 viscose rayon workers exposed to carbon disulfide were individually matched with workers from the local paper mill. There was no significant difference between the exposed and control groups with regard to smoking habits, physical activity, obesity, or drug treatment. Carbon disulfide concentrations in workplace air were 10-30 ppm during the 1960s

20-60 ppm during the 1950s and higher in earlier time periods. The incidence of mortality due to coronary heart disease was 29/343 in the exposed group versus 11/343 in the control group. Periodic health surveys during the study revealed an increased incidence of angina and increased blood pressure compared to a well-matched control group (Tolonen et al. 1979). The incidence of deaths from coronary heart disease appeared to be much greater during the first 5 years as reported in interim results of the same cohorts, but the numbers were too small to draw any conclusions (Hernberg et al. 1970, 1973).

Increased mortality from cardiovascular causes was noted in a 1975-1985 epidemiological study of 251 workers exposed to carbon disulfide compared to 124 nonexposed workers in two viscose rayon factories in Czechoslovakia (Balcarova and Halik 1991). The workers (spinners) were exposed to "high" levels of carbon disulfide with estimated concentrations ranging from less than 9.6 to 48 ppm. An increased incidence of myocardial infarction was also noted in the highly exposed group compared to controls. However, this study should be interpreted with caution since scanty data were provided regarding methods employed.

In a study conducted on Egyptian workers employed in a viscose rayon factory, Kamal et al. (1991) found a significantly higher prevalence of pathological changes revealed by electrocardiogram (ECG). The study was conducted on 253 workers exposed to 20-45 ppm of carbon disulfide for 4-29 years; the control group consisted of 99 workers. No association was found between the duration of exposure and ECG activity; this finding indicates that the duration of exposure to carbon disulfide may not be a major risk factor unless there are other predisposing factors. The study was limited, however, because the exposure concentrations documented in factory records may not have been representative of the actual exposures.

Among men who had been exposed to carbon disulfide for 5 or more years between 1942 and 1967, the incidence of angina was 25% compared to 13% in unexposed controls, and a significant increase in blood pressure was seen (Hernberg et al. 1971, 1976; Tolonen et al. 1975). Nonfatal first cardiac infarctions were more frequent in the exposed group (11) than in the control group (4). The relative risk of a fatal myocardial infarction was 4.8 times greater among those exposed to carbon disulfide; 16 of 343 men died of coronary heart disease within the 5-year period compared to 3 of 343 men in the control group ($p<0.007$) (Hernberg et al. 1973; Tolonen et al. 1975). In a subsequent study, the original relative risk estimates were adjusted for potential confounding effects of hypertension and

aging. After these adjustments, carbon disulfide exposure yielded a relative risk of 2.3 for coronary disease mortality (Nurminen et al. 1982). Thus, although the prognosis for exposed workers was better with improved occupational hygiene and with a reduction in the length of exposure over a lifetime, there was apparently some increased risk attributable to carbon disulfide exposure in this cohort. However, there were no adjustments for possible concomitant exposures to other chemicals.

A follow-up study of 343 Finnish viscose rayon workers was performed to examine the incidence of cardiovascular mortality from 1967 to 1982 (Nurminen and Hernberg 1985). Exposure to carbon disulfide varied greatly (approximately 22 ppm to <l0 ppm), with a decrease in exposures after 1972. Within the first 5 years of follow-up (1967-1972), there was a 4.7-fold increase in ischemic and heart disease mortality compared with a cohort of paper mill workers. In the period of 1972-1974, the relative risk ratio was 3.2. After all workers with high coronary risk factors were removed from exposure (19% of the cohort was exposed in 1977 compared to 53% in 1972), the risk of cardiovascular death was reduced to a ratio of 1.0 in the years 1974-1982. This study indicates that the cardiotoxic effects of carbon disulfide may be reversible with removal of individuals from the toxic environment. Caution must be used in interpreting these data because of the increase in the incidence of cardiovascular events in the aging cohort population and the possibility that carbon disulfide accelerates death in high-risk individuals.

In a study of Japanese viscose rayon workers, no effects were noted on blood pressure or on the incidence of angina. The exposed group comprised 420 rayon filament workers; 390 controls were obtained from a local cuprammonium rayon factory (Sugimoto et al. 1978). Mean carbon disulfide air levels were below 20 ppm at the time of the study (about 1975); these levels had been higher (15-30 ppm) during the 1950s. These observations suggest that ethnic variation or other demographic factors (e.g., dietary habits) may affect the response to carbon disulfide. Cardiovascular effects on 50 viscose rayon workers occupationally exposed to an average range of 3.2-9.6 ppm carbon disulfide for 3-12 years were compared to a pair-matched control group from unexposed departments of the plant (Cirla and Graziano 1981). On ophthalmoscopy, two control and two exposed workers suffered minor vascular changes. Both systolic and diastolic mean blood pressures were higher in the control group. Differences were not statistically significant ($p \geq 0.10$). The use of a questionnaire, confirmed by electrocardiography, determined one case of arrhythmia and one case of coronary heart disease in the exposed group, with no cases reported for the control group. The one case of coronary heart disease suggests there is some cardiac effect of carbon disulfide, but no general conclusions can be reached by

this study. The results further suggest that occupational exposure below 9.6 ppm (maximum exposure concentration) for up to 12 years does not cause recognizable health damage. This interpretation must be viewed with caution because the maximum exposure duration to carbon disulfide was only 12 years, with most workers between 6 and 9 years at risk. In addition, workers were exposed to mean average concentrations of 3.2-8 ppm carbon disulfide. If carbon disulfide exerts effects through an arteriosclerotic process, this duration of exposure may not have been adequate to observe cardiovascular effects. Also, the study does not include the turnover rate due to death or attrition among workers, some of which could be attributable to cardiovascular effects.

In another study, the effects of carbon disulfide on the cardiovascular system of 1,498 rayon workers were evaluated in comparison to 481 acetate workers (Lieben et al. 1974). An electrocardiogram, blood pressure level, total cholesterol level, and occupational exposure history were obtained for each worker. The only statistically significant finding was a higher average blood pressure level (140/87) in workers in the rayon plants than in workers in the acetate plants (135/83). However, blood pressure readings did not differ significantly among workers exposed to high, medium, and low concentrations of carbon disulfide, which suggests the lack of a dose response. Although no data were presented, there could have been confounding exposures to other chemicals. Increased retinal arterial pressure but not brachial arterial pressure was observed in viscose rayon workers exposed to carbon disulfide at average concentrations of 64-161 ppm with peaks of 289 ppm for l-9 years (Maugeri et al. 1967). A correlation between effects and duration of exposure was not detected.

Physical examinations were completed both before and after 114 workers were exposed to carbon disulfide for 5 years (Chrostek-Maj and Czeczotko 1995a). Cardiovascular effects, as assessed by blood pressure and electrocardiograms, were not observed. Exposure concentrations were stated as a mean of 0-21 ppm and a median of 0-l.1 ppm. Carbon disulfide metabolites in the urine ranged from 0 to 950 mg/L. Cardiovascular effects, as assessed by blood pressure, blood coagulation, and measurement of serum creatine kinase activity, were not observed in 247 workers exposed to carbon disulfide at a median concentration of 4 ppm for a median duration of 4 years (Drexler et al. 1995b).

As described in Section 2.7, an interaction between carbon disulfide exposure and consumption of an atherogenic diet may lead to enhanced cardiotoxicity in rats. Rats administered carbon disulfide at 16 ppm and greater for up to 6 months exhibited concentration-related structural and functional changes (distention of the lumen, attenuation of myocardial vessels, irregular thickening of the aorta

wall, as well as microscopic histological changes). Although an increase in the enzyme activity (fructose-1,6-phosphatase, glutamate dehydrogenase, and glucose-6-phosphate dehydrogenase) was reported at the lowest concentration (3.2 ppm), the statistical significance of this finding was not reported. Also, no structural changes were seen at 3.2 ppm. However, when rats exposed to the same concentration of carbon disulfide were administered an atherogenic diet, there was an increase in mortality, a decrease in albumin and increase in globulin fractions in the serum, and serious metabolic and structural changes in the myocardium and the aorta (Antov et al. 1985).

Rats chronically administered 321.1 ppm carbon disulfide (5 hours a day, 6 days a week, for 15 months) did not develop any gross or histological lesions in the aorta; however, lipid droplets were occasionally noted on histological examination of the coronary arteries (Wronska-Nofer et al. 1980). In this same study, rats simultaneously fed an atherogenic diet had more advanced lipid infiltrates of the coronary arteries, which suggests that carbon disulfide may have an accelerating effect on atherosclerotic changes induced by dietary hypercholesterolemia. Thus, carbon disulfide may have promoted the development of atherosclerosis and coronary heart disease via altered cholesterol metabolism within the arterial wall.

Male Wistar rats exposed to 803 ppm for 18 hours showed reduced cardiac and respiratory rates and severe narcosis (Tarkowski and Sobczak 1971). However, this study used only six or seven animals and only one dose was tested.

Several studies have shown that carbon disulfide causes vascular changes in various organs of experimentally exposed animals. Acute inhalation (2 days) of 1,285 ppm in phenobarbitone-pretreated rats resulted in myocardial lesions characterized by necrosis, interstitial edema, and cellular infiltrate (Chandra et al. 1972). This effect was not observed in rats treated with carbon disulfide alone.

Gastrointestinal Effects. Nausea and vomiting were reported in approximately 50% of 123 persons following an accidental release of carbon disulfide in India (Kamat 1994). Gastrointestinal symptoms are also common among heavily exposed workers with carbon disulfide poisoning. In one study, 28% of the workers in a viscose rayon plant had a prevalence of symptoms (Vigliani 1954), and in another, viscose workers (n=l00) exposed to 1.9-26.4 ppm carbon disulfide in combination with 1.0-4.0 ppm hydrogen sulfide for 3 months to 17 years complained of stomach distress and impaired appetite (Rubin and Arieff 1945). Significant associations with nausea,

vomiting, and flatulence were also found in 119 carbon disulfide-exposed workers in a cross-sectional study by Vanhoorne et al. (1992b). Workers were exposed to l-36 ppm for an average of 4.2 years. These studies, however, are of limited value because of fluctuating exposure concentrations, concomitant exposure to other chemicals, and the fact that the reported symptoms are nonspecific and may have several etiologic agents.

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to carbon disulfide.

Hematological Effects. Relative to 18 unexposed persons, fibrolytic activity was decreased in 57 workers exposed to carbon disulfide at 59-169 ppm for 2-8 years (Visconti et al. 1967). Serum plasmin concentrations decreased with increasing exposure duration. Red blood cell and white blood cell counts were not significantly different from preemployment values in 114 workers exposed to carbon disulfide at 0-21 ppm for 5 years (Chrostek-Maj and Czeczotko 1995a).

In animals, there is limited information that hematological effects occur following inhalation exposure. Brieger (1949) studied the bone marrow of four rabbits acutely exposed (6 hours/day for 12 days) to 1,100 ppm carbon disulfide and found no hematological effects. In the same study, longer exposure (6 hours/day for 48 days) to 300 ppm resulted in significantly increased pseudo-eosinophilic cells with a corresponding reduction in lymphocytes. It can be concluded that carbon disulfide increases the relative number of cells in the granulocytic series in the bone marrow. In another study, eight dogs exposed to 400 ppm carbon disulfide for 8 hours a day, 5 days a week, for 11 weeks did not exhibit adverse hematological effects or adverse alterations in blood chemistry except for a slight decrease in the serum albumin level after 2 weeks (Lewey et al. 1941). Significant depression in erythrocyte counts, total hemoglobin, and hematocrit were noted in mice exposed to 800 ppm, 6 hours daily, 5 days a week, for 90 days (Toxigenics 1983c). These studies suggest that hematologic effects are not consistent across species. Furthermore, these effects are likely to be dose- and duration-dependent. These changes do not correlate with clinical changes in the animals.

Hepatic Effects. Male volunteers exposed for 6 hours to graded concentrations (10-80 ppm) of carbon disulfide showed inhibition of oxidative demethylation of orally administered amidopyrine (Mack et al. 1974). In another study, Vanhoorne et al. (1992b) reported significantly increased liver size and γ-glutamyltransferase (GGT) activity in 119 carbon disulfide exposed workers compared to

79 controls. Workers had been exposed to l-36 ppm for a mean of 42 years. However, since adequate exposure data were not presented, this study should be interpreted with caution.

There is some evidence for increased serum cholesterol levels in workers exposed for prolonged periods to carbon disulfide levels in the range of 20-60 ppm, but most of these studies did not use an adequately matched control group (UK/HSE 1981). This study also measured total cholesterol levels and did not differentiate between high- and low-density cholesterol. Therefore, no interpretation is possible. An examination of total lipids, total and free cholesterol, and triglycerides in serum was conducted in workers with chronic exposure to carbon disulfide in a viscose rayon factory in Yugoslavia with time-weighted average (TWA) exposures of 4 ppm (n=58) or 18.5 ppm (n=102) over 17 years. Levels were compared to a nonexposed group of 41 workers (Krstev et al. 1992). Although there was a lack of dose response, i.e., total lipid and triglyceride levels were similar regardless of exposure, these levels were higher in exposed than in nonexposed workers. Again, no definitive conclusion can be established. In another study, workers chronically exposed to carbon disulfide in a viscose factory were classified by exposure duration of less than (n=17) or greater than (n=17) 20 years (El-Sobkey et al. 1979). Thirteen workers served as controls, and carbon disulfide concentrations ranged from 0.008 to 0.02 ppm for exposed workers. Exposed workers had significantly lower exposure duration-related mean values of thyroxine and higher mean values of free, esterified, and total cholesterol than controls. The study authors suggested that depression of serum thyroxine may be a key manifestation in hypercholesterolemia among carbon disulfide-exposed workers. The small sample size, lack of information on other exposures, and poorly characterized exposure concentrations preclude establishing conclusions from this study.

A significant positive trend for low-density lipoprotein cholesterol (LDL-Ch), total cholesterol, and diastolic blood pressure in workers exposed to carbon disulfide was observed in a cross-sectional study by Egeland et al. (1992). The Egeland study used existing data (Fajen et al. 1981) on 165 carbon disulfide-exposed workers and 245 unexposed controls recruited in 1979. The persons using medications to control ischemic heart disease and hypertension, as well as those using corticosteroid or thyroid medications, were excluded. Affected workers had been exposed for at least 1 year in a viscose rayon factory to a median 8-hour TWA of 7.6 ppm. The increases observed in total cholesterol were attributed to increases in LDL-Ch since there was no apparent effect on high-density lipoprotein cholesterol (HDL-Ch) ; triglyceride and fasting glucose levels were not associated with carbon disulfide exposure. Although these findings of an increased risk of arteriosclerotic heart

disease have been attributed to increased LDL-Ch, the correlation between carbon disulfide exposure and arteriosclerotic heart disease is not definitive in this study because of possible selection bias, cumulative exposure uncertainties, lack of control for coronary disease risk factors such as diet and exercise, and a limited statistical power to detect small changes in LDL-Ch.

Increased levels of LDL-Ch and apolipoprotein B as well as increased systolic and diastolic blood pressure indicative of increased coronary risk were noted in 115 carbon disulfide-exposed workers in a Belgian viscose rayon factory when compared to a control group of 76 workers (Vanhoorne et al. 1992a). Although these biochemical changes associated with cardiovascular disease were noted, no significant increases in prevalence of angina, myocardial infarction, or ischemia, as indicated by ECG changes, were found. In addition, elevated levels of apolipoprotein Al, which is a protective factor for coronary risk were also noted in exposed workers; this too may be related to the toxic effects of carbon disulfide exposure. Although the length of exposure was not specified, the authors noted that conditions in the plant had not changed since 1932 and that concentrations ranged from 1 to 36 ppm depending on job type. This study should be interpreted with caution since simultaneous exposure to low levels of H_2S also occurred. In addition, there may have been selection bias. Only 46% of those eligible for the referent group participated while referents with health complaints may not have participated, resulting in an underestimation of risk. Relative to preemployment values, increased triglycerides and β-lipoproteins were observed in workers exposed to 0-21 ppm carbon disulfide for 5 years (Chrostek-Maj and Czeczotko 1995a). A similar effect was not observed in 62 unexposed workers 5 years after a preemployment physical examination.

Compared to age-matched controls, an increase in total cholesterol, HDL-Ch, and LDL-Ch was observed in women 40-49 years of age and 50-59 years of age (Stanosz et al. 1994b). The women were exposed to carbon disulfide at 5-7 ppm for 0.5 to greater than 20 years. Only HDL cholesterol and LDL cholesterol were increased when the values were examined by duration of carbon disulfide exposure. Rather than being a hepatic effect, the investigators suggest that the effect may be on hormone production by the ovaries resulting in altered lipid metabolism.

No effects on serum cholesterol levels were noted in workers chronically exposed to l0-30 ppm carbon disulfide (Hernberg et al. 1971), and several studies failed to observe increased serum cholesterol levels in workers exposed to carbon disulfide at concentrations below 20 ppm. In an occupational study, 35 workers chronically exposed to carbon disulfide concentrations ranging from

6.4 to 12.8 ppm for 5-20 years exhibited a statistically significant reduction in blood cholesterol levels; a nonsignificant reduction in total lipid levels was also observed. This study is of limited value because of the small sample size and the likelihood of concurrent exposure to other chemicals (Sidorowicz et al. 1980). Another study of 70 men exposed to carbon disulfide in a viscose plant who were matched to unexposed men working in a different division of the plant found no statistically significant differences in blood lipid profiles (total cholesterol, HDL-Ch, and triglycerides) (Franco et al. 1982). Carbon disulfide concentrations were less than 11.2 ppm from 1972 to 1979. Workers (n=420) in a rayon filament factory chronically exposed to carbon disulfide (unspecified concentrations) for 4-25 years also exhibited no difference in total serum cholesterol, triglycerides, and β-lipoprotein in comparison to controls (n=390) (Sugimoto et al. 1978).

Only transient effects on liver metabolism have been observed in animals following inhalation exposure to carbon disulfide. Acute inhalation (8 hours) of 20 ppm carbon disulfide produced a reversible inhibition in oxidative drug metabolism by female rat liver microsomes and an increase in total hepatic lipid content (Freundt et al. 1974a). Rats exposed to much higher concentrations (642 ppm) of carbon disulfide for 4 hours exhibited no histological evidence of liver damage (Magos and Butler 1972; Magos et al. 1973). However, hepatotoxicity characterized by hydropic degeneration in parenchymal cells of the centrilobular zone was observed in rats pretreated with phenobarbitone to induce the liver mixed-function oxidase system and subsequently exposed to 642 ppm for 4 hours (Magos and Butler 1972; Magos et al. 1973). Starvation further potentiated the phenobarbitone-induced liver lesions in rats subsequently treated with carbon disulfide.

In mice, intermediate-duration inhalation exposures at a concentration of 482 ppm for up to 23 days (4 hours a day, 5 days a week) have resulted in a marked reduction in cytochrome P-450 and cytochrome c-reductase content after 2-3 days. The level returned to normal by the 23rd day of treatment. A significant decrease in uridine diphosphate-glucuronyl (UDP-glucuronyl) transferase was also noted, as well as a significant increase in lipid peroxidation (Jarvisalo et al. 1977a). Rabbits exposed to 300 ppm for 30 minutes a day for 120 days failed to develop any histopathologic alterations of the liver (Tsuyoshi 1959). However, because of the small sample size (n=3), the conclusions are preliminary.

The effect of carbon disulfide on lipid metabolism in the rat has been extensively studied (Wronska-Nofer 1972, 1973; Wronska-Nofer et al. 1978, 1980). Serum cholesterol, phospholipids, and

triglyceride levels were significantly elevated after exposure to 161-176.6 ppm carbon disulfide for 5 hours a day, 6 days a week, for 2 months or more (Wronska-Nofer 1972, 1973). A small but significant ($p<0.05$) increase was also noted in rats exposed to 74 ppm for 5 hours a day, 6 days a week, for 8 months (Wronska-Nofer 1973). Rats maintained on a Murigran chow *ad libitum* diet and exposed to 321 ppm carbon disulfide 5 hours a day, 6 days a week, for 6 months showed an increase in the rate of cholesterol influx from serum into the aorta wall. Those rats exposed under the same conditions for 8 months showed a slightly enhanced rate of aortic cholesterol synthesis (Wronska-Nofer and Parke 1978). Rats maintained on an atherogenic diet (2% cholesterol, 0.15% thiouracil) and exposed to 321 ppm carbon disulfide for 5 hours a day, 6 days a week, for 6 months had markedly increased serum and aortic cholesterol levels. These results suggest that carbon disulfide may increase arteriosclerotic changes resulting from diet-induced hypercholesterolemia (Wronska-Nofer et al. 1980).

Total serum cholesterol and fatty acids became elevated, but cholesterol esters decreased, in dogs administered 400 ppm carbon disulfide and simultaneously fed high-fat diets (Lewey et al. 1941). These data suggest that carbon disulfide, in conjunction with a high-fat diet, may lead to increases in cholesterol levels above those expected from high-fat diets alone. Rabbits (n=ll) exposed to higher concentrations of carbon disulfide (750 ppm) for 6 hours a day for 5 months exhibited a transient elevation in total serum cholesterol which was associated with weight loss (Cohen et al. 1959).

Thus, carbon disulfide does affect liver enzymes, particularly those related to lipid metabolism. The increases in serum cholesterol that are sometimes seen following carbon disulfide exposure may be a result of increased hepatic cholesterol synthesis.

Renal Effects. In a study with viscose rayon workers, there was a slight but statistically significant increase in the mean plasma creatinine concentration compared with the control group (Hernberg et al. 1971). However, the study authors concluded that all values were within reference ranges. Urinalysis did not reveal any effects on kidney function relative to preemployment values in 114 men exposed to carbon disulfide at 0-21 ppm for 5 years (Chrostek-Maj and Czeczotko 1995a).

Inhalation exposure of mice to 800 ppm for 6 hours a day, 5 days a week, for 90 days produced nephropathy (Toxigenics 1983c). Rabbits exposed to 300 ppm carbon disulfide for 30 minutes a day for 120 days failed to develop any histopathological alterations of the kidney (Tsuyoshi 1959). This study is of limited value because of the small sample size (n=3). An autopsy report of rabbits

(11 males) exposed to graded concentrations of 250-750 ppm carbon disulfide intermittently over a period of 38 weeks revealed an increased incidence of chronic interstitial nephritis (Cohen et al. 1959).

Endocrine Effects. The available human studies provide conflicting evidence on the adverse effects of carbon disulfide on thyroid function. However, these studies were limited by possible exposure to other chemicals, small sample size, and lack of quantification of precise exposure concentrations.

The effects of chronic exposure to carbon disulfide on serum thyroxine and cholesterol levels were studied in 50 workers employed in a viscose rayon factory (El-Sobkey et al. 1979). The control group consisted of 13 workers. The carbon disulfide concentration varied from 0.0083 to 0.02 ppm and the exposure duration from less than 20 years to greater than 20 years. An association was found between the lower serum thyroxine levels and serum concentrations of free and esterified cholesterol in exposed workers. According to the study authors, depression in serum thyroxine levels is related to metabolic disturbances leading to hypercholesterolemia among carbon disulfide-exposed workers.

In a study by Lancranjan et al. (1972), 109 workers exposed to carbon disulfide for 7-31 years were examined for thyroid function. A group of 40 workers served as controls. The exposure concentrations varied from 19 to 29 ppm and from 72 to 96 ppm. The study authors concluded that carbon disulfide did not induce thyroid alterations or disorders of lipid metabolism. In another study, the effect of long-term exposure to carbon disulfide (10-36 years) was studied in 15 exposed and 16 age-matched controls (Wagar et al. 1981). The exposure levels ranged from 10 to 51 ppm. No disturbance was noted in either thyroid function or serum prolactin values. Serum cortisol was also unchanged.

Urinary excretion of 17-hydroxycorticosteroids (formed from precursors of adrenal origin) and 17-ketosteroids (from both adrenal and gonadal sources) was reduced in workers exposed to carbon disulfide at 59-169 ppm for up to 8 years (Cavalleri et al. 1967). In a study that was designed to examine the effects of carbon disulfide on the sympathetic/adrenal system, decreased diurnal urinary excretion of adrenaline, decreased plasma dopamine, and increased serum β-hydroxylase activity were observed in women occupationally exposed at 5-7 ppm for periods ranging from 6 months to greater than 20 years (Stanosz et al. 1994a). Blood pressure was not significantly affected in this study, although among exposed women, a negative correlation was observed between daily urinary excretion of adrenaline and systolic blood pressure.

No studies were located regarding endocrine effects in animals after inhalation exposure to carbon disulfide.

Ocular Effects. Ophthalmological changes of various types, such as increased frequency of microaneurysms, related to the duration and intensity of exposure, have been found in Japanese workers. Severe ocular effects characterized by dot hemorrhages or microaneurysms of the retina were observed in Japanese workers exposed to carbon disulfide at levels between 5 and 15 ppm after 1955 and between 15 and 30 ppm earlier. The mean duration was 17 years in the first study (Sugimoto et al. 1978; Tolonen et al. 1976) and 10.8 years in the second study (Sugimoto et al. 1976) at concentrations averaging ≥20 ppm (high group) or <20 ppm (low group). Retinopathy, characterized by microaneurysms, was observed in 35% (43/124) of those exposed to air concentrations above 20 ppm carbon disulfide and in 23% (29/127) of those exposed to below 20 ppm, as compared to 4% (2/49) of the controls. These increases were statistically significant. The incidence and severity were shown to increase with longer durations of exposure to carbon disulfide. The retinopathy was not age related, the incidence being 37%, 29%, and 35% in exposed workers in the age groups 30-39, 40-49, and 50-59 years, respectively (Sugimoto et al. 1976). Although other exposures were not discussed, concurrent exposures to other chemicals may have also occurred.

A subsequent collaborative study was conducted to assess the incidence of retinopathy among groups of workers in Finland and Japan exposed to similar levels of carbon disulfide. The comparison showed that the differences in values obtained in the two studies were true differences and were not caused by interobserver variation (Sugimoto et al. 1977, 1978; Tolonen et al. 1976). Retinal red dots (microaneurysms and/or small hemorrhages) were observed in 25% (103/419) of the Japanese workers with chronic exposure to mean atmospheric levels of carbon disulfide of 15-35 ppm during the 1950s and below 20 ppm (5-15 ppm) since 1955, as compared to 4% (15/391) of the controls. However, no significant increase in the incidence of retinopathy was noted in Finnish workers exposed to 5-10 ppm carbon disulfide and to higher concentrations prior to 1970 (20-60 ppm during the 1950s and l0-30 ppm during the 1960s): 4% (7/188) were affected compared to 3% (2/76) of the controls. Thus, the prevalence of retinopathy among 419 Japanese workers exposed to 5-20 ppm carbon disulfide was high, whereas the incidence among 188 Finnish workers exposed to about 5-30 ppm was not greater than expected. The high prevalence among the Japanese workers may be explained by biased selection of exposed workers, different actual exposures, or the fact that no attempt was made in either study to account for the known risk factors for retinopathy (i.e., diabetes, hypertension). The

Finnish study may have selected for these findings. No data were presented regarding other possible exposures.

In studies of Finnish workers, 100 males with the longest and most marked history of exposure and 97 unexposed males were chosen for a neuro-ophthalmological investigation (Raitta and Tolonen 1975; Raitta et al. 1974, 1981). The 100 men had been exposed for between 1 and 27 years to 10-40 ppm; they were selected from the same cohort previously described by Hernberg et al. (1970). Corrected visual acuity, visual field, eye motility, pupillary reactions, and biomicroscopy were normal in all eyes examined. No retinopathy was detected in either group of individuals. However, delayed peripapillary filling of the choroid (both circumferential and segmental) occurred in 68 exposed and 38 unexposed eyes, a significant difference ($p \leq 0.01$). This was attributed to possible hemodynamic effects of carbon disulfide exposure. In addition, the mean widths of eight retinal vessels and the smallest vein were significantly greater in the exposed group, again attributed by the study authors to hemodynamic alterations with exposure. The study was limited by the difficulty in characterizing exposure levels because of the possibility that individual exposure varied widely and erratically over periods of time. However, a follow-up study validated the hypothesis that the delayed peripapillary filling of the choroid was related to the cardiovascular effects of carbon disulfide (Raitta and Tolonen 1975). In this follow-up, 38 male viscose rayon workers exposed to carbon disulfide were compared to 40 unexposed workers (previously examined neuro-ophthalmologically). Measurements were taken by oculosphygmography and were combined with individual electrocardiograms to statistically analyze the characteristics of the ocular pulse wave. Results showed that the exposed group of workers had a significantly lower pulse wave than that of the unexposed group, suggesting an increased rigidity of the ocular vascular bed in the viscose rayon workers. This study provided further evidence that carbon disulfide was not retinopathic in this Finnish cohort and in addition suggested a possible mechanism for the ocular effects of carbon disulfide.

To determine the effect of carbon disulfide exposure on retinal vasculature, American subjects from a viscose rayon plant (156 exposed, 233 unexposed) underwent pupillary dilation (with a short-acting mydriatic), direct ophthalmoscopy, and retinal photography with monochromatic light (NIOSH 1984a). Photographs were read by an ophthalmologist and rated as normal, as having definite or uncertain microaneurysms, or as having definite or uncertain hemorrhages. Subjects were categorized by job and characterized as having definitely low (DL<3 ppm), moderate (M=3-7.1 ppm), or definitely high exposure (DH>7.1 ppm). Retinal microaneurysms and hemorrhages were more prevalent in the

combined exposed groups than in the comparison group ($p \leq 0.04$). There was a concentration-related increase in the incidence of both definite and uncertain microaneurysms with exposure to carbon disulfide. No such trend was apparent for hemorrhages, nor for definite aneurysms alone. The combined exposed groups had almost 20% retinal microaneurysms (both definite and uncertain) compared to 7.5% for the comparison groups (significant, $p \leq 0.01$). The combined exposed groups had 10.5% retinal hemorrhages (both definite and uncertain) compared to 3% for the comparison group (significant, $p \leq 0.01$). The difficulties in characterizing dose levels and measuring the number of aneurysms by photography limit interpretation of the findings. Evidence regarding the occurrence of retinopathies due to carbon disulfide exposure is not uncomplicated. It appears that there may be a group of individuals, both Eastern and Western, who are genetically predisposed to respond with retinopathy to low levels of carbon disulfide exposure (NIOSH 1984a; Sugimoto et al. 1976, 1977). Conflicting evidence shows no retinopathy with exposure to slightly higher levels of carbon disulfide in the Finnish population (Hernberg et al. 1970; Raitta and Tolonen 1975; Raitta et al. 1974; Sugimoto 1977; Tolonen 1975; Tolonen et al. 1976). The differences in response by various populations have not been resolved.

Thirty workers in a viscose rayon plant were divided into two groups based on carbon disulfide exposure concentrations. The control group was exposed to average concentrations of 3.2 ppm, while the exposed workers experienced average levels of 16-32 ppm. Pigmentary changes and microvascular retinal lesions were observed in both groups (DeLaey et al. 1980; DeRouck et al. 1986). The study authors concluded that carbon disulfide affects several ocular structures and functions at low exposure levels. However, this study is limited by a small sample population, probable concomitant exposure to other chemicals, and an inappropriately chosen control group that was also exposed to carbon disulfide. In another study, viscose workers (n=l00) intermittently exposed to 1.9-26.4 ppm carbon disulfide in combination with 1.0-4.0 ppm hydrogen sulfide for 3 months to 17 years complained of burning of the eyes (Rubin and Arieff 1945). This study is of limited value because of fluctuating exposure concentrations, concomitant exposure to hydrogen sulfide and other chemicals, and lack of a control group.

Adverse ocular effects in workers of a viscose silk plant exposed 6 hours a day, 5 days a week, for 0.5-30 years to less than 3.2 ppm carbon disulfide were reported by Szymankova (1968). Disturbances were manifested as vascular or inflammatory degenerative changes in the retinas of 12

out of 75 (16%) of the exposed workers, which disappeared in 11 workers following cessation of carbon disulfide exposure.

Four female monkeys exposed to 256 ppm for 6 hours a day, 5 days a week, for 5-13 weeks suffered permanent visual impairment with degeneration of retinal ganglion cells compared to one control (Merigan et al. 1988). Visual acuity thresholds in two macaque female monkeys were severely disrupted after 5 weeks of intermittent exposure (6 hours a day, 5 days a week) to 256 ppm carbon disulfide. One monkey showed some recovery at 16 weeks postexposure; the other showed no improvement (Merigan et al. 1985). The observed effects were secondary to the effects on the optic nerve.

Body Weight Effects. Significant associations with anorexia were found in a cross-sectional study of 119 workers exposed to l-36 ppm carbon disulfide over a mean of 4.2 years (Vanhoorne et al. 1992b). This study, however, did not provide adequate exposure data. No information was located regarding effects on body weight in humans after inhalation exposure to carbon disulfide.

Female Wistar rats exposed to 800 ppm for 15 weeks (5 days a week, 6 hours a day) showed a 10% decrease in body weight gain (Hirata et al. 1992b). Male Long-Evans rats showed a 6-8% decrease at lower concentrations (350-600 ppm) following inhalation exposure for 10 weeks (Tepe and Zenick 1984). During a 14-day exposure (10 hours/day) to carbon disulfide at 600 ppm, male rats lost 14% of their body weight (Wilmarth et al. 1993). This concentration also resulted in a narcotic-like stupor in the exposed rats. Contrary to these findings, male Long-Evans rats that were exposed to 500 ppm for 5 or 12 weeks (5 days a week, 6 hours a day) showed no significant changes in body weight gain (Clerici and Fechter 1991). Female Long-Evans rats exposed to 800 ppm for 11 weeks (7 days a week, 7 hours a day) had a 15% decrease in body weight gain; this effect was not seen at 400 ppm (Rebert and Becker 1986). Inhalation exposure of Fischer 344 and Sprague-Dawley rats at 800 ppm for 90 days (5 days a week, 6 hours a day) caused a 16-30% decrease in body weight gain in both sexes (Toxigenics 1983a, 1983b). The percent of decrease in body weight gain depended on the strain of rat used (Toxigenics 1983a, 1983b). Inhalation exposure of Wistar rats to 546 ppm for 8 months or to 482 ppm for up to 14 months produced decreases in body weight (Szendikowski et al. 1974; Wronska-Nofer 1973).

Other Systemic Effects. Workers exposed for less than 5 years to TWA concentrations of 4.8-8 ppm had significantly elevated plasma sodium and chloride ions and decreased erythrocyte potassium and calcium (Pines 1982). However, the large variance in the electrolyte measurements among workers, the concomitant exposure to other chemicals, the fluctuating exposure concentrations, and the lack of a dose response for blood electrolyte alterations limit the value of this study.

Animal studies include a necropsy report on 10 male rabbits exposed to graded concentrations of 250-750 ppm carbon disulfide intermittently for 38 weeks that revealed increased adrenal weight, hyperplasia of adrenal cortex, and mild hemosiderosis of the spleen (Cohen et al. 1959). No information was given as to whether the controls underwent a sham exposure process, thereby controlling for the stress of the exposure procedure.

2.2.1.3 Immunological and Lymphoreticular Effects

The only study located that specifically addressed a possible immunological effect of carbon disulfide exposure in humans reported data that indicated that the β-lipoprotein isolated from carbon disulfide-exposed workers (presumably exposed via inhalation) is antigenically identical to lipoproteins isolated from healthy nonexposed controls (Bobnis et al. 1976). The authors concluded that these findings suggested no immunologic component involved in the increase of arteriosclerotic lesions found in carbon disulfide-exposed workers. There are no further data to either support or refute this conclusion.

No studies were located regarding immunological or lymphoreticular effects after carbon disulfide exposure in either humans or animals.

2.2.1.4 Neurological Effects

The primary target of carbon disulfide appears to be the nervous system. Neurophysiological and behavioral effects as well as pathomorphology of peripheral nervous system structures have been reported in humans as well as animals. Acute exposure to high concentrations of carbon disulfide can result in fainting and loss of consciousness. These effects were observed in 36-39% of 123 persons exposed to carbon disulfide following an accidental release of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon factory in India (Kamat 1994).

Most information available on neurotoxic effects of carbon disulfide in humans comes from occupational epidemiology studies. These exposures are considered to occur via inhalation, although some dermal exposures could have conceivably occurred, especially under conditions that may have prevailed 40-50 years ago. Separating possible effects of concomitant exposures to other chemicals can also present a problem. An examination of 118 male workers in a viscose rayon plant exposed for a median length of 15 years to carbon disulfide at an estimated average concentration of between 10 and 20 ppm revealed that the carbon disulfide-exposed workers had reduced maximal motor conduction velocity of the median, ulnar, deep peroneal, and posterior tibia1 nerves when compared to the controls (workers in a paper mill) (Seppalainen and Tolonen 1974). Individuals working in the plant before 1960 were exposed to higher levels (20-40 ppm) of carbon disulfide than were those working after this time. Furthermore, follow-up examination of these workers indicated that removal from the exposure environment did not lead to improvement of the nerve conduction velocity. However, it was noted that when individuals were removed from carbon disulfide exposure for l0-15 years, there was an equal division of people with either normal or decreased conduction velocities compared to a greater percentage of decreased velocities in individuals absent for 0-4 years. The authors of this study had earlier reported on neurophysiological findings in 36 workers exposed to high levels of carbon disulfide and described diminished nerve conduction velocities indicating polyneuropathy in many subjects (Seppalainen et al. 1972). Polyneuritis was reported to be present in almost all workers occupationally exposed to carbon disulfide for an average of 40 months at unspecified concentrations (Lancranjan et al. 1972). Overt polyneuropathy was reported in 9 of 17 male workers exposed to 150-300 ppm carbon disulfide for greater than 2 years, while 19 workers exposed to 15-150 ppm also had some symptoms of polyneuropathy (Chu et al. 1995). Nerve conduction velocities were significantly different in subjects with overt polyneuropathy when compared to subjects with subclinical effects and in subjects with subclinical effects when compared to unexposed controls.

Three groups of grain workers in three different work facilities (grain inspectors, malt laboratory workers, and grain elevator workers) showed various neurological effects. Their symptoms included distal sensory shading indicated by decreased sensitivity to pinprick and light touch, intention tremors, resting tremors, and nerve conduction abnormalities. The authors of this study concluded that the similarities of these symptoms to those reported in viscose rayon workers implicate carbon disulfide. The 21 subjects in this study were, however, self-selected, no controls were used, and no measurements of actual exposure to carbon disulfide were made (Peters et al. 1988). All individuals reported being able to smell the fumigant mixtures of carbon disulfide and carbon tetrachloride, and it

is suggested that at this point safe levels had been exceeded. Possible contributions to effects from the other components of the pesticide were not considered. It has been suggested that the symptoms of the grain workers exposed to the fumigant mixtures of carbon disulfide and carbon tetrachloride resemble those of patients with idiopathic Parkinson's disease (Matthews et al. 1990). Clinically, Peters et al. (1982) described loss of associated movements, cogwheeling, and atypical tremor in the grain workers resembling early Parkinsonism.

Clinical neurological examination of 16 men formerly exposed to carbon disulfide for at least 10 years revealed abnormalities in 15. Cerebral computerized tomography (CT-scans) showed signs of atrophy in 13, and neuropsychological examination indicated brain organic changes in 13. The authors of this study believed that long-term exposure to carbon disulfide involved a risk of developing toxic encephalopathy (Aaserud et al. 1988). Exposures were assumed to be between 9.6 and 19 ppm, with occasional higher exposures. There was no quantitation of individual exposures, however, and no adjustment was made to account for other possible occupational exposure or for lifestyle factors. CT-scans also revealed evidence of brain atrophy in 12 of 20 workers exposed to carbon disulfide at 0-21 ppm for 5 years (Chrostek-Maj and Czeczotko 1995b). The changes were observed most frequently in the frontal lobe. CT-scans were only completed in the 20 individuals with the worst psychiatric effects. Exactly what was being measured in the psychiatric examinations was not clear. In this study, psychiatric exams were completed before and 5 years after the start of exposure. In the exposed group of 114 men, the prevalence of "pseudoneurotic" symptoms increased from 8.4% to 43%. A similar increase was not observed among 62 unexposed control workers. Peters et al. (1988) noted that magnetic resonance imaging findings in 2 out of the 3 grain storage workers were indicative of central demyelination.

Regional cerebral blood flow was examined using Doppler ultrasound in 15 workers exposed to 3.2-28.9 ppm carbon disulfide for a mean of 20 years (Aaserud et al. 1992). Studies were performed 4 years after exposure ended. Asymmetrical blood flow patterns were observed in 8/14 workers, all of whom had encephalopathies consistent with carbon disulfide exposure. However, when the results were corrected to adjust for a possible influence of pCO_2, the values did not differ between the exposed workers and referents. No clear conclusions about this study can be made because of the small number of exposed workers, lack of a current exposure group, possible selection bias, and age variation between cohort and referents.

A study was conducted on 81 patients who had worked in environments containing toxic chemicals, who exhibited chronic carbon disulfide poisoning, and who showed evidence of polyneuropathy (Vasilescu 1976). The patients showed decreased conduction velocity in sensory nerves. These decreases were greater than those seen in the motor nerve velocities and, in some patients, occurred before any clinical signs. No measurements of actual exposure were given, however, and no estimates were made on the duration of occupational exposures or the chemicals that may have contributed to the total exposure history of the patients. Carbon disulfide was one of the major contaminants.

Workers exposed to carbon disulfide (n=145) were evaluated for its effects on the peripheral nervous system and compared to a group of nonexposed artificial fiber plant workers (n=212) located on the same premises (Johnson et al. 1983). The mean exposure period was 12.1 ± 6.9 years (mean ± SD), and individuals were divided into three groups based on previous exposure histories, job descriptions, and current carbon disulfide levels established on the basis of 8-hour personal monitors. The median carbon disulfide level for the comparison group was 0.2 ppm, while the median carbon disulfide levels of exposed individuals were 1, 4.1, and 7.6 ppm. The mean exposure concentration of all groups considered together was 7.3 ± 17.2 ppm (mean f SD) (23 mg/m^3), ranging from 0.6 to 16 ppm (1.9-50 mg/m^3). Carbon disulfide levels showed variability during breakdown periods at the plant which occurred “infrequently” but exposed some workers to brief periods of high carbon disulfide concentrations. Surface electrodes were used to measure maximum motor conduction velocity (MCV) in the ulnar and peroneal nerves and sensory nerve conduction velocity (SCV) in the sural nerve. There was a dose-related reduction in motor nerve conduction velocities in the calves and ankles, which was statistically significant in the high-concentration exposure group and in the average exposure group. However, the reductions were within the range of normal values. The study authors considered this to indicate minimal neurotoxicity. A chronic-duration MRL of 0.3 ppm was established for this effect, using 7.6 ppm as the LOAEL. In addition, reductions in the peroneal nerve conduction velocity appeared to be related to the workers’ cumulative exposure to carbon disulfide. This study reported health-related effects at average levels of exposure much less than those usually reported for occupational cohort studies. However, potential coexposure of these viscose rayon workers to hydrogen sulfide, tin oxide, zinc oxide and sulfate, sodium hydroxide, sulfuric acid, and lead may account for a portion of the toxicity response.

Mental performance and personality disorders were examined in 17 long-term workers from each of two rayon factories, one group with a long history of relatively high exposures to carbon disulfide

(mean concentration of carbon disulfide measured after 1971 was 57.8 ppm) and the other with lower exposures (mean concentration of carbon disulfide measured after 1971 was 19.3 ppm). Twenty-one test variables were used: 3 intelligence tests, 6 personality tests, and 12 ability measurements. The workers from the factory with the highest exposure measurements showed more anxiety, introversion, and depression than the other workers. They also did significantly worse on tests designed to measure number facility, sustained attention, speediness, and carefulness. There were, however, no estimates of individual exposure and no control for concomitant exposure to other chemicals (Foa et al. 1976). Lack of attention and reduced perceptive ability were observed in viscose rayon workers exposed to carbon disulfide at 0.6-2.6 ppm with peaks of 11.2 ppm (Cassitto et al. 1993). The investigators suggest the effects may be a result of transient peak concentrations of carbon disulfide rather than the low concentrations. Other studies have indicated that grain storage workers had intense exposures interspersed often with periods of more minimal contact with anti-weevil chemicals (Matthews et al. 1990; Peters et al. 1982, 1986a, 1986b, 1988).

Behavioral examinations (psychological tests, psychomotor tests, and cognitive-perceptual tests) of 131 workers in a rayon plant who were exposed to carbon disulfide were compared to those of 167 workers who worked in textile plants that manufactured other synthetic fibers. Exposure and companion (control) groups and exposure levels are the same as those described for the Johnson et al. (1983) study. The workers completed a checklist of symptoms characteristic of various neurobehavioral syndromes. The results showed no behavioral changes of any major significance. The rayon workers did report symptoms of neurobehavioral ailments, however. Workers were classified individually according to job title and the past and present exposure levels for individuals in that job title. The exposures measured in the plant were generally below 20 ppm, suggesting that these levels may be too low to identify behavioral changes (Putz-Anderson et al. 1983).

A study of neuropsychological variables in carbon disulfide-exposed workers investigated 120 workers selected on the basis of age not exceeding 50 years and an absence of family or personal history of nervous disorders. The test battery consisted of three intelligence tests, three personality questionnaires, a test of memory involving measures of perception, recognition, and free recall, and two performance measures. Workers were grouped according to exposure categories (none; low, about 20 ppm; medium, between 20 and 38 ppm; and high, greater than 38 ppm). The no-exposure and low-exposure categories were combined for analysis. Differences in the groups' measurements were statistically analyzed and revealed decreased intelligence scores, performance, and memory and

increased fatigue and depression in the workers with higher exposures. These changes were dose related, although the exposure variable was categorical and not quantitative (Cassitto et al. 1978; Cirla et al. 1972). Neurological effects in 50 viscose rayon workers occupationally exposed to an average concentration ranging from 3.2 to 9.6 ppm carbon disulfide for 3-12 years were compared to a pair-matched control group from unexposed departments of the plant (Cirla and Graziano 1981). There were no significant differences in the exposed and control groups for three considered parameters: maximum and minimum conduction velocity and residual latency on the peroneal nerve. Psychological examinations of 25 pairs showed no apparent differences between the exposed and control groups. Electromyography and clinical diagnosis revealed only one exposed worker with minimal neuropathy. Furthermore, four exposed and two unexposed workers were clinically diagnosed with the nontoxic syndrome and radiculopathy, and no cases of polyneuropathy were found in either group. Clinical diagnosis of central impairment was found in two exposed workers and no unexposed workers with psychoorganic syndrome. The number of neuropathy or psychoorganic cases was not significant enough to associate exposure to carbon disulfide with adverse neurological effects. Furthermore, the absence of significant differences in peripheral nerve motor conduction velocity and psychological parameters lends particular weight to the conclusion that occupational exposure below 9.6 ppm (maximum exposure concentration) for up to 12 years does not cause recognizable adverse neurological health effects.

In a cross-sectional study of the chronic effect of carbon disulfide exposure on the central nervous system, researchers measured the brain stem auditory evoked potential (BAEP) in Japanese spinning workers from a viscose rayon factory (Hirata et al. 1992a). The workers were divided into three groups depending upon length of exposure: 34 current workers exposed for more than 240 months, 24 current workers exposed for 24-84 months, and 16 former workers exposed for more than 120 months. The 39 unexposed controls were workers in a nylon filament factory. The TWA exposures ranged from 3.3 to 8.2 ppm (mean 4.76 ppm). The latencies of the three main components of BAEP increased compared to those in the control group. The significantly higher interpeak latencies in workers exposed to carbon disulfide for more than 240 months suggest that chronic exposure to carbon disulfide involves the auditory ascending tract in the brain stem. Despite long exposure, BAEP parameters in workers exposed to more than 120 months were not significantly higher than those of the control group.

Neuropsychological and neuropathological examinations were performed on 16 viscose rayon workers in Norway exposed to 3.2-28.9 ppm for an average of 20 years (Aaserud et al. 1990). Workers were also exposed to hydrogen sulfide (80 mg/m^3) while working. The clinical tests performed included electroencephalograms (EEG), electromyograms (EMG), computerized axial tomography (CAT) scans, and motor and sensory neurography. Workers complained of dyspnea, tiredness, nausea, decreased memory, and irritability. Major neurological deficits occurred in 6/16 workers, while minor deficits occurred in 9/16. Pathological changes in EEG patterns were noted in two workers while six showed pathological changes in EMG. Decreased nerve impulse conduction and motor, sensory, and mixed motor/sensory neuropathies were demonstrated. Cerebral or cerebellar atrophy was noted in 13/16 workers, and neurophysiological examinations showed psychomotor retardation and coordination difficulties. Study deficiencies were the lack of a control group and confounding factors in the subjects, including alcohol abuse.

The neurotoxic effects after 10 years or more of long-term, low-level occupational exposure to carbon disulfide in workers at a viscose rayon plant were examined by assessing markers of the peripheral and autonomic nervous system (Ruijten et al. 1990, 1993). Reinvestigation of 44 of 45 exposed and 31 of 37 matched control workers revealed changes in the motor nerve conduction velocity (Ruijten et al. 1993). The exposure concentration in the two studies varied from 1 to 30 ppm. For peripheral nerves, a decrease in the conduction velocity in both fast and slow motor nerve fibers (peroneal nerve) was observed in exposed workers. Sensory conduction velocities were reduced and the refractory period of the sural nerve was increased. The effects on the sural nerve were pronounced. A small decrease in conduction velocities in the absence of symptoms of neuropathy and decreased response amplitudes suggest a mild presymptomatic nerve impairment.

The relationship between electric impulse transmission and visual stimuli was examined in a group of 21 patients with chronic carbon disulfide exposure in a rayon production plant for 20-36 years and control groups of 25 or 36 healthy unexposed males (Sikora et al. 1990). A significant correlation was observed in latency and amplitude of response. The correlations suggest cerebral dysfunction of the visual pathway and diminished ability to transform visual information to motor reaction at the level of the cortical association center. The study was limited by the lack of quantification of exposure levels and the variability in responses in the exposed group.

Finger tremor accompanying voluntary movement was studied in 19 control subjects and 19 grain workers exposed to carbon disulfide-based fumigants for 13.5 years (Chapman et al. 1991). Comparison was made between finger tremor detected using computerized techniques and Parkinsonian tremor detected visually on neurological examination. The measurement of amplitude and frequency provided a more accurate diagnosis than the visual observation. The distribution of tremor frequency power in the grain workers was reminiscent of tremor in idiopathic Parkinson's disease. These findings suggest that the measurement of subtle tremor frequency changes may provide an early indication of chronic carbon disulfide poisoning. However, the study was limited by the lack of exposure concentrations and the use of only symptomatic cases.

Animal studies on the neurotoxicity of carbon disulfide have usually been done in rats and provide histopathologic and neurochemical data that support a neurotoxic effect for carbon disulfide. In general, the doses used in these animal studies are considerably higher than the occupational exposures seen in epidemiological studies.

In a study that examined effects in rats (Wistar) and mice (H strain) exposed to carbon disulfide for 4 hours, rats appeared to be more sensitive than mice (Frantik et al. 1994). The concentration that resulted in a 30% inhibition of electrically evoked seizure discharge was 1,370 ppm in male rats and 2,600 ppm in female mice.

Short-term exposures to inhaled carbon disulfide in rats have shown consistent results with respect to brain chemistry changes and sensory and motor nerve conduction alterations. Rats exposed for 4 hours a day for 10 days at 642 ppm showed decreased noradrenaline, increased dopamine, and elevated tyrosine in the brain (Magos and Jarvis 1970). The authors proposed that changes in tyrosine, dopamine, and noradrenalin may be caused by a feedback mechanism in which the increase in dopamine prevents a conversion of tyrosine to dopamine. No relationship to clinical signs or behavioral effects was noted. Further work at the same exposure level indicates that the noradrenaline concentration remains significantly decreased for at least 20 hours after a l-hour exposure, but the dopamine levels return to normal (Magos et al. 1974). Female rats exposed to 777.1 ppm for 12 hours showed swollen brain mitochondria and elevated brain adenosine triphosphate (ATP) levels compared to controls (Tarkowski et al. 1980). However, no histopathological changes were noted in brain tissue. Under the same experimental conditions, rats exposed to 257 ppm for 5 hours a day, 5 days a week,

for 10 months showed biochemical changes that involved uncoupling of oxidative phosphorylation (Tarkowski et al. 1980).

Mice exposed to 0, 120, 580, 2,200, or 3,700 ppm carbon disulfide for 1 hour showed no behavioral changes at 120 ppm but did at 580 ppm and higher (Liang et al. 1983). The same study investigated an intermediate-duration exposure as well. Mice were exposed to 0, 260, 580, or 840 ppm carbon disulfide for 4 hours a day, 5 days a week, for approximately 30 days. No changes were observed at 260 ppm, but concentration-related decreased responses to operant behavior were observed at 580 ppm and at 840 ppm. The study is limited because there is no indication of the number of animals used. For the 30-day exposure study, there was no truly unexposed control, since some of the mice from the acute phase were used. The same mice were used for all doses in the intermediate phase, with l0-14 days between each dose exposure. It is therefore unclear whether the effects noted were due to the dose or were a cumulative effect of previous doses.

Male Wistar rats were exposed to 578 ppm carbon disulfide for 10 months. Also reported in the same paper were results from an 18-hour exposure to 803 ppm. The rats developed different signs of poisoning depending upon the type of exposure: the l0-month exposure caused loss of motor equilibrium, muscular weakness, and hind-limb paresis, and the acute dosing caused severe narcosis, reduced cardiac and respiratory rate, straightening of hind limbs, and lower body temperature. However, brain mitochondria in both groups of animals exhibited the same types of disturbances in oxidative phosphorylation-uncoupling of oxidative phosphorylation, decreased phosphorus-oxygen (P:O) ratio, and a lower ATP-inorganic phosphorus (ATP-P_i) exchange rate (Tarkowski and Sobczak 1971). Although this study used only six or seven animals and one dose, the results were consistent and do not conflict with other information regarding the effects of carbon disulfide on metabolism.

A narcotic-like stupor was observed during carbon disulfide exposure of rats at 600 ppm 10 hours a day for 14 days (Wilmarth et al. 1993). By the end of the study, mild ataxia and moderate hind-limb splay were also observed. Neurobehavioral effects were observed in rats exposed to 642.2 ppm of carbon disulfide for 4 hours a day, 5 days a week, for 6 weeks. No changes were seen after 3 weeks of exposure, but hind-limb extensor responses and motor coordination were impaired after 6 weeks of exposure. Recovery had occurred by 3 weeks after cessation of exposure. The carbon disulfide-exposed rats were stimulated less than the air-ventilated controls by 3 mg/kg of *d*-amphetamine, suggesting that repeated exposure to carbon disulfide affects the availability of brain

noradrenaline for release. This effect also disappeared by 3 weeks after exposure (Tilson et al. 1979). These results are consistent with those of Magos and Jarvis (1970) and Magos et al. (1974) (discussed above) in which noradrenaline was shown to be decreased in the brains of acutely exposed rats.

Motor capacity (static endurance and dynamic performance at forced motor activity) was studied in a total of 96 albino rats that were repeatedly exposed to 0 (42 males/group), 48, 385, or 770 ppm carbon disulfide via inhalation (18 males/group) (Frantik 1970). Acute toxicity was measured 0-60 minutes after termination of exposure, and chronic toxicity was measured 48-72 hours postexposure. After initial exposure to the 770-ppm dose there were reductions in spontaneous motor activity (60%), conditioned avoidance, and motor performance. Effects persisted for 24 hours but disappeared completely 3 days postexposure and failed to reappear after repeated experiments. Symptoms of motor impairment were observed after a variable latent period and were related to exposure concentration (385-ppm dose, 18 weeks; 770-ppm dose, 8 weeks). On the average, motor capacity (maximum speed and endurance at dynamic performance) was reduced by 40-50% at the 385-ppm dose and by more than 80% at the 770-ppm dose. Motor function recovered during the first 8 weeks. The study is limited by the lack of quantitative measurement of nervous system impairment.

Neuromuscular and sensory effects were evaluated in Long Evans rats exposed to 500 ppm (6 hours a day for 5 or 12 weeks) using an acoustic startle test (Clerici and Fechter 1991). Neuromuscular integrity was shown to be compromised based on auditory startle reflex amplitude; animals showed a 70% recovery 4 weeks postexposure. No clinical signs of neurotoxicity or changes in hearing function or acoustic tone thresholds were noted. Use of a single exposure concentration precluded assessment of dose response.

The chronic effect of carbon disulfide exposure on the central nervous system was examined by auditory brainstem responses (ABR) in female JCl Wistar rats (Hirata et al. 1992b). Rats were exposed by inhalation to 200 or 800 ppm, 6 hours a day, 5 days a week, for 15 weeks. Auditory responses were measured before exposure, every 3 weeks during exposure, and in weeks 2 and 6 after exposure. The delayed latencies of ABR were observed at 800 ppm suggesting a conduction dysfunction. The transient delay of ABR responses at 200 ppm indicated only slight conduction dysfunction. Rats recovered 2-6 weeks after carbon disulfide exposure.

Neuropathology has been investigated in several studies of carbon disulfide exposure in rats. The results are consistent with regard to effect but not with regard to the dose required to produce the effect. Axonal swellings, demyelination at axonal enlargements, swelling of nerve terminals at neuromuscular junctions, muscle atrophy and degeneration, damage of the terminal axons, myelin indentation, fiber breakdown, and distended mitochondria have been reported (Jirmanova and Lukas 1984; Juntenen et al. 1977; Szendzikowski et al. 1974). These experiments used only one dose, which varied between 482 and 770.7 ppm, and a control group; therefore, no dose response can be established using only these data. A study by Rebert and Becker (1986) attempted to establish a temporal dose-response relationship for peripheral nerve conduction activity. Their work with rats showed that visual-evoked potentials and conduction time in peripheral nerves and in brainstem auditory pathways were longer in animals exposed to 800 ppm, 7 hours a day, 7 days a week than in those exposed to 400 ppm for the same duration (11 weeks). The potentials in the groups exposed to lower levels were longer than in the controls, although the differences were not statistically significant. Four female monkeys exposed to 256 ppm for 6 hours a day, 5 days a week, for 5-13 weeks suffered permanent visual impairment with degeneration of retinal ganglion cells (Merigan et al. 1988). Visual acuity thresholds in two macaque female monkeys were severely disrupted after 5 weeks of intermittent exposure (6 hours a day, 5 days a week) to 256 ppm carbon disulfide. One monkey showed some recovery at 16 weeks postexposure; the other showed no improvement (Merigan et al. 1985).

Neurological effects such as hind-limb motor difficulties, reduced nerve conduction velocity, and degeneration of nerve fibers were seen in rats exposed to 700 ppm of carbon disulfide for 5 hours a day, 5 days a week, for 12 weeks (Colombi et al. 1981). These pathologies continued to 3 weeks postexposure but were slightly improved 6 weeks after carbon disulfide exposure. The improvement continued up to the 18th week of recovery, suggesting that the process may be reversible. This study was limited by the use of a single carbon disulfide dose. In another study, paralysis of hind limbs was observed in Wistar rats exposed to 546 ppm for 8 months (5 hours a day, 6 days a week); this effect was not seen at 321 ppm (Wronska-Nofer 1973).

Morphological changes in the peripheral nerve and the spinal cord were studied in rats and mice exposed to 50, 300, or 800 ppm carbon disulfide, 6 hours a day, 5 days a week, for 90 days (Gottfried et al. 1985; Toxigenics 1983a, 1983b, 1983c). Rats exposed to 50 ppm showed no changes in any parameters; rats exposed to 300 ppm showed only occasional swelling of axons in dorsal corticospinal

fibers of the lumbar spinal cord; and rats exposed to 800 ppm showed extensive neurofilamentous axonal swelling in the spinal cord. Neurofilamentous axonal swelling was particularly seen in the distal portion of long fiber tracts, including prominent swellings in the dorsal ascending sensory fibers, whereas it was only intermittently seen in the dorsal corticospinal fibers. In addition, extensive peripheral nerve changes were seen at the level of the posterior tibia1 nerve. The sciatic nerve showed no appreciable loss and only occasional axonal swelling. Ultrastructurally, the axonal swellings contained abundant disorganized neurofilaments, decreased microtubules, and thin or absent myelin. Brain and body weight were decreased in proportion to the concentration, with the decrease in brain weight statistically significant in the 800-ppm group.

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

2.2.1.5 Reproductive Effects

Data on reproductive effects of carbon disulfide in humans come from studies of occupational cohorts that are exposed primarily via inhalation to carbon disulfide in the workplace. These studies are limited by generally poor exposure measurements, concomitant exposures to other chemicals, and occasionally the lack of appropriate control groups. Nonetheless, the data provide some evidence that carbon disulfide may act on the reproductive system.

In some studies, effects in females included an increased incidence of spontaneous abortion at levels as low as about 2 ppm (6-7 mg/m^3) (Heinrichs 1983; Wang and Zhao 1987). Other epidemiological studies have not corroborated these reports, however. In a community study of spontaneous abortion, occupation, and air pollution, the study authors found no relationship between carbon disulfide concentrations and miscarriage rates (Hemminki and Niemi 1982). Another study reported that women exposed to 0.5-4.7 ppm (1.7-14.8 mg/m^3) had significantly more menstrual disorders than nonexposed women; however, there was no increase in the rate of spontaneous abortion, stillbirth, premature delivery, or congenital malformation (Zhou et al. 1988). Increased rates of menstrual disorders and toxemia of pregnancy were also reported in workers exposed to 12-18 ppm carbon disulfide (Cai and Bao 1981). However, concomitant exposures to other chemicals were not considered.

Researchers examined 15 men exposed in a viscose plant to unspecified concentrations of carbon disulfide and hydrogen sulfide for 10-36 years and compared them with 16 age-matched controls (Wagar et al. 1981). The carbon disulfide concentrations at the viscose plant were below 10 ppm just prior to the study, but the levels had been higher previously. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were significantly increased in the exposed workers. The study authors concluded that this was a sign of primary gonadal insufficiency. However, no changes were seen in serum testosterone or thyroid functions. Exposures were not well characterized, only a small number of workers were examined, and semen analysis was not performed; these limitations preclude developing conclusions from this study. In a National Institute for Occupational Safety and Health (NIOSH) study of 434 American workers, semen quality was evaluated in 86 exposed and 89 unexposed workers (Meyer 1981). The duration of carbon disulfide exposure ranged from 12 months to 257 months. Statistical analysis of sperm count, ejaculation volume, and morphology patterns showed no statistically significant difference between the exposed and unexposed groups. Exposures were not well characterized and were designated as definitely high (DH=18 workers), moderate (M=27 workers), definitely low (DL=22 workers), and difficult to quantify or other (O=19 workers). A further limitation was the possibility that the poor rate of worker participation (50%) in the semen sampling could have affected the results. In another workplace study, men (n=116) exposed to carbon disulfide at 0.3-9.6 ppm or >9.6 ppm complained of decreased libido and impotence more frequently than 79 unexposed controls (Vanhoorne et al. 1994). Reproductive histories did not reveal any effects on fertility. Examination of semen from 43 exposed workers and 35 controls did not show any adverse effects.

A review of some reports from the USSR and other Eastern Bloc countries indicates that, although these studies may be deficient in design and reporting of data, there is enough evidence to warrant concern about the effects of carbon disulfide on the female reproductive system. Reported adverse effects included menstrual disturbances, endocrine alterations, and increased incidence of miscarriage (Zielhuis et al. 1984).

Effects in males occupationally exposed to carbon disulfide have included teratospermia, decreased sperm motility, hypospermia, and decreased libido (Lancranjan 1972). Studies have stressed the importance of assessing the statistical power of a test for comparing sperm count or morphology (Wyrobek 1983) and the need for more clearly defining the link between changes in morphology and altered fertility (Schrag and Dixon 1985). The relationship between paternal exposure to carbon

disulfide and pregnancy was studied in 540 workers and their wives (NIOSH 1983). Patterns of fetal loss, number of births, and time between live births were examined by comparing pregnancies conceived after paternal exposure to carbon disulfide to pregnancies occurring in an unexposed population. Several statistical analyses showed no relationship between carbon disulfide exposure and fertility measures.

Evidence in animals supports the effect of carbon disulfide on the reproductive system. Male Long-Evans rats exposed to 600 ppm carbon disulfide 5 or 6 hours a day, 5 days a week, for 10 weeks showed significant alterations in copulatory behavior and a decrease in ejaculated sperm counts by the 4th and 7th weeks of exposure, respectively. Caudal epididymal sperm counts were not depressed and testes appeared histologically normal. The plasma testosterone levels were significantly reduced. Exposed rats had significantly reduced weight gains during the experiment (Tepe and Zenick 1984; Zenick et al. 1984).

The NOAEL and LOAEL values for reproductive effects in animals are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.6 Developmental Effects

Developmental effects of carbon disulfide have been reported in several animal studies. Inhalation exposure of pregnant rats did not produce congenital malformations at levels of 3.2 and 0.01 ppm. However, administration of 3.2 ppm was also associated with viability impairments and retardation of morphological and sensory development, and 0.1 ppm was associated with behavioral changes (Tabacova and Balabaeva 1980b). To study the reproductive toxicity and teratogenicity of carbon disulfide, other inhalation exposure studies of pregnant Wistar or Sprague-Dawley rats and New Zealand white rabbits exposed to 20 or 40 ppm carbon disulfide were conducted (Hardin et al. 1981; NIOSH 1980). Results showed an absence of maternal toxicity, fetal toxicity, and teratogenicity. These studies were limited by the absence of information regarding exposure conditions and the presentation of results as a summary of 10 tested chemicals with little experimental detail.

A developmental study was conducted using New Zealand White rabbits, which are more sensitive than rats to the effects of carbon disulfide (PA1 1991). In this study, rabbits (24/group) were exposed by inhalation to 0, 60, 100, 300, 600, or 1,200 ppm, 6 hours a day, on gestation days 6-18. Animals

were evaluated on day 29. Maternal toxicity was observed as reduced body weight gain and adverse clinical signs (ataxia, lowered food consumption, wheezing) in the 1,200-ppm group, with some sporadic hematologic alterations at 600 ppm (for example, decreased hematocrit on gestation day 19). These effects were not seen in an initial range-finding study in which rabbits were exposed to 1,000 ppm carbon disulfide. Embryotoxic effects (reduced mean fetal body weight) and post-implantation loss were seen in the 600- and 1,200-ppm exposure groups. Teratogenic effects (increased cumulative skeletal and visceral malformations) were also seen at 1,200 ppm carbon disulfide. In this study the NOAEL for developmental effects was 300 ppm and the NOAEL for maternal toxicity was 600 ppm because of the lack of biological significance in the hematologic findings at this exposure concentration.

In another developmental study, B6C3Fl mice and Fischer and Sprague-Dawley rats were exposed to 0, 49, 297, or 798 ppm carbon disulfide 6 hours a day, 5 days a week, for 90 days (Toxigenics 1983a, 1983b, 1983c). Examination of the mice and rats that received the highest dose revealed maternal toxicity but no developmental toxicity.

Based on data from mice exposed for 10 minutes to 750 ppm ^{14}C-labelled carbon disulfide, carbon disulfide and its metabolites pass the placenta at all stages of gestation and localize selectively in tissues reported to be target organs for the effects of this chemical (brain, blood, liver, and eye) (Danielsson et al. 1984). This finding provides evidence that carbon disulfide or its metabolites may exert effects directly on the embryo.

Maternal reproduction and fetal parameters were evaluated for groups of 20-23 pregnant Sprague-Dawley rats exposed to 0, 100, 200, 400, and 800 ppm carbon disulfide, 6 hours a day, during days 6-20 of gestation (Saillenfait et al. 1989). Maternal toxicity for the animals exposed to 400 or 800 ppm was evidenced by the significant decrease in maternal body weight gain (19% and 31% decrease) of the exposed rats compared to the controls ($p \leq 0.01$). When gravid uterine weight was subtracted from the dam's body weight gain, the maternal weight was significantly reduced by 56% and 144% versus the controls. Exposure to 400 or 800 ppm carbon disulfide resulted in a significant reduction of fetal body weights for both sexes; the respective weight reduction from control for males was 7% and 14% ($p \leq 0.0l$), and for females was 6% and 20% ($p \leq 0.01$). Clubfoot was the only external malformation occurring at higher frequency than in the controls; however, the one fetus affected in one litter at 400 ppm and the seven fetuses affected in five litters at 800 ppm were not

sufficient for statistical significance. Incidence of unossified stemebrae was significantly increased in groups exposed to 800 ppm ($p \geq 0.0l$), but no other soft tissue anomalies or major skeletal anomalies were present in any treated groups.

Behavioral and neurotoxic effects in the offspring of rats exposed via inhalation have been reported. Perinatal mortality was shown to be dose related to prenatal carbon disulfide exposure levels (225 and 642 ppm) in rats (Lehotzky et al. 1985). Exposure to 642 ppm carbon disulfide throughout pregnancy for 2 hours daily produced no malformations of fetuses in rats or mice but did increase the death rates of the embryos at all stages of intrauterine development (Yaroslavski 1969).

In a developmental study in which only the dams of both F_0 and F_1 generations were dosed during gestation, the F_1 offspring of albino rats were exposed for 8 hours daily throughout pregnancy to 64.2, 32.1, 3.2, or 0.01 ppm carbon disulfide and were postnatally exposed to the same exposure concentrations for the same duration (Tabacova et al. 1983). Both the F_1 and F_2 generations showed a marked increase in malformations and behavioral and learning changes at the highest concentration, but the F_2 generation also showed the same effects at the two lower concentrations. Maternal toxicity and teratogenic effects were observed in offspring of dams of all generations exposed to 32 or 64 ppm carbon disulfide. The two lower exposure levels, although nonteratogenic, provoked functional and behavioral disturbances of varying degree. The study authors suggested that carbon disulfide causes preconditioning to increased effects in the next generation. There are, however, no other data that support a conditioning effect of carbon disulfide. This study is limited by the lack of information on chemical exposure (including chemical purity, atmosphere verification, or analytical techniques) on the control animals, and on the methods used to select F_1 and F_2 generations. In addition, there was in excess of a 300-fold difference in concentration below the lowest treatment level (0.01 ppm) and the next higher level (3.2 ppm); there was not a clear dose response; there was no information on mode of control exposure; there was a lack of concurrent controls (separate control animals were used for higher and lower exposure animals); animal diet and housing conditions were not specified; and there was a lack of information regarding the significance of the increased behavioral effects measured in exposed animals (reduced exploratory activity and “increased emotional activity”). Finally, the low effect levels found in this study were not substantiated in subsequent inhalation studies by Saillenfait et al. (1989) and PAI (1991). However, the design of the Tabacova et al. (1983) study was significantly different from both the Saillenfait et al. (1989) and PAI (1991) studies. The lack of

substantiation of the Tabacova et al. (1983) study may be due to differences in the designs of the Saillenfait et al. (1989) and PAI (1991) studies.

All reliable LOAEL values for developmental effects are recorded in Table 2-l and plotted in Figure 2-1.

2.2.1.7 Genotoxic Effects

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

Carbon disulfide failed to produce significant chromosomal aberrations in the bone marrow of rats inhaling 20 and 40 ppm for either acute or intermediate periods (NIOSH 1980).

Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

There is no definitive evidence for an increased cancer potential from carbon disulfide in humans. The number of deaths due to neoplasms was compared in a cohort of rayon plant workers versus paper mill workers, and no significant differences in mortality were found between the years 1967 and 1982 (Nurminen and Hernberg 1985). Thus, there appears to be no association between occupational exposure to carbon disulfide and cancer in this study. (This information was reported incidentally in another study examining cardiovascular mortality, described in Section 2.2.1.2.) On the other hand, data supporting an increased odds ratio for lymphocytic leukemia in rubber workers exposed to several different kinds of solvents including carbon disulfide have been reported (Arp et al. 1983). Categories of exposure were based on process descriptions for the person's job classification and not on ambient air measurements. Jobs incorporating benzene use were specifically excluded. The odds ratio was not, however, statistically significant at $p<0.05$. Data did indicate a statistically significant ($p<0.00l$) odds ratio for exposure to carbon disulfide and development of lymphocytic leukemia when researchers carefully analyzed specific exposures in the group to solvents other than benzene (carbon disulfide, toluene, xylene, naphtha, ethanol, acetone, hexane, phenol, trichloroethylene, trichloroethane, and 13 others). This odds ratio was higher than that detected for benzene, which was not statistically

significant (Checkoway et al. 1984). Deaths from lymphatic leukemia in this same cohort were also shown to be associated with exposure to carbon disulfide (Wilcosky et al. 1984). There are several potential confounding factors in these analyses. All three reports are based on nested case-control studies from the same cohort of rubber workers. The small number of cases for each particular type of cancer examined and the large number of solvents used in the analysis indicate a need for cautious interpretation. Many of the solvents were used in mixtures so that identifying a single causal agent is not possible. Confounding factors from nonoccupational or other occupational exposures were not taken into account. In addition, the system designed to estimate historical exposures may have had weaknesses, specifically that the designation of "permitted to use" may not indicate actual use.

No studies were located regarding cancer in animals after inhalation exposure to carbon disulfide.

2.2.2 Oral Exposure

Humans are not likely to be exposed to significant quantities of carbon disulfide in food or water. Most information on the effects of oral exposure to carbon disulfide is derived from studies in animals. These studies are summarized in Table 2-2 and Figure 2-2, and the findings are discussed below. All doses are expressed as mg/kg/day.

2.2.2.1 Death

First-hand reports of death from oral exposure to carbon disulfide are very rare. Three case reports cited in Gosselin et al. (1984) indicate that half an ounce (concentration not specified) caused death following ingestion.

In mice, Gibson and Roberts (1972) determined the median oral lethal dose (LD_{50}) over a 24-hour period to be 3,020 mg carbon disulfide/kg. In Wistar rats, a single dose of carbon disulfide administered by gavage at doses up to 632 mg/kg did not cause death, nor were deaths noted in the rats after a 4-week (5 days a week) administration of 253 mg/kg/day carbon disulfide, also by gavage (Hoffmann and Klapperstück 1990). No deaths were noted after a 4-week (5 days a week) administration of 253 mg/kg/day carbon disulfide by gavage to Wistar rats (Hoffmann and Muller 1990).

2.2.2.2 Systemic Effects

The systemic toxicity of carbon disulfide after oral exposure is manifested primarily in the liver, mainly as enzymatic disruptions. Animal data show that hepatic toxicity is potentiated by pretreatment with agents that induce hepatic microsomal enzymes. Cardiovascular effects have also been reported, although these data are limited.

No studies were located regarding respiratory, gastrointestinal, renal, dermal, or ocular effects in humans or animals after oral exposure to carbon disulfide. Information on cardiovascular, hematological, musculoskeletal, hepatic, and body weight effects is presented below. The highest NOAELs and a reliable LOAEL for hepatic and cardiac effects in mice and rats, respectively, for the acute-duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to carbon disulfide.

Anesthetized male Wistar rats acutely exposed to 632 mg carbon disulfide/kg (0.5 mL/kg) had a significantly reduced occurrence of some cardiac arrhythmias after coronary occlusion by surgical ligation when compared to controls (Hoffman 1987). In addition, the heart rate measured before the procedure was significantly lower in exposed rats than in the controls (Hoffman 1987).

Exposure of anesthetized male rats at a lower gavage dose (253 mg/kg/day) daily for 4 weeks produced a decrease in left ventricular systolic pressure and changes in an electrocardiograph (Hoffmann and Klapperstück 1990). When coronary ligation was performed 1 hour after exposure, these animals showed an earlier appearance of cardiac arrhythmias, delay in acotroitine-induced arrhythmia, and decreased survival. No exposure-related effects occurred at a lower dose level (126 mg/kg). This study also examined the effects of a single gavage administration of carbon disulfide on ECG parameters in anesthetized rats. Changes in ECG were noted at 373 and 506 mg/kg; dose response was noted in QT_c. Heart rate was decreased at 632 mg/kg. No significant changes in contractile force of the left ventricle were noted.

In conscious male Wistar rats administered 506 mg/kg carbon disulfide once by gavage, blood pressure was decreased ($p \leq 0.001$) 5 hours after treatment, but this change was reversible in another 5 hours

TABLE 2-2. Levels of Significant Exposure to Carbon Disulfide - Oral

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Wistar)	Once (GO)	Cardio	253 M	506 M (decreased blood pressure)		Hoffman and Klapperstuck 1990
2	Mouse (ddY)	1-14 d 1x/d (GO)	Hepatic		3[b] M (decrease in P-450 and drug metabolizing enzymes)		Masuda et al. 1986
3	Rabbit (New Zealand White)	14 d Gd 9-19 (GO)	Bd Wt			25 F (decrease in body weight gain - 43%)	Jones-Price et al. 1984b
	Neurological						
4	Rat (outbred Zealand White)	10 d Gd 6-15 (GO)		200 F		400 F (hindlimb paralysis)	Jones-Price et al. 1984a
5	Rat (Sprague-Dawley)	Once (G)			300 M (significant decreases in norepinephrine in the midbrain, hypothalamus, and medulla oblongata)		Kanada et al. 1994
6	Rabbit (New Zealand White)	14 d Gd 9-19 (GO)				25 F (hindlimb paralysis)	Jones-Price et al. 1984b
	Developmental						
7	Rat (outbred)	10 d Gd 6-15 (GO)		100 F	200 F (reduced fetal weight)		Jones-Price et al. 1984a

TABLE 2-2. Levels of Significant Exposure to Carbon Disulfide - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
8	Rabbit (New Zealand)	14 d Gd 9-19 (GO)				25 F (increased percent resorptions)	Jones-Price et al. 1984b
	INTERMEDIATE EXPOSURE						
	Systemic						
9	Rat (Wistar)	4 wk 5d/wk 1x/d (GO)	Cardio	253 M			Hoffmann and Klapperstuck 1990
			Bd Wt	126 M	253 M (decreased body weight - 10%)		

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute Minimal Risk Level (MRL) of 0.01 mg/kg/day for carbon disulfide; the LOAEL of 3 mg/kg/day was divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for interhuman variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); F = female; Gd = gestation day; GO = gavage - oil; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; wk = week(s); x = time(s)

Figure 2-2. Levels of Significant Exposure to Carbon Disulfide – Oral

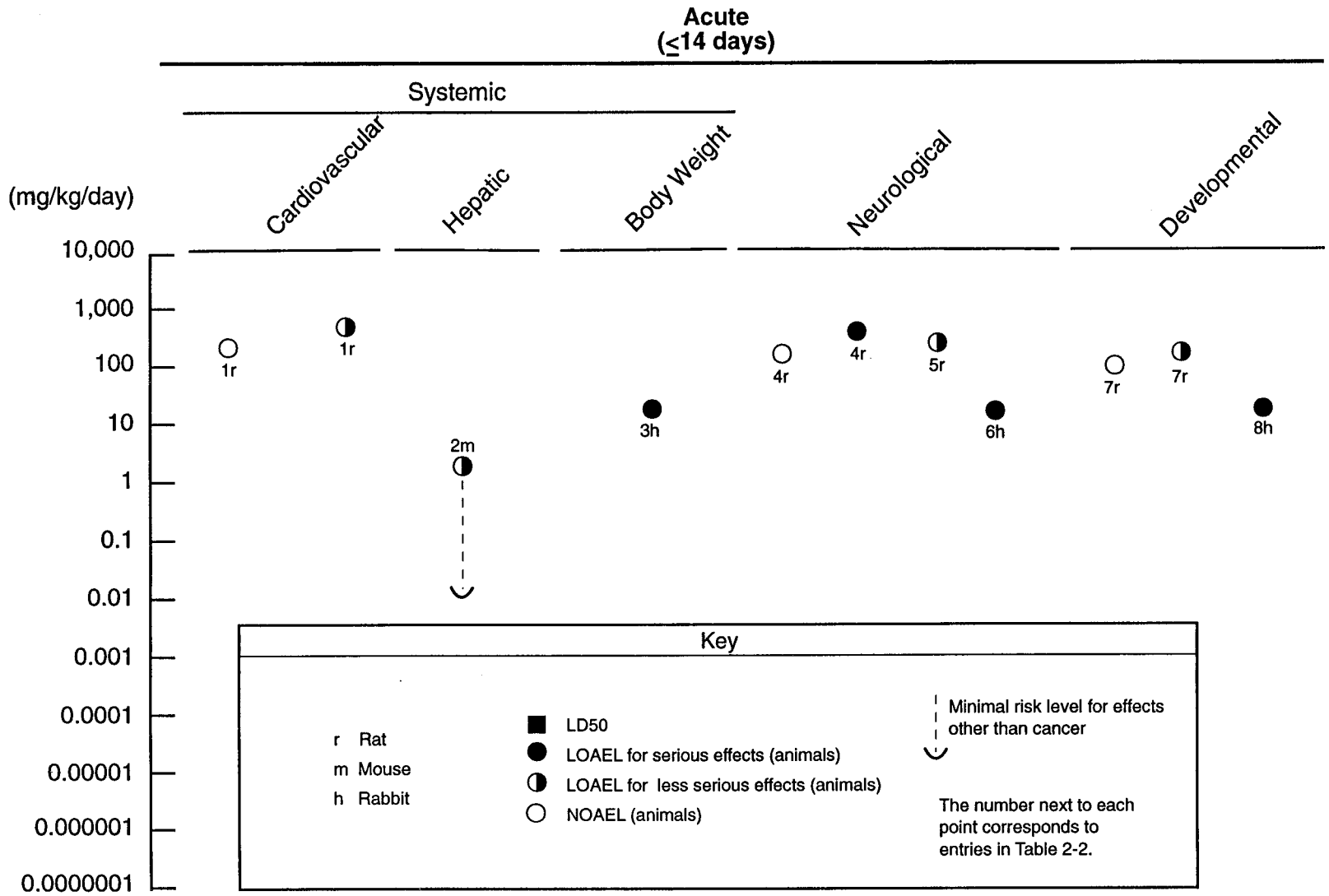
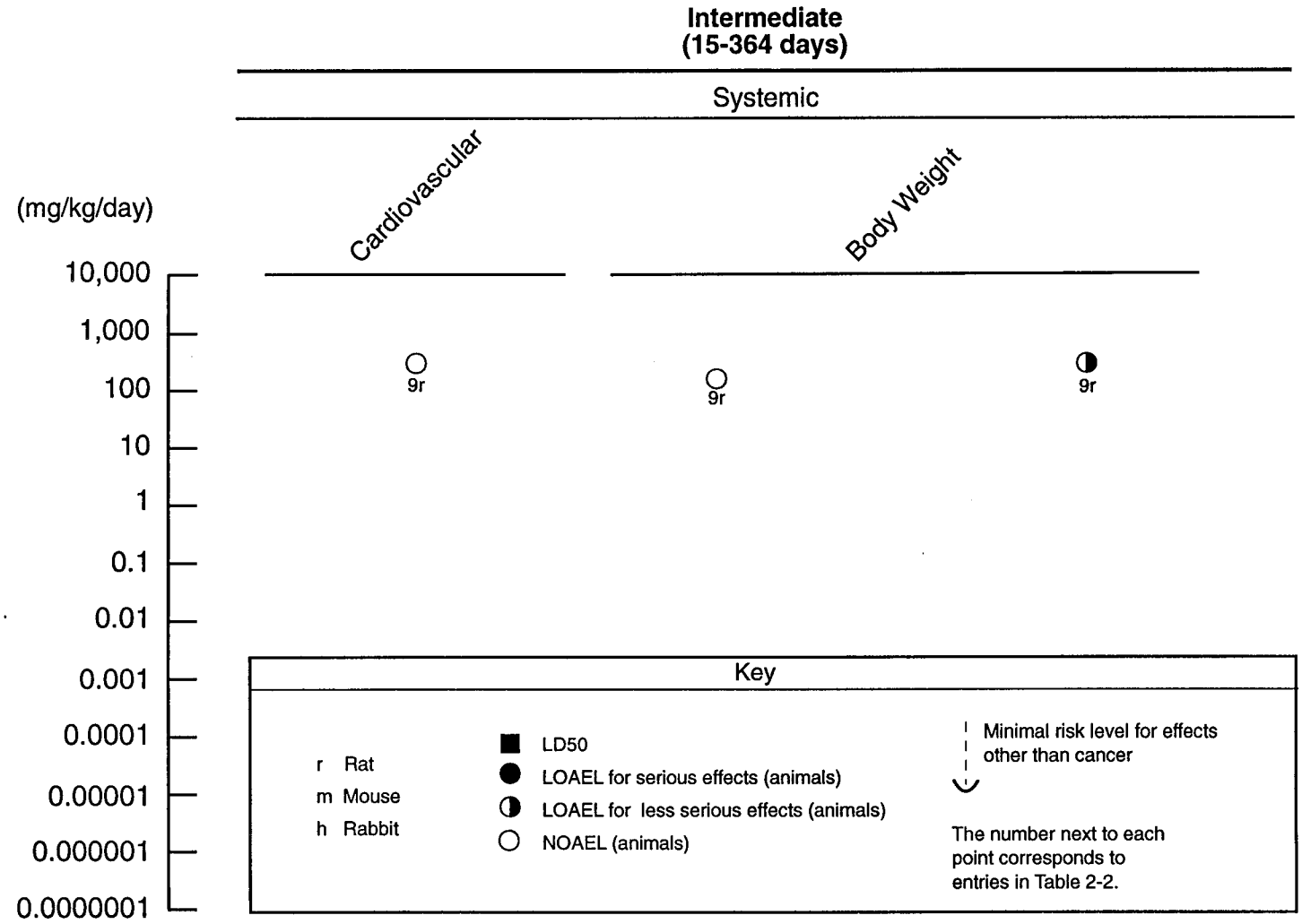


Figure 2-2. Levels of Significant Exposure to Carbon Disulfide – Oral (continued)



(Hoffmann and Klapperstück 1990). The NOAEL was 253 mg/kg. In subacute experiments, no effects on blood pressure or heart rate were observed in rats after receiving carbon disulfide at 126 or 253 mg/kg/day for 4 weeks; however, the study authors did not examine the effects on these parameters at 506 mg/kg/day.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to carbon disulfide.

In a study by Pilarska et al. (1973), rats administered 25 mg carbon disulfide/kg/day for 60 days developed normochromic and normocytic anemia, eosinopenia, and an increase in reticulocyte cell numbers. No changes in leukocyte or platelet numbers were observed, however. No other studies were found on the hematological effects in animals after oral exposure to carbon disulfide.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to carbon disulfide.

In rats treated by gavage with carbon disulfide in olive oil at 12.5 mg/kg/day for 1, 2, or 4 weeks, a decreased response of the ancoccygeus muscle to noradrenaline was observed relative to vehicle-treated controls (Gandhi and Venkatakrishna-Bhatt 1993). The response of the muscle to noradrenaline was tested *in vitro* in a muscle bath. The reduction in sensitivity to noradrenaline increased with increasing duration of exposure. The investigators suggested that the mechanism of decreased noradrenaline responsiveness may be a result of a block of calcium influx, a delay of the calcium efflux, an inhibition of the uptake of calcium, a decreased sensitivity to calcium by the muscle, or a combination of these mechanisms.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to carbon disulfide.

In animals, oral exposure to carbon disulfide does not appear to cause significant liver toxicity, although the data are limited. Male mice orally exposed to 3-300 mg/kg/day (1-14 days) have shown rapid, reversible, dose-related suppression of hepatic microsomal enzymes (Masuda and Yasoshima 1988; Masuda et al. 1986). An acute-duration MRL of 0.01 mg/kg/day was derived based on dose-dependent decreases in the activities of liver microsomal drug-metabolizing enzymes in mice (Masuda

et al. 1986). The LOAEL for this effect was 3 mg/kg/day. The following enzyme activities were decreased: hydroxylation of aniline, *O*-dealkylation of *p*-nitroanisole, 7-ethoxycoumarin and 7-ethoxyresorufin, *N*-demethylation of *N,N*-dimethylaniline, NADPH-cytochrome P-450 reductase activity, and P-450-associated peroxidase activity. In addition, a decrease in cytochrome P-450 content and total heme content was observed. The inhibition of enzyme activities was reversible. There were no effects on the activities of NADH-ferricyanide reductase, NADPH-cytochrome c reductase, flavin-containing monoxygenase, UDP-glucuronyltransferase, glucose-6-phosphatase, heme oxygenase, and glutathione *S*-transferase. Also, the content of cytochrome b_5 was not altered. In rats and mice, single oral doses of 1,263 mg/kg/day have caused necrotic lesions, suppression of microsomal enzymes, fat accumulation, and increased liver weight. These effects are significantly enhanced by pretreatment with a microsomal enzyme inducer such as phenobarbitone (Bond and DeMatteis 1969; Bond et al. 1969; El-Masry et al. 1976; Freundt et al. 1974a). These studies are limited by the use of an unspecified or small number of animals and, in the case of Bond et al. (1969), by the lack of quantitative analysis.

In sheep pretreated with DDT, a dose of 63.2 mg carbon disulfide/kg/day was reported to cause hepatic lesions accompanied by microsomal enzyme suppression and increases in total liver water and electrolytes (Wilkie et al. 1985). This study is of limited value because of its lack of controls to eliminate or account for possible synergistic effects of carbon disulfide and DDT.

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

In a study by Hoffmann and Klapperstuck (1990), male Wistar rats that had been administered 253 mg/kg/day carbon disulfide for 4 weeks by gavage showed a 10% decrease in body weight compared to the controls. In a developmental toxicity study, gestational exposure to 25 mg/kg/day for 10 days produced a 19% decrease in the maternal body weight gain of female New Zealand rabbits (Jones-Price et al. 1984b).

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 carbon disulfide.

2.2.2.4 Neurological Effects

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

Compared to untreated rats, significant decreases in noradrenaline in the midbrain, hypothalamus, and medulla oblongata were observed in rats 2 hours after they received a single gavage dose of 300 mg carbon disulfide/kg body weight (Kanada et al. 1994). 3,4-Dihydroxyphenylalanine was significantly increased in the midbrain, as was dopamine in the medulla oblongata. In the hippocampus, no change in acetylcholine was observed. Clinical signs were not reported in this study. Hind-limb paralysis was noted in New Zealand rabbits and outbred rats administered 25 and 400 mg/kg/day, respectively, for l0-14 days during gestation (Jones-Price et al. 1984a, 1984b).

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after oral exposure to carbon disulfide.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to carbon disulfide.

Administration of carbon disulfide by oral gavage at doses up to 150 mg/kg/day to New Zealand white rabbits (gestational days 9-19) and 600 mg/kg/day to albino rats (gestational days 6-15) resulted in signs of maternal toxicity, such as transient hind-limb paralysis and loss of body weight over the period of gestation (Jones-Price et al. 1984a, 1984b). In rabbits, there were statistically significant, dose-related increases in the number of resorptions: mean values of 12, 43, 42, and 61 resorption at 0, 25, 75, and 150 mg/kg/day, respectively. Also, dead fetuses were found: 13%, 33%, 43%, and 62% dead/litter for each respective dose group ($p<0.00l$). Average fetal body weight was decreased significantly in each dose group: 45.5, 45.2, 41.6, 39.5 g/litter for each dose group, respectively. In rabbits, there was a significant increase in the number of malformed fetuses in 150-mg/kg/day (high-dose) animals; however, there was no characteristic pattern of carbon disulfide-related malformations.

Males were affected to a greater extent than females. The teratogenic effect of carbon disulfide appears to be more severe in males at the 150-mg/kg/day dose than in females (when separated by dose, $p<0.036$ for males and $p<0.481$ for females), whereas the percentage of live fetuses and the average fetal body weight are not sex-dependent. In contrast, rats exhibited only decreased average fetal weight at doses greater than 200 mg/kg/day (moderate dose). No increase in resorption, fetal deaths, or malformations was found in carbon disulfide-treated rats.

The highest NOAEL and a reliable LOAEL for developmental effects in rats and rabbits are recorded in Table 2-2 and are plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after oral exposure to carbon disulfide.

Genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

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

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to carbon disulfide.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or ocular effects in humans or animals following dermal exposure to carbon disulfide.

Dermal Effects. Dermal contact with carbon disulfide is important in the occupational setting (Dutkiewicz and Baranowska 1967). Viscose rayon workers have been reported to develop serious blisters that progressed to hemorrhagic blisters covered by a thin membrane. These blisters appeared on the fingers in spite of wearing rubber gloves (Hueper 1936).

The implication of carbon disulfide as the causative agent is supported by experiments in rabbits whose ears were dermally exposed to carbon disulfide. Blisters similar to those found in the rayon workers developed on the animals' ears despite protective covering (Hueper 1936).

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

2.2.3.3 Immunological and Lymphoreticular Effects
2.2.3.4 Neurological Effects
2.2.3.5 Reproductive Effects
2.2.3.6 Developmental Effects
2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

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

2.3 TOXICOKINETICS

The available data from human and animal studies indicate that carbon disulfide is extensively and rapidly absorbed via inhalation, oral, and dermal routes. Absorbed carbon disulfide is distributed throughout the body. Because of its lipophilic nature, its distribution is greatest in organs such as the brain and liver where it is metabolized to thiocarbamates. Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate which either spontaneously degrades to atomic sulfur and carbonyl sulfide or hydrolyzes to form atomic sulfur and monothiocarbonate. Carbonyl

sulfide is converted to monothiocarbonate by carbonic anhydrase. Monothiocarbonate degrades to generate carbonyl sulfide or forms carbon dioxide and hydrogen sulfide. Unlike in animals, oxidation of sulfur to inorganic sulfate does not contribute significantly to the metabolism of carbon disulfide in humans. Despite the differences in the metabolism of carbon disulfide between animals and humans, dithiocarbamates are the common metabolites formed in these species after reaction with amino acids. These metabolites contribute in part to the neurotoxic effects of carbon disulfide.

The kidneys are the primary route of excretion of carbon disulfide metabolites. Conjugation of carbon disulfide or carbonyl sulfide with endogenous glutathione results in formation of thiozolidine-2-thione-4-carboxylic acid and 2-oxythiazolidine-4-carboxylic acid, respectively, which are excreted in the urine. The unmetabolized carbon disulfide is excreted unchanged in the breath, and small amounts (<l%) have been detected in the urine.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Studies conducted on human subjects reported rapid and extensive absorption of inhaled carbon disulfide. Rapid absorption was demonstrated in a study conducted on volunteers exposed to 17-51 ppm for l-4 hours (Teisinger and Soucek 1949). The amounts of carbon disulfide retained in the body and excreted by the lungs and kidneys were determined by measuring the carbon disulfide in inspired and expired air, blood, and urine during and after completion of the experiment until it disappeared from the urine and blood. About 80% of the inhaled carbon disulfide was retained during the first 15 minutes of exposure which decreased to about 40% after 45 minutes and remained at that level for the rest of the exposure period. The degree of retention did not depend on the exposure concentration. Only 5% of the retained carbon disulfide at the end of the exposure period was subsequently eliminated in the exhaled air. About 0.06% of the retained carbon disulfide was excreted unchanged in the urine and was detectable 24 hours after exposure. In another retention study involving exposure to vapor for an unspecified period (Soucek 1957), about 10-30% of the retained carbon disulfide was exhaled, and less than 1% was excreted in urine as carbon disulfide. The concentration of inhaled carbon disulfide was not reported. About 70-90% was metabolized.

Studies in animals indicate that carbon disulfide is rapidly absorbed following inhalation exposure. Absorption of carbon disulfide was studied by evaluating pulmonary and urinary excretion of carbon disulfide during and after exposure. Studies in rabbits indicate that an equilibrium concentration of carbon disulfide is reached after inhalation exposure to 20-150 ppm for 1.5-2.0 hours (Toyama and Kusano 1953). About 70-80% of the inhaled carbon disulfide was absorbed. After termination of exposure, 15-30% of the absorbed carbon disulfide was excreted through the lungs and less than 0.1% by the kidneys. In dogs exposed to 25-60 ppm carbon disulfide, equilibrium concentrations in blood were attained after 0.5-2.0 hours (McKee et al. 1943). Desaturation of blood carbon disulfide was almost complete within the first 30-60 minutes after exposure. Approximately 8-13% of the retained carbon disulfide was exhaled, less than 0.5% was excreted in the urine, and none was excreted in the feces. Excretion in the urine occurred within 2 hours of exposure. Freundt et al. (1975) observed that an equilibrium concentration of carbon disulfide in blood was attained after exposure of rats to 400 ppm carbon disulfide for 1 hour. Equilibrium was reached in liver and blood between 1 and 8 hours after exposure. Elimination of free carbon disulfide from these tissues was rapid, with an estimated half-life in the blood of 35 minutes and in the liver of approximately 1 hour.

The data presented above indicate that carbon disulfide is absorbed by humans and animals following inhalation exposure and reaches equilibrium rapidly (0.5-8 hours) across a wide range of doses and exposure durations.

2.3.1.2 Oral Exposure

No studies were located regarding absorption of carbon disulfide following oral exposure of humans. In rats, intragastric administration of 10 mg/kg ^{14}C-carbon disulfide resulted in exhalation of 63% of the dose within 4 hours as unchanged carbon disulfide (DeMatteis and Seawright 1973). It is evident from these results that a large fraction of orally administered carbon disulfide is absorbed by rats.

2.3.1.3 Dermal Exposure

Dermal exposure of humans to aqueous solutions of carbon disulfide resulted in significant absorption through the skin. A series of experiments were performed to investigate the rate of absorption of carbon disulfide by immersion of the hand in aqueous solutions of increasing concentrations (0.33-1.67 g/L) for 1 hour (Dutkiewicz and Baranowska 1967). Absorption was calculated indirectly

by determining carbon disulfide elimination by the lung or directly by measuring carbon disulfide concentration in the solutions before and after immersion of the hand. Rates of absorption of carbon disulfide, determined from analysis of the solutions, ranged from 0.232 to 0.789 mg/cm^2/hour and were about 10 times higher than rates calculated from lung excretion of carbon disulfide. In the former case, 25% of the absorbed dose was exhaled in the desaturation period; in the latter, only 3% was eliminated in the expired air. These findings suggest that carbon disulfide excretion varies with the route of absorption. This study provided only brief details of the experimental procedure, and therefore factors other than absorption through the skin (e.g., evaporation) may have accounted for the reduced carbon disulfide concentration noted at the end of the experimental period. Nevertheless, these results suggest that rapid absorption of carbon disulfide can occur in humans through skin. Occupational exposure of persons with pathological skin conditions has also been noted to increase the dermal absorption of carbon disulfide (Drexler et al. 1995a).

The limited information available on skin absorption in animals indicates that carbon disulfide is appreciably absorbed. Exposure of rabbit skin to high concentrations of the vapor (800 ppm and above) for 1 hour resulted in detectable amounts of carbon disulfide in the breath (Cohen et al. 1958). A linear relationship was noted between the dermal exposure concentration and the amount of carbon disulfide exhaled. No detectable carbon disulfide was found in the breath of rabbits exposed to 150 ppm vapor by skin contact for 6 hours (Cohen et al. 1958).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

Absorbed carbon disulfide is taken up by the blood (McKee et al. 1943) and is distributed throughout the body (Brieger 1967). Because of the lipophilic nature of carbon disulfide, distribution is greatest to lipid-rich tissues and organs such as the brain and liver where it is metabolized to dithiocarbamate (Santodonato et al. 1985). Milk from nursing mothers occupationally exposed to carbon disulfide was found to contain an average of 12.3 μg carbon disulfide/l00 mL (Cai and Bao 1981). Exposure concentrations of carbon disulfide ranged from 9.3 to 21.1 ppm for a 6.5-hour period. Exposure to 7.4-40 ppm for a shorter duration (2-4 hours) resulted in a lower average milk concentration of 6.8 μg/l00 mL.

The distribution of carbon disulfide following inhalation exposure has been studied in rabbits and rats (Toyama and Kusano 1953). In rabbits, blood equilibrium concentrations of carbon disulfide were reached after exposure to 20-150 ppm for 1.5-2.0 hours. In rats exposed to 60-350 ppm carbon disulfide, distribution was primarily to the brain, kidney, and liver. In contrast to rabbits, blood equilibrium concentrations for various carbon disulfide exposures in rats were not determined. Although carbon disulfide was rapidly eliminated from rat tissues during the first 6-8 hours after exposure, low concentrations of carbon disulfide were still detected in the tissues 20 hours after exposure. A separate study reported that equilibrium concentrations of carbon disulfide in blood were attained in dogs after 0.5-2.0 hours of exposure to 25-60 ppm carbon disulfide (McKee et al. 1943). Desaturation was largely complete within the first 30-60 minutes after inhalation exposure. Anesthetized male Sprague-Dawley rats exposed to 640 ppm carbon disulfide had an exponential increase in carbon disulfide in the blood which reached an apparently steady state after 90 minutes of exposure. After discontinuation of exposure, the blood concentration decreased rapidly, with elimination half-lives reported to be 6 and 85 minutes for the fast and slow components, respectively. In all tissues except fat, the carbon disulfide concentration approached steady state within 4-5 hours of exposure. Loss of free carbon disulfide was rapid from all tissues except the liver and kidneys, which retained 25% and 29%, respectively, at 8 hours postexposure (McKenna and DiStefano 1977a).

Inhalation exposure of pregnant mice to carbon disulfide during gestation resulted in rapid absorption and distribution of carbon disulfide and its metabolites in embryonic and fetal tissues within 1 hour (Danielsson et al. 1984). Pregnant mice were exposed via inhalation to 25 microcuries (μCi) ^{35}S- or ^{14}C-carbon disulfide for 10 minutes on day 9, 14, or 17 of gestation. The levels of ^{35}S-labelled metabolites in the embryonic neuroepithelium were higher in the fetal brain than in the maternal brain during early gestation (day 9). The concentrations in the fetal brain, eyes, and skeleton exceeded that of other fetal organs during mid-gestation (day 14). In late gestation (day 17), the levels in the fetal and maternal brain were relatively low, but high uptake of radioactivity was seen in the placenta, fetal blood, liver, and eyes. During early gestation, the distribution of ^{14}C-labelled metabolites was similar to that of ^{35}S-labelled metabolites with an immediate higher uptake in the embryo (including neuroepithelium) than in the maternal serum. On days 14 and 17 of gestation, radioactivity was present in the ventricle of the fetal brain. High levels were detected in the fetal liver and blood at late gestation (day 17). In contrast to ^{35}S-labelled metabolites, ^{14}C-labelled metabolites were retained longer (up to 24 hours) in the fetal brain and liver. High concentrations of ^{14}C-labelled metabolites were also seen in the fetal urinary tract. Thus, the distribution pattern varied with the age of the

conceptus and also with the radiolabel of carbon disulfide. These results indicate that carbon disulfide and its metabolites pass through the placenta at all stages of gestation and localize selectively in various tissues of the body.

2.3.2.2 Oral Exposure

No studies were located regarding distribution of carbon disulfide in humans or animals following oral exposure.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of carbon disulfide in humans or animals following dermal exposure.

2.3.2.4 Other Routes of Exposure

The distribution of free carbon disulfide and bound carbon disulfide liberated by acid hydrolysis was investigated in the tissues of white rats after a large, single subcutaneous dose (approximately 361 mg/kg) of carbon disulfide (Bartonicek 1957, 1959). Results of these studies indicate that following absorption, free carbon disulfide is rapidly removed from the blood and tissues. Negligible blood levels were present 11 hours after the dose was administered (Bartonicek 1957, 1959). Initially, free carbon disulfide accumulated in the blood, adrenals, and brain, but levels in the organs rapidly decreased, and only very small amounts were present after 10-16 hours.

A similar rapid reduction of free carbon disulfide levels in the blood was noted when radiolabelled ^{35}S-carbon disulfide was administered parenterally to guinea pigs (Strittmatter et al. 1950). About 20-50% of intracardially injected ^{35}S-carbon disulfide was retained; the amount of material retained depended on the concentration of dose administered. The largest amount of radiolabel appeared in the liver (0.43 μg) and the least amount in the brain (0.05 μg) at 1.5 hours following injection. Only 10% of the labelled compound remained in the tissues after 48 hours. Urinary and fecal excretion was not reported. In guinea pigs exposed to carbon disulfide vapors (13.6-25.7 ppm), the brain and blood contained more ^{35}S-label relative to the liver. Forty-eight hours later, 30-50% of ^{35}S-label remained in the tissues such as blood, liver, brain, kidney, and skin. The urinalyses revealed that urinary ^{35}S-label

was about 30% of the retained sulfur, with about 85% or 90% of it appearing in the first 24-hour output, the larger part of the metabolized material in the urine being excreted as inorganic sulfate. The feces contained about 5-15% metabolized ^{35}S-label, the amount of which increased with the increasing dose of carbon disulfide.

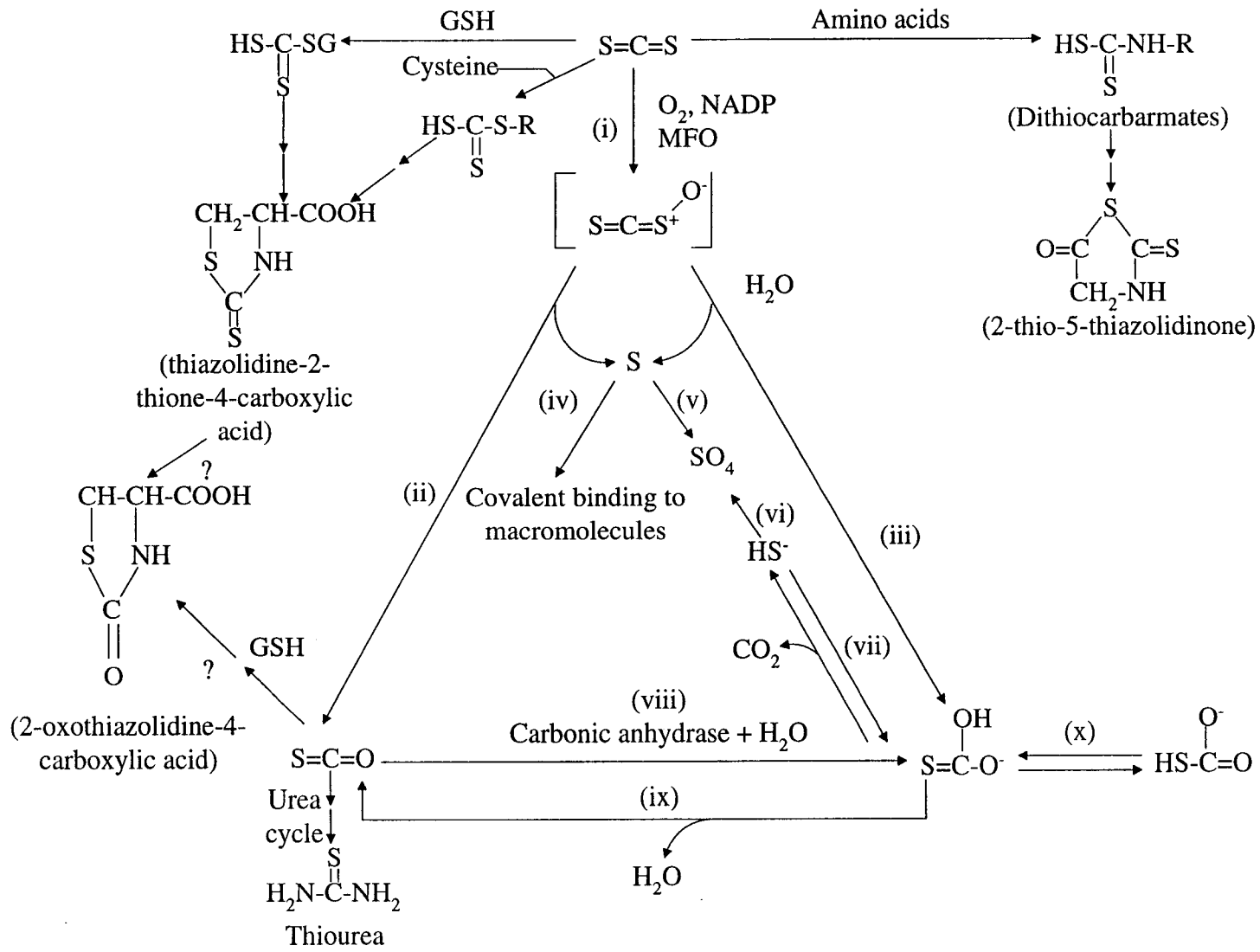
Only metabolites of carbon disulfide were found 3 hours after a dose of ^{14}C- or ^{35}S-labeled carbon disulfide was intraperitoneally administered (Snyderwine and Hunter 1987). Distribution varied with the age of the rat and the radiolabel injected. Following intraperitoneal administration of ^{14}C-carbon disulfide, 4-9% of the dose was metabolized to carbon dioxide depending on age. Significantly more carbon disulfide was metabolized to carbon dioxide by 30- and 40-day-old rats than by 1-20-day-old rats. The biotransformation products of carbon disulfide which were covalently bound remained in tissues from rats of all ages. Twenty-four hours after dosing with ^{35}S-labeled carbon disulfide, up to 13 times more labeled metabolites were covalently bound in organs from l-day-old rats than in similar organs from 40-day-old rats.

The data presented above indicate that the absorbed carbon disulfide is rapidly distributed via blood to other tissues irrespective of the route of exposure.

2.3.3 Metabolism

Limited information is available on the biotransformation of carbon disulfide in humans, and the metabolic products of carbon disulfide are not completely known. In animals and humans the proposed metabolic pathways involved in the metabolism of carbon disulfide (Beauchamp et al. 1983) are depicted in Figure 2-3, reactions i-x. Reaction i has been demonstrated in *in vivo* animal studies and in *in vitro* assays. Reactions ii-v are proven by *in vitro* studies, while products of reactions vi-ix are the results of proposed metabolic pathways of carbon disulfide in animals and humans. Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate (reaction i). The intermediate may either spontaneously degrade to atomic sulfur and carbonyl sulfide (reaction ii) or hydrolyze to form atomic sulfur and monothiocarbonate (reaction iii). The atomic sulfur generated in these reactions may either covalently bind to macromolecules (reaction iv) or be oxidized to products such as sulfate (reaction v). The carbonyl sulfide formed in reaction ii may be converted to monothiocarbonate by carbonic anhydrase (reaction viii). Monothiocarbonate may further spontaneously degrade in reaction ix, regenerating carbonyl sulfide or forming carbon dioxide and

FIGURE 2-3. Proposed Metabolic Pathways for Carbon Disulfide*



*Source: Beauchamp et al. 1983

sulfide bisulfide ion (HS^-) (reaction vii). The HS^- formed in reaction vii can subsequently be oxidized to sulfate or other nonvolatile metabolites (reaction vi).

Dithiocarbamates are the products of the reaction of carbon disulfide with amino acids (Brieger 1967). *In vitro* studies demonstrated that carbon disulfide readily combines with the amino acids in human blood, the half-life of this reaction being approximately 6.5 hours (Soucek 1957). Thiocarbamide has been found in the urine of exposed workers (Pergal et al. 1972b). After inhalation exposure of male subjects, up to 90% of the retained carbon disulfide was metabolized while the remainder was eliminated unchanged by various routes (McKee et al. 1943). High levels of thiocarbamide and trace amounts of 2-thio-5-thiazolidinone were identified by chromatographic analysis of the urine of workers exposed to carbon disulfide by inhalation (Pergal et al. 1972a, 1972b). Van Doorn et al. (1981a, 1981b) reported conjugation of carbon disulfide or carbonyl sulfide with endogenous glutathione to yield thiazolidine-2-thione-4-carboxylic acid and 2-oxythiazolidine-4-carboxylic acid, respectively. High concentrations (approximately 320 mM) of thiazolidine-2-thione-4-carboxylic acid (TTCA) were detected in the urine of women exposed to approximately 32 ppm (100 mg/m^3) carbon disulfide through inhalation (refer to Figure 2-3).

In contrast to the results obtained in animals, oxidation to inorganic sulfate does not appear to contribute significantly to the metabolism of carbon disulfide in humans. A marked increase in inorganic sulfate excretion in the urine was noted in a case study of a young worker with signs of carbon disulfide poisoning because of exposure to high levels of the vapor; no increase was noted in the amount of inorganic sulfate excreted in the urine (Djerassi and Lumbroso 1968). However, exact dose, mode of exposure, and duration were not presented in the study.

Carbon disulfide is oxidized by the liver mixed-function oxidase (MFO) system to carbonyl sulfide, which then undergoes further desulfurization, releasing elemental sulfur. This reaction has been shown to occur in vitro (Dalvi et al. 1974; DeMatteis 1974). *In vivo* studies in rats using ^{14}C-labelled carbon disulfide demonstrated that significant amounts (80%) of $^{14}CO_2$, are exhaled after exposure to carbon disulfide. Following intraperitoneal administration of approximately 100 mg carbon disulfide/kg, about 5% of the total dose was excreted in the breath as carbon dioxide. This amount was increased to 13% in animals pretreated with phenobarbital to induce liver microsomal enzymes (DeMatteis and Seawright 1973). Snyderwine and Hunter (1987) found that 4-9% of an intraperitoneally administered dose of ^{14}C-carbon disulfide was excreted as $^{14}CO_2$ in expired air, with 30- and 40-day-old rats

excreting more (9% versus 4%) $^{14}CO_2$, than 1-20-day-old rats. This was attributed to the increased hepatic MFO of carbon disulfide to carbon dioxide in 30-40-day-old rats.

The metabolic formation of carbonyl sulfide from carbon disulfide was confirmed in an in *vivo* study (Dalvi and Neal 1978). After intraperitoneal injection of ^{14}C-carbon disulfide in nonpretreated rats, carbonyl sulfide was excreted by the lung in greater quantities than carbon dioxide. Pretreatment with phenobarbital, however, resulted in a greater amount of excretion of carbon dioxide than carbonyl sulfide. In both experiments, excretion of ^{14}C-carbonyl sulfide and carbon dioxide accounted for 14-43% of the total administered radioactivity, with about twice as much carbon dioxide. These results indicate that phenobarbital treatment caused induction of cytochrome P-450 which catalyzed the conversion of carbon disulfide to carbonyl sulfide faster in pretreated rats than in rats not pretreated with phenobarbital. The role of the cytochrome P-450 monooxygenase system in catalyzing carbonyl sulfide formation was also confirmed by *in vitro* studies (Dalvi et al. 1974, 1975). The rate of carbonyl sulfide formation was NADPH-dependent and increased with microsomes obtained from phenobarbital-treated rats.

In a study designed to examine the effect of P-450 induction on the metabolism of carbon disulfide to TTCA, rats were treated with nothing, ethanol, phenobarbital, 3-methylcholanthrene, or phenobarbital and ethanol before being exposed to carbon disulfide at 50 ppm for 6 hours (Kivisto et al. 1995). After 7 days the pretreatment regimens were repeated in the same rats, and the rats were again exposed to carbon disulfide at 500 ppm for 6 hours. None of the inducers had any effect on urinary excretion of TTCA. About 7.6% and 2.3% of the dose was excreted as TTCA at 50 and 500 ppm, respectively, suggesting saturation. However, the investigators speculated that saturation may not have occurred because the physical activity level of the rats was reduced at 500 ppm suggesting that carbon disulfide uptake at 500 ppm may also have been reduced because of the lowered respiratory rate. They also note that the saturation observed in rats is not likely to occur in humans at the prevailing occupational exposure concentrations. Saturation of TTCA production was observed in an oral study in rats (Kivisto et al. 1995). In rats treated with a single gavage dose of 1, 10, 30, or 100 mg/kg, 4.6%, 2.4%, 1.7%, and 0.8%, respectively, of the dose was excreted in the urine as TTCA.

The effect of P-450 induction or glutathione depletion on carbon disulfide metabolism to TTCA in rats following oral exposure has also been studied (Kivisto et al. 1995). The rats were pretreated with nothing, acetone, phenobarbital, 3-methylcholanthrene, or three inhibitors of glutathione production,

namely phorone, diethylmaleate, or buthionine sulfoximine, before being given a single gavage dose of carbon disulfide at 26-34 mg/kg. Phenobarbital decreased the output of TTCA by 21% during the first 12 hours of the urine collection. None of the other P-450 inducers had any effects on TTCA excretion, and the investigators suggested that the effect of phenobarbital may have been a result of cytochrome P-450 aggregation. Buthionine sulfoximine, an inhibitor of glutathione production, reduced the total output of TTCA by about 40%. Phorone and diethylmaleate pretreatment, which transiently reduce glutathione, decreased TTCA excretion.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Following inhalation exposure, the primary route of excretion of unmetabolized carbon disulfide in humans is exhalation. In one study it was estimated that 6-10% of the carbon disulfide that was taken up was excreted by the lungs (McKee et al. 1943). In a study conducted on humans, carbon disulfide levels in the exhaled breath decreased rapidly on cessation of exposure (Soucek 1957). The excretion by the lung accounted for 10-30% of the absorbed carbon disulfide. Less than 1% was excreted unchanged in the urine. The remaining 70-90% of the dose was metabolized. The details regarding carbon disulfide exposure levels were not available. A correlation was established between carbon disulfide exposure of rayon workers and urinary excretion of a metabolite or metabolites that catalyzed the reaction of iodine with sodium azide (Djuric 1967). This test indicated exposures to carbon disulfide above 16 ppm but failed to identify specific urinary metabolites. The failure to detect carbon disulfide exposure below 16 ppm may be because of interference with the reaction by dietary sulfur containing compounds.

In dogs exposed to 25-60 ppm carbon disulfide for 0.5-2.0 hours, approximately 8-13% of the carbon disulfide that was taken up was exhaled; less than 0.5% was excreted in the urine (McKee et al. 1943). Experimental details and control information are limited in this study. Inhalation exposure of rabbits to 20-150 ppm carbon disulfide for 1.5-2 hours resulted in excretion of 15-30% of the absorbed carbon disulfide via the lung and less than 0.1% by the kidney after termination of exposure (Toyama and Kusano 1953).

In guinea pigs, carbon disulfide metabolites are excreted as inorganic sulfur compounds in the urine (Strittmatter et al. 1950). Inhalation exposure to 14 ppm ^{35}S-carbon disulfide for 8 hours or to 26 ppm ^{35}S-carbon disulfide for 40 hours resulted in excretion of the administered dose mainly in the urine (63%) and expired air (30%) within 48 hours of exposure. The metabolized material was excreted in the urine predominantly in the form of inorganic sulfur compounds; some organosulfur derivatives were also present. Most of the unmetabolized carbon disulfide was excreted in the expired air.

The studies discussed above indicate that the lungs are the primary route of excretion of unmetabolized carbon disulfide in humans and animals exposed by inhalation, whereas the kidneys are the primary route of excretion of carbon disulfide metabolites.

2.3.4.2 Oral Exposure

No studies were located regarding excretion of carbon disulfide in humans after oral exposure.

Rats administered 10 mg ^{14}C-carbon disulfide/kg by gavage excreted 63% of the dose as unchanged carbon disulfide in the breath (DeMatteis and Seawright 1973).

2.3.4.3 Dermal Exposure

Following dermal exposure of humans to aqueous solutions of carbon disulfide of increasing concentrations (0.33-1.67 g/L) for 1 hour, only 3% of the absorbed carbon disulfide was eliminated by the lungs (Dutkiewicz and Baranowska 1967). For details and study limitations, see Section 2.3.1.3.

Exposure of rabbit skin to high concentrations of carbon disulfide vapor (800 ppm and above) for 1 hour resulted in detectable amounts of carbon disulfide in the breath of animals (Cohen et al. 1958). A linear relationship was noted between the exposure concentration and the amount of carbon disulfide in the exhaled breath.

2.3.4.4 Other Routes of Exposure

Appreciable amounts of absorbed carbon disulfide are excreted unchanged in breath regardless of the route of exposure (refer to Sections 2.3.4.1, 2.3.4.2, and 2.3.4.3). Small amounts of carbon disulfide

are excreted in the sweat and saliva of exposed individuals. In mice injected intraperitoneally with 30-42 μg of ^{35}S-carbon disulfide, about 13-23% of the radiolabel was excreted via the lung (Strittmatter et al. 1950). Rats receiving 10 mg ^{14}C-carbon disulfide/kg by intraperitoneal injection excreted about 70% of the dosed material as unchanged carbon disulfide in the breath (DeMatteis and Seawright 1973). Rats receiving 19 mg/kg ^{14}C-carbon disulfide intraperitoneally excreted 58-83% free carbon disulfide in expired air in the 3 hours following dosing (Snyderwine and Hunter 1987). Younger rats expired significantly more free carbon disulfide than older rats. In another study (Dalvi and Neal 1978), intraperitoneal administration of ^{14}C-carbon disulfide to rats resulted in excretion of carbonyl sulfide by the lungs in greater quantities than carbon dioxide. Pretreatment of rats with phenobarbital, however, resulted in a greater amount of excretion of carbon dioxide than carbon disulfide. In both experiments, excretion of ^{14}C-carbonyl sulfide and carbon dioxide accounted for 14-43% of the total administered radioactivity, with about twice as much carbon dioxide.

2.4 MECHANISMS OF ACTION

Despite the apparent differences between animals and humans in the metabolism of carbon disulfide, dithiocarbamates are the common metabolites formed. These may in part account for the neurotoxic effects of carbon disulfide. Formation of dithiocarbamates has been demonstrated in both *in vitro* and *in vivo* studies in these species. Metabolism studies in animals clearly indicate that carbon disulfide is metabolized by two distinctly different pathways (see Figure 2-3): it can form dithiocarbamates and glutathione conjugates, or it can be catalyzed by the monooxygenase system to generate reactive sulfur. However, the relative contributions of each of these metabolic pathways to the development of the acute and chronic toxicity of carbon disulfide remain to be determined. Both metabolic pathways suggest several potential mechanisms of toxicity. Formation of dithiocarbamates may in part account for peripheral neurotoxicity. Furthermore, the nonenzymatic reaction of carbon disulfide with free amino groups suggests a potential interaction with biological macromolecules such as proteins and nucleic acids. In contrast, the formation or generation of reactive sulfur may inhibit the microsomal monooxygenase system and disturb the metabolism of other endogenous and exogenous compounds.

In the paragraphs that follow, the two metabolic pathways are discussed in detail. The relative importance of these two pathways is also discussed.

Carbon disulfide combines readily with the amine groups of amino acids to produce dithiocarbamates, which are water-soluble metabolites. Such reactions have been demonstrated with free amino groups in serum *in vivo* (Cohen et al. 1958). The formation of acid-labile carbon disulfide, readily destroyed at low pH, was also consistent with significant *in vivo* formation of dithiocarbamate metabolites (McKenna and DiStefano 1977a; Snyderwine and Hunter 1987). Following absorption of inhaled carbon disulfide by the lung, free carbon disulfide is distributed to various tissues where it is either eliminated, primarily by the lung, or further metabolized to acid-labile carbon disulfide metabolites. The formation of acid-labile carbon disulfide metabolites may continue to increase at steady-state concentrations of carbon disulfide as long as free carbon disulfide is available to the tissue and amine substrates are available. This was demonstrated in rats exposed to 640 ppm carbon disulfide for 8 hours in which acid-labile carbon disulfide metabolites continued to accumulate in several tissues after steady-state levels of carbon disulfide were reached (McKenna and DiStefano 1977a).

The formation of the carbon disulfide metabolite, dithiocarbamate, may explain the mechanism of carbon disulfide-induced neurotoxic effects. For example, McKenna and DiStefano (1977b) found a decrease in the activity of copper-requiring enzyme, dopamine-β-hydroxylase, in response to increased inhalation exposure to carbon disulfide (0.1-2.0 mg/L for 8 hours). The effect of carbon disulfide was attributed to the formation of dithiocarbamates, which complex with copper, since in vitro inhibition of purified dopamine-β-hydroxylase by carbon disulfide was dependent on preincubation with amines capable of dithiocarbamate formation (McKenna and DiStefano 1977b). The inhibition of dopamine-β-hydroxylase decreased progressively with increasing Cu^{++} concentration, and equimolar concentrations of Cu^{++} and inhibitor were without effect, suggesting that the inhibition occurred through the binding of enzymic copper.

An alternative mechanism proposed to explain the neurotoxic effect of carbon disulfide is the formation of a dithiocarbamate derivative of pyridoxamine, a form of vitamin B_6, with carbon disulfide (Vasak and Kopecky 1967). Investigators postulated that transaminases and amine oxidases would be inhibited because these enzymes require the pyridoxamine phosphate form of vitamin B_6, as a cofactor. In subsequent *in vivo* studies, evidence of altered tryptophan metabolism (consistent with an inhibition of B_6-requiring enzymes) appeared to support this hypothesis (Abramova 1966). Furthermore, supplementation of the diet with vitamin B_6, delayed some of the neurotoxic effects of carbon disulfide (Teisinger 1974). However, continued research could not detect any change in the tissue content of vitamin B_6 in rats after chronic exposure to carbon disulfide (Okayama et al. 1988), and in a later

study the authors suggested that the abnormalities in tryptophan metabolism caused by exposure to carbon disulfide were not related to vitamin B_6 deficiency (Okayama et al. 1988). In this instance, they studied the activities of enzymes in the kynurenine pathway in rats chronically exposed to carbon disulfide. Increased activities of L-tryptophan-2,3-dioxygenase, kynurenine-3-hydroxylase, and kynureninase in the high-concentration group (800 ppm) were found; this may indicate activation of the kynurenine pathway upon carbon disulfide exposure rather than vitamin B_6 deficiency (Okayama et al. 1988).

The effects of carbon disulfide on the microsomal drug metabolizing system were demonstrated in rats by Freundt et al. (1975). Exposure of rats by inhalation to low concentrations of carbon disulfide (20-400 ppm for 8 hours) inhibited microsomal drug metabolism. This was reflected by increased hexobarbital sleep times. Pretreatment of rats with SKF-525A, an inhibitor of cytochrome P-450-mediated metabolism, reduced the liver damage from carbon disulfide in phenobarbital-pretreated animals (Bond and DeMatteis 1969).

Valentine et al. (1993) have shown that the initial dithiocarbamate protein adduct decomposes to isothiocyanate derivatives which then react with protein nucleophiles resulting in crosslinking. The crosslinking of protein in the nerve axons to cause their ultimate degeneration is correlated with the crosslinking of spectrin, a blood cell membrane protein. This suggests that the latter can be used as a biomarker of adverse effect of nerve damage.

Acute intraperitoneal injections of 504 mg/kg carbon disulfide for 3 consecutive days in male rats caused a significant decrease in aminopeptidase activity in the thalamus and cerebellum of the brain (de Grandarias et al. 1992). The role of aminopeptidase activity in carbon disulfide neurotoxicity was postulated.

It has been postulated that carbon disulfide cardiotoxicity may be mediated by disruption of the energy supply in the heart (Klapperstuck et al 1991). The mechanism for carbon disulfide acceleration of arteriosclerotic plaque formation involves direct injury to the vessel epithelium and changes in lipid metabolism.

It has been postulated that the reduced thyroid activity in workers exposed to carbon disulfide may be related to a central involvement of catecholamine metabolism (Cavalleri et al. 1978).

2.5 RELEVANCE TO PUBLIC HEALTH

There are substantial data available on which to base conclusions regarding the potential health effects of carbon disulfide exposure in residents near hazardous waste sites and occupationally exposed individuals. The principal adverse health effects noted in humans exposed via inhalation are neurotoxic and cardiovascular effects (Aaserud et al. 1988, 1990; Egeland et al. 1992; Hernberg et al. 1971; Hirata et al. 1992a; Lancranjan 1972; Ruijten et al. 1990, 1993; Sikora et al. 1990; Tolonen et al. 1979; Vanhoorne et al. 1992a). Most of the human data are derived from occupational studies. Although these are somewhat limited by the difficulties in estimating the exact dose for individuals and by the fact that most industrial environments have multiple types of exposures, these data are consistent with effects noted in experimental animal studies. In addition, the toxicokinetic studies and the derivations of mechanisms of action are consistent with these observations. Specific health effects are discussed in greater detail below.

Minimal Risk Levels for Carbon Disulfide

A User's Guide has been provided at the end of the profile (see Appendix A) to aid in the interpretation of MRLs.

Inhalation

- An MRL of 0.3 ppm was derived for chronic exposure to carbon disulfide. This MRL was derived based on peripheral neuropathy (reduced motor nerve conduction velocity) in humans after prolonged occupational exposure to carbon disulfide. A LOAEL of 7.6 ppm was established for this effect. Although a dose response was achieved, the effect was considered minimal since the reductions in motor nerve condition velocity were within a range of clinically normal values. This concentration was divided by an uncertainty factor of 30 (3 for use of a LOAEL, 10 for human variability) to yield the calculated MRL of 0.3 ppm.

No acute-duration or intermediate-duration inhalation MRLs were derived since potential MRL values would not have been as protective of human health as the chronic-duration MRL. In addition, there is greater confidence in the chronic-duration MRL since it is based on human data.

Oral

- An MRL of 0.01 mg/kg/day was derived for acute exposure to carbon disulfide. This MRL was derived based on the inhibition of enzyme activities, specifically decreases in the activities of several hepatic microsomal cytochrome P-450-dependent drug-metabolizing enzymes and cytochrome P-450 content. A LOAEL of 3 mg/kg/day was established for this effect. Also, the effect was minimal since the inhibition of enzyme activities was selective and reversible. This dose was divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for interhuman variability) to yield the calculated MRL of 0.01 mg/kg/day.

No intermediate- or chronic-duration oral MRLs were derived because of a lack of reliable LOAELs and a lack of definite target organs.

Death. Several epidemiology studies have reported increased mortality due to cardiovascular disease in cohorts of workers occupationally exposed to carbon disulfide (Balcarova and Halik 1991; Hernberg et al. 1970, 1973; Swaen et al. 1994; Tolonen et al. 1975, 1979). It is not possible, however, to draw accurate or definitive conclusions from these reports regarding the levels of exposure associated with an effect. This is because of the wide range of exposure levels that workers experience and the difficulty in estimating exact levels from area sampling done at different times and with different methods. Moreover, historical exposure data are lacking in most of the studies, and occupational studies frequently encompass concomitant exposures to other chemicals. However, decreased survival associated with ventricular fibrillation in rats has been noted following oral exposure to carbon disulfide (Hoffman 1987). There are no studies available that address death following dermal exposure in humans or animals.

Systemic Effects. The systemic toxicity of carbon disulfide is manifested primarily as cardiovascular, hepatic, and ocular effects in both humans and animals following inhalation exposure.

Respiratory Effects. Some transient respiratory effects have been noted in humans briefly exposed to unspecified amounts of carbon disulfide (Kamat 1994; Spyker et al. 1982). Limited studies were available that specifically address respiratory function changes in exposed animals. Decreased

respiratory rate was noted in rats exposed to 803 ppm carbon disulfide for 18 hours (Tarkowski and Sobczak 1971).

Cardiovascular Effects. Several studies of occupationally exposed individuals have documented an increased incidence of elevated blood pressure (Egeland et al. 1992; Hernberg et al. 1971; Tolonen et al. 1979; Vanhoorne et al. 1992a). This indicates that an elevation in blood pressure could be one mechanism for the adverse cardiovascular effects of carbon disulfide. Concomitant occupational exposure to hydrogen sulfide may be a confounding factor. At relatively low concentrations (median 1.1 or 4 ppm) cardiovascular effects have not been observed in humans occupationally exposed to carbon disulfide (Chrostek-Maj and Czeczotko 1995a; Drexler et al. 1995b). A transient decrease in blood pressure was reported in rats administered 506 mg/kg carbon disulfide once by gavage (Hoffmann and Klapperstück 1990). ECG changes were seen at 373 and 506 mg/kg, while heart rate decreased at 632 mg/kg. The other studies on animals attest to the adverse effects of carbon disulfide on the cardiovascular system (Antov et al. 1985; Chandra et al. 1972; Wronska-Nofer et al. 1980). These effects included lipid droplet infiltration in the coronary arteries, metabolic and structural changes in the myocardium and the aorta, and myocardial lesions characterized by necrosis, interstitial edema, and cellular infiltrate.

Gastrointestinal Effects. Gastrointestinal symptoms are commonly reported in workers exposed to carbon disulfide (Rubin and Arieff 1945; Vanhoorne et al. 1992b; Vigliani 1954). These symptoms are subjective, however, and cannot be identified with a specific exposure level. No animal studies have been done that address effects on the gastrointestinal system.

Hematological Effects. Fibrolytic activity was decreased in workers exposed to carbon disulfide at 59-169 ppm for 2-8 years (Visconti et al. 1967). Red blood cell and white blood cell counts were not significantly different from preemployment values in workers exposed to carbon disulfide for 5 years (Chrostek-Maj and Czeczotko 1995a).

There are limited animal data concerning hematological effects after inhalation exposure. No hematological effects were seen in rabbits acutely exposed to 1,100 ppm carbon disulfide for 12 days (Briegor 1949). However, exposure to 300 ppm for 48 days resulted in significant increase in pseudo-eosinophils. Significant decreases in erythrocyte counts, total hemoglobin, and hematocrit were noted in mice exposed to 800 ppm for 90 days (Toxigenics 1983c). Animal studies also show an elevation

of serum lipids following carbon disulfide exposure (Wronska-Nofer 1972, 1973; Wronska-Nofer et al. 1980). Vascular arteriosclerotic changes may be a unifying mechanism for the toxic effects caused not only on the cardiovascular system but also in the liver and kidney (Beauchamp et al. 1983).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after exposure to carbon disulfide by any route. In rats treated by gavage with carbon disulfide, a decreased response of the ancoccygeus muscle to noradrenaline was observed relative to the vehicle-treated controls (Gandhi and Venkatakrishna-Bhatt 1993). The response of the muscle to noradrenaline was tested *in vitro.*

Hepatic Effects. In humans, inhalation or oral exposure to carbon disulfide causes inhibition of microsomal enzymes and increased liver size (Mack et al. 1974; Vanhoorne et al. 1992b). Similar changes were seen in animals without accompanying histological evidence of liver damage (Magos and Butler 1972; Magos et al. 1973; Tsuyoshi 1959). However, hepatoxicity characterized by hydropic degeneration in parenchymal cells of the centrilobular zone was observed in rats pretreated with phenobarbital to induce the liver mixed-function oxidase system. Effects on lipid metabolism, for example, increased levels of serum cholesterol, total lipids, and triglycerides, have been seen in workers exposed by inhalation to carbon disulfide (Krstev et al. 1992; Stanosz et al 1994b; UK/HSE 1981). Serum cholesterol, phospholipids, and triglyceride levels were significantly elevated in rats following inhalation exposure to carbon disulfide (Wronska-Nofer 1972, 1973). One postulated mechanism involved in the toxicity of carbon disulfide is its suppressive effect on the microsomal hepatic enzyme system (Mack et al. 1974). Many of the hepatic effects of carbon disulfide as well as the interactions of other chemicals with carbon disulfide would then result from the loss of the capability to detoxify other harmful chemicals.

An acute-duration oral MRL was calculated on the basis of decreases in the activities of several hepatic microsomal cytochrome P-450-dependent drug metabolizing enzymes and cytochrome P-450 content in mice after acute exposure to 3 mg/kg/day (Masuda et al. 1986). The calculated acuteduration oral MRL is 0.01 mg/kg/day.

Renal Effects. Although several reviews of the literature on carbon disulfide refer to renal effects in humans, no studies were located that specifically address renal toxicity (Beauchamp et al. 1983; WHO 1986). Rabbits exposed to a wide range of levels of carbon disulfide for 38 weeks showed an

increased incidence of chronic interstitial nephritis (Cohen et al. 1958). Nephropathy was observed in mice exposed via inhalation at 800 ppm for 90 days (Toxigenics 1983c). Because of limited available data, the significance of these findings in animals with regard to adverse effects in humans is not known.

Endocrine Effects. The available data in humans provide conflicting evidence regarding the adverse effects of carbon disulfide exposure on thyroid function (El-Sobkey et al. 1979; Lancrajan et al. 1972; Wagar et al. 1981). Based on decreases in the urinary excretion of products of adrenal/gonadal or adrenal/sympathetic origin, exposure of workers to carbon disulfide may affect adrenal gland function (Cavalleri et al. 1967; Stanosz et al. 1994a). These studies also involved possible exposure to other chemicals and did not identify the precise exposure level.

Dermal Effects. Dermal effects are limited to a report of blisters on the hands of viscose rayon workers presumably due to carbon disulfide exposure. This is borne out by studies in rabbits in which similar blisters could be induced by carbon disulfide exposure (Hueper 1936). This indicates that dermal contact from either occupational exposure or from contaminated soil or water near hazardous waste sites could cause adverse effects.

Ocular Effects. Ophthalmological changes of various types including fundus anomalies, retinal microaneurysms, retinopathy, and burning eyes have been reported in workers occupationally exposed to carbon disulfide (DeLaey et al. 1980; NIOSH 1984a; Raitta et al. 1974, 1975; Rubin and Arieff 1945). These findings suggest that chronic low-level occupational exposure to carbon disulfide or exposure at hazardous waste sites could result in ophthalmological changes.

Body Weight Effects. A 14-day exposure to carbon disulfide at a concentration that resulted in a narcotic-like stupor caused male rats to lose 14% of their body weight (Wilmarth et al. 1993). Inhalation exposure of rats for intermediate durations has been reported to produce a dose-dependent decrease in body weight gain. This effect varied depending upon the sex and the strain of rat used (Clerici and Fechter 1991; Hirata et al. 1992b; Tepe and Zenick 1984; Toxigenics 1983a, 1983b). However, the implications of these findings with regard to adverse effects in humans are unknown.

Other Systemic Effects. Workers occupationally exposed to carbon disulfide exhibited elevated plasma sodium and chloride levels and decreased erythrocyte potassium and calcium levels (Pines

1982). In animals, increased adrenal weight, hyperplasia of adrenal cortex, and mild hemosiderosis of the spleen have been observed (Cohen et al. 1959). However, the value of these studies is limited by confounding factors, lack of dose-response relationships, and limitations in study design.

Immunological and Lymphoreticular Effects. The only study available (Bobnis et al. 1976) reported no immunological component involved in the increase of arteriosclerotic lesions found in carbon disulfide-exposed workers.

Neurological Effects. The primary target organ for carbon disulfide toxicity is the nervous system. In humans, behavioral changes, neurophysiological changes, and neuropathology have been demonstrated (Aaserud et al. 1988, 1990; Chrostek-Maj and Czeczotto 1995b; Chu et al. 1995; Hirata et al. 1992a; Lancranjan 1972; Ruijten et al. 1990, 1993; Sikora et al. 1990). Some studies have noted that these changes were reversed following removal from exposure; other studies have indicated no improvement. One of the major problems has been in establishing levels of exposure associated with the onset of symptoms.

In occupational settings, exposure levels vary daily and over the course of years. Improved technology and an awareness of the harmful effects of carbon disulfide exposure have resulted in a marked decrease in the ambient levels in the workplace from approximately 60 ppm to about 10 ppm. Concomitant exposures to other chemicals remain a problem. Animal data suggest serious neurological effects from carbon disulfide exposure, but most of the studies are single-dose studies using levels of carbon disulfide one to two orders of magnitude larger than those levels seen in occupational settings. Because of difficulties in accurately measuring low concentrations of carbon disulfide in environmental settings and in assessing subtle behavioral or neurological effects, it is hard to draw conclusions about levels or durations of exposure that represent no-effect levels for neurotoxicity in humans. The mechanism of neurotoxicity appears to involve effects on both the axons and the myelin sheaths. In an *in vitro* assay, De Caprio et al. (1992) demonstrated formation of protein-bound isothiocyanate adducts in carbon disulfide-treated peptides and protein. Based on these findings, the study authors proposed that a direct reaction of carbon disulfide with neurofilament lysineamino moieties was a step in the mechanism of neuropathy. Valentine et al. (1993) have shown that the protein in degenerated axons caused by carbon disulfide exposure is greatly crosslinked. Externally, the nerves show swellings paranodally, retraction of myelin from specific nodes, and degeneration of the distal axon (especially long ones). The isothiocyanate adducts formed from

dithiocarbamate adducts cause crosslinking of the nerve proteins, leading to the phenomena observed in electron micrographs.

Valentine et al. (1995) postulated that covalent crosslinking of low molecular weight neurofilament triplet proteins by dithiocarbamates proceeded through liberation of carbon disulfide.

A chronic-duration inhalation MRL was calculated on the basis of reduced motor nerve conduction velocity in humans after occupational exposure to an average of 7.6 ppm carbon disulfide, for individuals working approximately 8 hours a day, 5 days a week, for 12.1 years (the mean exposure period) (Johnson et al. 1983). The calculated chronic-duration inhalation MRL is 0.3 ppm.

Reproductive Effects. Birth defects have been reported in newborns of women workers exposed to carbon disulfide (Bao et al. 1991). However, no definite conclusion can be made because of the inadequacy of available data (lack of exposure analysis and dose-response assessment) since the study was reported only as an abstract. Decreased sperm count and decreased libido in men and menstrual irregularities in women exposed in the workplace have been the most frequently reported effects. In exposed women, possible disruptions of the neurohormonal-endocrine balance necessary for normal ovarian and uterine cycles may lead to amenorrhea, abnormal menstrual cycles, and even sterility (WHO 1979; Zielhius et al. 1984). However, community and workplace studies have not shown a decreased fertility rate, an increase in the time between live births, or an effect on semen quality with carbon disulfide exposure (Hemminki and Niemi 1982; Meyer 1981; NIOSH 1983; Vanhoorne et al. 1974; Zhou et al. 1988). Potential reproductive effects are supported by animal studies, primarily in male rodents, that report decreased sperm count, abnormal coital behavior, and reduced plasma testosterone (Tepe and Zenick 1984; Zenick et al. 1984). These effects are supported by data from intraperitoneal exposures to rats. No effects on the testicular structures were seen at 6.25 mg/kg/day administered for 60 days. Some disorganization of seminiferous tubules was seen at 25 mg/kg/day for 60 days, and a decrease in spermatogenesis was seen at 25 mg/kg/day for 120 days (Gondzik 1971).

Developmental Effects. Although developmental effects have been seen in the offspring of women exposed to carbon disulfide in the workplace (Bao et al. 1991), the data are inadequate to draw any definitive conclusion. The Bao et al. (1991) study was reported as an abstract, and there was a lack of exposure analysis and dose-response assessment. There are reports of congenital malformations in the offspring of rats exposed via inhalation (Tabacova and Balabaeva 1980a), but the general finding in

the animal studies of developmental effects is one of increased embryotoxicity (Lehotzky et al. 1985; Yaroslavskii 1969). In a preliminary study reported only in abstract form, increases in early resorptions and decreases in viable fetuses were observed in New Zealand white rabbits exposed by inhalation to 600 or 1,200 ppm (Gerhart et al. 1991). No effects were noted at 300 ppm. Pharmacokinetic studies indicate that carbon disulfide and its metabolites pass the placenta at all stages of gestation and localize in the recognized target organs for this chemical (brain, blood, liver, and eyes) (Danielsson et al. 1984), but the levels at which these exposures could produce effects in humans is not identifiable at this time.

Genotoxic Effects. There are some data on *in vitro* genotoxicity of carbon disulfide. Haworth et al. (1983) evaluated carbon disulfide at five doses both with and without S9 activation in the *Salmonella*/microsome assay. All results were negative. Other studies in *Salmonella typhimurium* and *Escherichia coli,* both with and without activation, were also negative (Donner et al. 1981; Hedenstedt et al. 1979). *Salmonella* strain TA98 was used in a host-mediated assay investigating the ability of carbon disulfide to induce reverse mutations. Carbon disulfide was not considered to be an active mutagen in this test; however, the results are equivocal in this regard (NIOSH 1980). A response curve suggestive of carbon disulfide-induced unscheduled deoxyribonucleic acid (DNA) synthesis was observed in human WI-38 cells, but the results failed to meet the criteria indicative of a positive response (NIOSH 1980). Carbon disulfide was not considered to be an a mutagen in this test. Studies in lymphocytes (Garry et al. 1990) demonstrated a requirement for microsomal activation in the induction of dose-related ($p \leq 0.05$) increases in sister chromatid exchanges. This finding is consistent with past work on the cytochrome P-450 monooxygenase system that suggests a requirement for microsomal activation to form a highly reactive metabolite. Carbon disulfide was reported to inhibit the mutagenic activity of 1,2-dimethylhydrazine and azoxymethane in a host-mediated assay (Moriya et al. 1979). In this report, the authors suggested that this effect was due to carbon disulfide's prevention of *in vivo* oxidation of azomethane to azoxymethane. This would be consistent with the known suppressive effects of this chemical on the cytochrome P-450 system. Results of carbon disulfide on the *Drosophila* sex-linked recessive lethal test were also negative (Donner et al. 1981; NIOSH 1980). Carbon disulfide failed to produce significant chromosomal aberrations in the bone marrow of rats inhaling carbon disulfide for either acute or intermediate periods (NIOSH 1980). There are no mutagenicity data on humans exposed occupationally to carbon disulfide, so at this time it is difficult to predict effects of carbon disulfide at the DNA level.

Cancer. There are no definitive data in humans or animals that indicate a carcinogenic potential for carbon disulfide. In the absence of positive genotoxic data, increased cancer risk does not appear to be an effect of exposure to carbon disulfide.

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (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 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 carbon disulfide are discussed in Section 2.6.1.

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

be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by carbon disulfide are discussed in Section 2.6.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.8, "Populations That Are Unusually Susceptible."

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Carbon Disulfide

Levels of carbon disulfide detected in exhaled breath, blood, urine, and milk as well as various metabolite concentrations in the urine of exposed individuals have been studied as biomarkers of carbon disulfide exposure.

Because of its solubility in lipids and affinity for proteins, environmental carbon disulfide is quickly absorbed in the blood and other body tissues through inhalation or cutaneous absorption. The absorbed carbon disulfide is then eliminated through the lungs unchanged as carbonyl sulfide or as carbon dioxide, or it is converted to water-soluble metabolites, which are excreted by the kidneys. Metabolites of carbon disulfide found in the urine are often utilized as biomarkers of exposure. Sulfates, for example, are nonspecific products of the metabolism of carbon disulfide and many other compounds. Some investigators have observed an increase in total urinary sulfate levels (specifically inorganic sulfates) in humans after exposure to 22 ppm of carbon disulfide vapor for 1.5 hours (McKee et al. 1943) suggesting that sulfate levels may be useful as a nonspecific biomarker of exposure, perhaps in combination with other markers. However, other researchers have made conflicting observations. More recently, certain specific dithiocarbamates have been used in more specific and reliable tests (discussed below) of carbon disulfide exposure.

Carbon disulfide is also found in the saliva and sweat of exposed individuals in small quantities. These measurements have not been shown to quantitatively correlate with carbon disulfide exposure. The concentration of carbon disulfide in the feces is very low and therefore has also not been routinely used as a biological marker of exposure (Djuric 1967).

Higher cholesterol levels (correlated with exposure levels), higher blood creatinine levels, marked disturbances of the hepatic cytochrome P-450 content and of the associated microsomal monooxygenase system, as well as the inhibition of succinic-oxidase enzyme activity, may also be considered as nonspecific biomarkers of carbon disulfide exposure. More research, however, needs to be done in order to determine whether a direct correlation exists between these parameters and carbon disulfide exposure.

The following paragraphs describe reasonably specific biomarkers for carbon disulfide exposure in humans that correlate with exposure levels to varying degrees.

Carbon disulfide levels eliminated by exhalation may yield some information about recent, short-term exposure; however, the breath test has not been widely used for monitoring occupational exposure because of several confounding factors. The first phase of carbon disulfide elimination by breath is very fast. Pollutant concentrations in the breath, therefore, may reflect variable uptake during the day but may not be a good reflection of the longer term uptake and exposure values for an entire working day. Results from breath tests for carbon disulfide exposure are further confounded by the fact that absorption and elimination rates of carbon disulfide by the lungs are variable among different individuals and within the same individual because of differences in activity and metabolic rates. Chronically exposed persons, for example, have a lower retention rate than those exposed for the first time (Campbell et al. 1985; Djuric 1967).

Because of these confounding factors, results of breath tests for carbon disulfide exposure do not correlate well with its environmental levels. However, one might be able to detect carbon disulfide exposure more reliably if measurements of carbon disulfide were made during a second slower elimination phase and if a more sensitive detection technique were used. A quadrupole mass spectrometer makes the measurement of carbon disulfide in exhaled breath much more sensitive, detecting exposure levels as low as 1 ppm. In an investigation by Campbell et al. (1985), the shortterm elimination of carbon disulfide was studied measuring uptake that had taken place l-2 hours before the test. Carbon disulfide levels in the breath were found to fluctuate, but the value of next-day tests measuring the slower elimination phase of carbon disulfide by the breath should be explored. Nevertheless, the use of exhaled carbon disulfide remains an equivocal biomarker of exposure.

Blood carbon disulfide levels may also serve as a biomarker of exposure. Data from blood tests, however, have not shown consistent correlation with carbon disulfide exposure levels in past studies. Blood carbon disulfide concentration after exposure may be a poor indicator of actual exposure because of rapid clearance from this tissue (Beauchamp et al. 1983; WHO 1979). A confounding factor in using blood carbon disulfide concentration as a biomarker of exposure is the fact that two different species of carbon disulfide exist in the blood. “Free” carbon disulfide is unbound carbon disulfide that is still dissolved in blood plasma; “bound” or “acid-labile” carbon disulfide refers to an appreciable amount of carbon disulfide dissolved in plasma lipids or bound to proteins in the blood. The equilibrium between free and acid-labile carbon disulfide in blood varies inter- and intraindividually, rendering blood carbon disulfide measurements less reliable for calculating exposure dose. Moreover, bound carbon disulfide’s rate of release into other body systems may vary also. A head-space gas chromatography detection technique used to measure acid-labile carbon disulfide may bear investigation. Carbon disulfide levels are usually measured in acid-treated whole blood; however, it is suspected that the greater percentage of carbon disulfide in the blood is bound to blood cell membranes because of its affinity for lipids and proteins (Campbell et al. 1985). This means that the lack of correlation of blood plasma levels of carbon disulfide with exposure data may be due to a significant portion of the carbon disulfide not being measured at all as it is not in the plasma.

Some investigators have measured levels of carbon disulfide in urine as a biomarker of exposure, but this is probably optimal only for measuring high exposure levels (Beauchamp et al. 1983). Good correlation between urinary carbon disulfide levels and work exposure was not found in any studies. The measurements of excreted or “free” carbon disulfide may have been confounded by the presence of various thiometabolites or “bound” species. Moreover, the volatility of carbon disulfide and individual variations in urinary flow rate and metabolism may have confounded results in these studies (Djuric 1967).

The concentration of crosslinked red blood cell spectrin has been suggested as a marker of nerve protein crosslinking damage that leads to slower conduction velocities and abnormal nerves due to protein adduct formation initially with dithiocarbamates which decompose to form isothiocyanate adducts (Valentine et al. 1993). These latter adducts can then cause the actual crosslinking of both spectrin and nerve protein. As new red blood cells must be made to replace the damaged spectrin, the crosslinking of this protein may serve as a longer term biomarker of carbon disulfide exposure.

The iodine-azide test measures possible carbon disulfide exposure, based on the decolorization of iodine in the presence of urinary carbon disulfide thiometabolites. The iodine-azide test is a means for determining longer term carbon disulfide exposure at air concentrations of 50 mg/m^3 (around 16-20 ppm) and above (Baselt 1980; Beauchamp et al. 1983; Campbell et al. 1985; Lieben 1974; WHO 1986). However, it is not a very sensitive test for exposure at lower concentrations of concern to OSHA. The iodine-azide test measures the catalysis of the reaction between iodine and sodium azide by the following metabolites of carbon disulfide acting as biomarkers of exposure: thiourea, which is the main urinary thiometabolite involved in this test, 2-thio-5-thiazolidinone, and a third unidentified metabolite. The latter two chemicals are present in very small quantities and are not as important in catalyzing the iodine-azide reaction (Beauchamp et al. 1983). However, even though exposure can be detected, the utility of such measurements is limited by the lack of the correlation between exposure and urinary carbon disulfide described in the previous paragraph; that is, level or duration of exposure cannot be quantified.

Measuring the total concentration of urinary thio compounds (including glutathione conjugates, mercapturic acids, and other sulfur-containing carbon disulfide metabolites) can serve as a good marker of exposure. The level of total thio compounds correlates with carbon disulfide exposure levels and is a more sensitive biomarker of exposure than the iodine-azide test (Beauchamp et al. 1983; Van Doorn et al. 1981a). This biomarker detects an exposure to 6 ppm carbon disulfide for 8 hours (Beauchamp et al. 1983). These compounds are not absolutely specific for carbon disulfide exposure.

TTCA is used as an indicator to assess the degree of occupational exposure to carbon disulfide (Thienpont et al. 1990). Although the detection limit was estimated at 0.05 mg of TTCA/g of urinary creatinine, the study did not provide data on carbon disulfide exposure levels.

In a rayon production factory, exposure to carbon disulfide was measured by personal air sampling and the excretion of TTCA in urine (Meuling et al. 1990). Based on the personal air-sampling, the TWA exposure level for carbon disulfide was 12.6 mg/m^3 (4 ppm). The study authors established a calculated biological limit value of 0.77 mg TTCA/g creatinine (0.57 mmol/mol creatinine) to correspond with 95% confidence, to a TWA air concentration lower than the threshold limit value (TLV) of 30 mg/m^3.

Measuring levels of one particular urinary thiometabolite of carbon disulfide may serve as an even more sensitive and specific biomarker of exposure. TTCA, which is a product of glutathione conjugation, is quantitatively related to carbon disulfide uptake (Beauchamp et al. 1983; Campbell et al. 1985; Drexler et al. 1994). Carbon disulfide exposure levels of 2.5 ppm and above are detected by this analytical method. Carbon disulfide exposure correlates well with urinary TTCA concentrations, especially once the metabolite levels are normalized to urinary creatinine content. It appears that TTCA levels may be used as a sensitive monitor of longer term exposure in workers (Beauchamp et al. 1983; Campbell et al. 1985; Drexler et al. 1994). One limitation of urinary TTCA levels is that this compound has been detected at low concentrations (range, 0.005-0.15 mg/g creatinine) in persons not exposed to carbon disulfide (Lee et al. 1995). The source of this TTCA is thought to be from dietary intake, especially the consumption of brassica vegetables (e.g., cabbage) (Simon et al. 1994). Therefore, in persons who eat large amounts of these vegetables, measurements of urinary TTCA may overestimate carbon disulfide exposure. Baseline sampling is therefore necessary to correct for nonworkplace exposure sources.

Biological monitoring for carbon disulfide exposure was performed using the iodine-azide test and TTCA test in urinalysis of workers with high exposure to carbon disulfide (36-46 ppm) (van Poucke et al. 1990). Based on the findings of the study, the specificity and the sensitivity were low for the iodine-azide test and high for the TTCA test. ACGIH (1986, 1994) has recommended a biological exposure index of end-of-shift urinary TTCA of 5 mg/g creatinine.

Therefore, it appears that because of the limitations in the methodology for measuring carbon disulfide in blood, breath, and urine of exposed individuals, direct measurement of this compound is not the most sensitive test for determining the extent of exposure (Beauchamp et al. 1983; Campbell et al. 1985; Djuric 1967; McKee et al. 1943; WHO 1979). For the present, the biomarker that correlates best with exposure is measurement of metabolites in the urine. The iodine-azide and TTCA tests can be conducted to measure urinary levels of carbon disulfide metabolites as they have been shown to correlate with exposure (Baselt 1980; Beauchamp et al. 1983; Campbell et al. 1985; Lieben 1974; WHO 1986), with the TTCA test being more sensitive and specific than the iodine-azide test.

For more information concerning absorption, distribution, metabolism, and excretion of carbon disulfide, refer to Section 2.3.

2.6.2 Biomarkers Used to Characterize Effects Caused by Carbon Disulfide

The battery of biomarkers discussed here may be used as indicators of probable carbon disulfide exposure. However, the physiological effects of carbon disulfide poisoning are numerous and range from mild to severe. Their utilization as biomarkers of effect are confounded by their occurrence in response to other epidemiological, nutritional, and environmental factors. Their significance as biomarkers is further reduced by the fact that these effects occur with great variance in the cohort exposed population.

The following are proposed as likely biomarkers of effect for carbon disulfide; however, more information about their possible correlation with actual carbon disulfide exposure and their reliability and consistency is necessary before they can be utilized to indicate level or duration of exposure or predict potential health effects.

Several neurological parameters may be useful as more specific biomarkers of polyneuropathy from carbon disulfide exposure. CT-scans, magnetic resonance imaging, and pneumoencephalography (PEG) may indicate early cerebral/cerebellar atrophy in humans (Beauchamp et al. 1983; Peters et al. 1988). EMGs have detected signs of neurogenic lesions in humans, and changes in brain EEG patterns in animals have accompanied carbon disulfide-induced central nervous system toxicity. Moreover, neurophysiological methods may be utilized to detect decreasing nerve conduction velocity, which is a biomarker of peripheral nervous system effects (WHO 1981).

Changes in lipid metabolism are the most obvious biomarkers of carbon disulfide's vasculopathic effects. Hypercholesterolemia (Toyama and Sakurai 1967) and high β-lipoproteins in the blood (Prerovska and Drdkova 1967) have been observed by investigators following long-term occupational carbon disulfide exposure. Elevated blood lipid concentrations following long-term carbon disulfide exposure in humans may be an appropriate indicator of ensuing arteriosclerosis, clinical vasculopathy, and increased risk of cardiovascular disease (El-Sobkey et al. 1979). However, the accuracy and reliability of this parameter as a potential biomarker of exposure for carbon disulfide are in question since many things can cause changes in lipid metabolism.

More specific blood lipid parameters, however, may prove to be useful in the future. Changes have been observed in lipid metabolism when a cytochemical enzymological examination of leukocytes and

platelets was carried out for over 600 exposed workers (Micu et al. 1985). Researchers found high levels of lymphocytic lipids and low levels of granulocytic lipids. Another investigator found elevated serum cholesterol and fatty acids and low cholesterol ester levels in an 11-week study of dogs. However, only the experimental animal group fed a high-fat diet showed altered lipid metabolism. The exposed groups on normal and high-carbohydrate diets had normal serum lipid content (Lewey et al. 1941).

In exposed women, possible disruptions of the neurohormonal-endocrine balance necessary for normal ovarian and uterine cycles may lead to amenorrhea, abnormal menstrual cycles, spontaneous abortions, and even sterility (WHO 1979; Zielhuis et al. 1984). Serum thyroxine levels, which decrease following carbon disulfide exposure, have also been suggested as a biomarker (Cavalleri 1975).

Higher plasma creatinine levels were observed among workers exposed to 4-18 ppm of ambient carbon disulfide. Creatinine level in plasma may be utilized as a nonspecific biomarker of short-term renal dysfunction (Hernberg et al. 1971). Another such biomarker of renal effects may be the blood sugar level. Higher than normal blood sugar levels in response to carbon disulfide exposure were observed in a chronic-duration human study (Hernberg et al. 1971) and an intermediate-duration dog study (Lewey et al. 1941).

In studying the effects of carbon disulfide exposure on enzyme systems of carbohydrate metabolism, McKee et al. (1943) observed that the succinic-oxidase system was inhibited. They noted a 10% decrease in the activity of this system. Carbohydrate metabolism is crucial in proper neural function; thus, succinic-oxidase activity may serve as an appropriate biomarker of nervous system effects (McKee et al. 1943).

In conclusion, the following summarizes possible correlative biological markers of early carbon disulfide poisoning: (1) electromyographical indications of neural lesions; (2) decreased neuromuscular conduction velocity; (3) abnormal lipid metabolism as indicated by hypercholesterolemia; (4) decreased steroid hormone levels (Lieben 1974); (5) low urinary thiamine levels; (6) high plasma creatinine levels; and (7) lower succinic-oxidase enzyme activity (Beauchamp et al. 1983; Hernberg et al. 1971; Lewey et al. 1941; WHO 1981). In addition, covalent crosslinking to erythrocyte spectrin may find application as a biomarker (Valentine et al. 1993). All of these are early indicators of central and peripheral nervous system, cardiovascular, endocrine, and reproductive

toxicity. Also, these biological markers are not specific for carbon disulfide. One or a combination of these markers may prove to be a useful biomarker for carbon disulfide effects. See Section 2.2 for other effects caused by carbon disulfide.

2.7 INTERACTIONS WITH OTHER SUBSTANCES

Many of the chemical interactions with carbon disulfide appear to be related to loss of microsomal cytochrome P-450. Carbon disulfide suppresses the hepatic cytochrome P-450 microsomal enzyme system. Elimination of phenazone, a drug often used in the study of hepatic microsomal enzyme activity, is significantly and reversibly inhibited in rabbits exposed to 193 ppm carbon disulfide for 5 hours a day, 6 days a week, for 6 months (Orzechowska et al. 1984). It has been proposed that the active sulfur atoms released following carbon disulfide metabolism suppress the cytochrome P-450 enzymes, thus inhibiting detoxification of other drugs or chemicals.

The influence of carbon disulfide exposure on the cardiovascular actions of adrenaline and noradrenaline was examined in urethane-anesthetized rats. Electrocardiographic change (T-wave elevation) consistent with slight myocardial ischemia was noted in anesthetized rats administered carbon disulfide (253 mg/kg/day) for 4 weeks and challenged with adrenaline or noradrenaline (Hoffmann and Muller 1990; Klapperstück et al. 1991). Carbon disulfide-exposed rats were more prone to ventricular arrhythmias (extrasystoles) and a prolongation of the PR interval than nontreated animals. Thus, the hypertensive adrenergic effects of these drugs appear to be enhanced in carbon disulfide-exposed rats. It was further shown by Klapperstück et al. (1991) that lactic dehydrogenase M isozyme activity was increased under these condition, while total lactate dehydrogenase activity was not, a change that has been associated with adaptation to anaerobic metabolism. Based on these findings, the study authors postulated that carbon disulfide cardiotoxicity may be mediated by disruption of the energy supply. Administration of up to 506 mg/kg carbon disulfide by gavage to urethane-anesthetized Wistar rats did not cause changes in the development of cardiac arrhythmias induced either by ischemia (coronary ligation) or by aconitine administration (Hoffmann and Klapperstuck 1990).

The combined effect of carbon disulfide exposure and ethyl alcohol has been examined to determine if carbon disulfide exposure results in the Antabuse syndrome, an intolerance to alcohol. The metabolism of Antabuse, disulfuram, or tetraethylthiuram disulfide (TETD) produces carbon disulfide

and diethylamine. The metabolites of Antabuse inhibit the enzymes necessary to metabolize ethyl alcohol (aldehyde dehydrogenase and catalase), which results in the Antabuse syndrome due to a buildup of aldehyde. Symptoms include a sensation of heat, a fall in blood pressure, nausea, and in extreme cases circulatory collapse (Djuric 1971). Research by Freundt et al. (1976) on rats and humans of the combined effects of carbon disulfide exposure and ethanol ingestion indicate that, at low (20 ppm) and medium (400 ppm) levels of carbon disulfide exposure and blood alcohol levels of approximately 0.75%, there is a carbon disulfrde inhibition of aldehyde dehydrogenase with an increase in acetaldehyde concentrations in the blood. However, these increased acetaldehyde concentrations were not considered great enough to indicate the Antabuse syndrome. The study authors asserted that the Antabuse syndrome is not likely to occur in subjects who have blood alcohol levels of up to 0.8% and are exposed to 10 ppm carbon disulfide.

The possible role of the ethanol-inducible isozyme of cytochrome P-450 in the metabolism of carbon disulfide has been examined (Snyderwine et al. 1988). Rats were administered various alcohols (methanol, ethanol, isopropanol, and isobutanol) by gavage. Eighteen hours after alcohol administration, rats were administered carbon disulfide intraperitoneally at doses of 1, 100, or 625 mg/kg. The results showed that pretreatment of rats with these alcohols enhances the metabolism of carbon disulfide by increasing the ethanol-inducible isoform of cytochrome P-450 with isopropanol being most potent. Furthermore, the study authors indicate that alcohol induction of P-450-dependent carbon disulfide metabolism per se is not sufficient to result in carbon disulfide-induced hepatic damage although it does lead to the loss of specific cytochrome P-450 function.

The chronic effect of carbon disulfide and ethanol was examined by Opacka et al. (1984). Rats were exposed to 257 ppm of carbon disulfide for 5 hours a day, 6 days a week, for 11 months, and 10% ethanol (in water *ad libitum* for the last 3 months, control water *ad libitum*). Control rats were exposed to filtered air. The behavior, memory, and learning ability of the ethanol-fed rats were adversely affected compared to controls. Additional studies indicate biochemical alterations in the central nervous system and increased β-glucuronidase activity; ultrastructural studies show degeneration in the peripheral nervous system, particularly in the myelin sheath. These authors reported that the effects from combined exposures are greater than those from each substance alone. Wronska-Nofer et al. (1986) investigated the hepatotoxicity of combined ethanol and carbon disulfide in the rat. Rats were chronically exposed to 482 ppm (1.5 g/m^3) of carbon disulfide 5 hours a day, 5 days a week, for 5 months and given a 10% ethanol solution as their sole source of fluids. Ethanol

increased the hepatotoxicity of carbon disulfide by potentiating the effects (further depressing the P-450 levels) of carbon disulfide on the activity of the microsomal cytochrome P-450 monooxygenases of the liver and on the hepatic endoplasmic reticulum (development of giant mitochondria and degranulation of the rough endoplasmic reticulum).

An occupational epidemiological study of grain workers indicates that there are neurotoxic effects from exposure to the 80/20 carbon tetrachloride/carbon disulfide fumigant. Most of the effects (dysfunction of the peripheral axons, auditory nerve, optic nerve, and extrapyramidal system, as well as altered behavior and cognition) also appear in viscose rayon workers exposed to carbon disulfide. However, a combined effect with carbon tetrachloride could not be ruled out. EPA banned the use of 80/20 carbon tetrachloride/carbon disulfide fumigants after June of 1986 because of the potential for combined and synergistic toxic effects on the nervous system (EPA 1989a). In sheep, combined treatment with carbon tetrachloride and carbon disulfide, whether given simultaneously or separated by 6 hours (carbon disulfide then carbon tetrachloride), is effective in controlling liver fluke (Seawright et al. 1972). Carbon tetrachloride can be hepatotoxic in sheep; when it is administered with carbon disulfide, however, the toxicity is significantly reduced. The mechanism by which carbon disulfide protects against carbon tetrachloride toxicity in rats was investigated by Seawright et al. (1980). Carbon tetrachloride requires microsomal metabolism in the liver to exert a toxic effect. Carbon disulfide is effective in decreasing microsomal metabolic activity through the loss of cytochrome P-450 and therefore decreases the microsomal metabolism of carbon tetrachloride.

Carbon disulfide potentiates the toxic effect of amphetamines in rats; this effect increases with duration of exposure to carbon disulfide (Caroldi et al. 1987). Freundt et al. (1974b) have found that the effect of sodium phenobarbital pretreatment on rat liver fat accumulation following exposure to carbon disulfide is dependent on the dose and method of ingestion of carbon disulfide. Fat accumulation is significant if the phenobarbital-treated rat is exposed orally to a very high dose of carbon disulfide (1,263 mg/kg body weight); however, inhalation of 20-200 ppm of carbon disulfide (8 hours for 2-7 days) in a phenobarbital-treated rat did not produce fat accumulation in the liver. Extrapolation to human beings is difficult, but the study authors asserted that the sensitivity of hepatic MFOs to carbon disulfide is similar qualitatively and quantitatively in rats and humans. They suggest that it is not hazardous for individuals without hepatic disease to take barbiturate-containing drugs and simultaneously work in 20-200 ppm carbon disulfide (Freundt et al. 1974b).

Carbon disulfide interacts with several organophosphorus compounds including the insecticides malathion and parathion. Metabolism of malathion and parathion requires cytochrome P-450 and is thus inhibited by carbon disulfide (Dalvi and Howell 1978). It is important to note that carbon disulfide would potentiate the toxic effect of compounds that require cytochrome P-450 microsomal metabolism for detoxification.

Oral administration of diethylthiocarbamate and carbon disulfide (separately) protects mice against chloroform-induced kidney injury (Masuda and Nakayma 1983a). The mechanism for protection is postulated to be the inhibition of the kidney microsomal mono-oxygenase system by the thiono-sulfur groups of diethylthiocarbamate and carbon disulfide because chloroform requires metabolic activation in the kidney to induce nephrotoxicity. Carbon disulfide also protects mice from 1,2-dimethyl-hydrazine-induced neoplasia of the large intestine (Wattenberg and Fiala 1978). 1,2-Dimethyl-hydrazine requires several steps of metabolic activation, including the *N*-oxidation of azomethane to azoxymethane. This step is inhibited by the loss of microsomal MFO activity caused by carbon disulfide.

In a well-conducted study by Antov et al. (1985), rats were administered carbon disulfide by inhalation for l-6 months with or without an arteriogenic diet (consisting of cholesterol, choleic acid, and vitamin D2), which was used to develop the sclerotic process. Concentrations of 16, 32, and 64 ppm carbon disulfide produced significant changes in the myocardium in a dose-response relationship. The administration of the atherogenic diet potentiated the cardiotoxicity and enhanced the sclerotic process. Although changes in enzyme activity (statistical significance not reported) were noted, no substantial structural changes occurred in the heart muscle or aorta at the lowest dose of carbon disulfide tested (3.2 ppm) without the atherogenic diet. Histological changes characterized by vacuolar dystrophy and interstitial fibrosis occurred at 16 ppm carbon disulfide and became more advanced at higher concentrations (64 ppm). The study authors concluded that carbon disulfide has an arteriogenic effect.

Carbon disulfide was reported to inhibit the mutagenic activity of 1,2-dimethylhydrazine and azoxymethane in a host-mediated assay (7-week-old ICR mice) (Moriya et al. 1979). These authors suggested that this effect is due to carbon disulfide’s prevention of in *vivo* oxidation of azomethane to azoxymethane.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to carbon disulfide than will most persons exposed to the same level of carbon disulfide in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters may result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects on clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, "Populations With Potentially High Exposure."

There are studies that have investigated particular metabolic traits that may result in hypersusceptibility to carbon disulfide (Djuric et al. 1973; Stokinger and Scheel 1973). The study conducted by Djuric et al. (1973) reported on 72 workers who had been divided into three groups: 18 exposed to carbon disulfide at levels below the industrial air limit of 20 ppm (60 mg/m^3) (controls), 21 who had been exposed to levels higher than 20 ppm but had shown no signs or symptoms of carbon disulfide intoxication (resistant), and 33 who had polyneuritis or other signs of overexposure and had been removed from exposure (susceptibles). All individuals were administered an oral dose of 0.5 g of disulfiram (Antabuse), a compound that produces carbon disulfide when metabolized. It was assumed that carbon disulfide and disulfiram are metabolized by the same or similar enzyme system, and determination of diethyl dithiocarbamates (DDC) in urine after disulfiram administration was used to evaluate the rate at which sulfur compounds are metabolized. The excretion of DDC was significantly lowest in the susceptible group (49.70 μg/mg creatinine) when compared to both the control (160.05 μg/mg creatinine) and resistant (90.04 μg/mg creatinine) groups. These results led to the suggestion that the reduced ability of the symptomatic workers to metabolize this compound would lead to hypersusceptibility to carbon disulfide and would thus be associated with the clinical signs observed in that group. No supporting data have been located, however.

The study authors (Djuric et al. 1973) suggested that carbon disulfide exposure causes a decrease in excretion of DDC, especially in once-poisoned workers; thus carbon disulfide exposure produced a

disturbance in the metabolism of sulfur compounds. They also suggested that in the susceptible worker group this decreased metabolic conversion appeared to persist even 5-10 years after exposure, and thus carbon disulfide exposure may have led to an irreversible metabolic disturbance. These authors did not speculate on the mechanism of actual metabolic inhibition, nor did they propose any genetic hypothesis. One study limitation included the problematic issue of whether it is possible to establish that a prior hypersusceptibility existed in testing workers who had been exposed for long periods of time and whose differences in metabolism may have related to the circumstances of their exposure rather than to a previous susceptibility.

Because it appears that one common mechanism of the cerebral, cardiovascular, and hepatic effects may be an acceleration of the arteriosclerotic process (see Section 2.4), individuals at risk for arteriosclerosis or those with early arteriosclerosis would probably be at increased risk for health effects following exposure to carbon disulfide (NIOSH 1978). The mechanism for carbon disulfide acceleration of arteriosclerotic plaque formation involves direct injury to the vessel endothelium and changes in lipid metabolism.

Three other groups are recognized as being unusually susceptible to carbon disulfide: alcoholics (including those treated with Antabuse), those with neuropsychic disorders, and those with vitamin B_6 deficiency (Djuric et al. 1973; Lefaux 1968; Peters et al. 1982). Carbon disulfide reduces the levels of vitamin B_6, which in turn upsets carbohydrate metabolism, particularly the cerebral carbohydrates (Lefaux 1968).

2.9 METHODS FOR REDUCING TOXIC EFFECTS

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

2.9.1 Reducing Peak Absorption Following Exposure

There are no specific methods available to reduce the absorption of carbon disulfide following exposure. Activated charcoal (Stutz and Janusz 1988) and gastric lavage or induced emesis using a saturated sodium bicarbonate solution to decrease gastric acidity and to preclude hydrogen sulfide formation (Dreisbach and Robertson 1987) have been suggested for treatment of carbon disulfide ingestion. However, the use of emetics is controversial (Bronstein and Currance 1988; Stutz and Janusz 1988). If contamination occurs via the skin, thorough washing with soap and water has been suggested. For eye contamination, flushing the eyes with copious amounts of water is the recommended treatment (Stutz and Janusz 1988).

Urea (0.5-1.5 g/kg) administered intravenously has been recommended to inactivate free carbon disulfide in the blood; intravenous administration of large doses of vitamin B_6 has also been recommended (HSDB 1995).

2.9.2 Reducing Body Burden

Unmetabolized carbon disulfide is exhaled unchanged in expired air (McKee et al. 1943), whereas metabolites are excreted primarily in the urine (Soucek 1957). Therefore, increasing ventilation and urinary output may be potential methods to facilitate mitigation of the effects of carbon disulfide. However, such methods have not been attempted with regard to carbon disulfide within a clinical setting. Removing the patient from the contaminated area followed by supportive care is the suggested basic form of treatment (Bronstein and Currance 1988; Dreisbach and Robertson 1987; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Morgan 1982; Stutz and Janusz 1988).

Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate which may either spontaneously degrade to atomic sulfur and carbonyl sulfide or hydrolyze to form atomic sulfur and monothiocarbamate (Beauchamp et al. 1983). Although phenobarbital pretreatment of rats can enhance the metabolism of carbon disulfide (Dalvi and Neal 1978), it can also enhance the hepatic toxicity of carbon disulfide. Another metabolic pathway for carbon disulfide is its conjugation with glutathione to form 2-thiothiazolidine-4-carboxylic acid and 2-oxothiazolidine-4-carboxylic acid,which are excreted in the urine (Van Doorn et al. 198la, 1981b). Although the extent to which glutathione

conjugation plays a role in the metabolism of carbon disulfide is unclear, it is conceivable that administration of cysteine may be utilized in the mitigation of the effects of carbon disulfide.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

There are no specific methods available that provide information on interfering with the mechanism of action for toxic effects of carbon disulfide. However, decreases in carbon disulfide toxicity may be brought about by other chemical substances. For example, the effects of carbon disulfide on the nervous system (the primary target of exposure to carbon disulfide) appear to be influenced by the mineral content of the diet. Rabbits given diet supplements of copper and zinc were exposed to high levels of carbon disulfide (1,100 ppm) and did not show the usual signs of exposure-weight loss, serum lipoprotein and total cholesterol increase, adrenal hypertrophy, and pathological changes in the brain and spinal cord-that appeared in controls on a normal salt diet (ACGIH 1986). A proposed biochemical basis for the antagonist effect of minerals is that carbon disulfide metabolites-dithiocarbamates- are metal chelating agents. The metal complex is more water soluble than the dithiocarbamate alone and thus is excreted faster than the dithiocarbamate. Additional minerals hasten the loss of the compound that causes the effects.

The formation of reactive intermediates resulting from the metabolism of carbon disulfide by the hepatic MFO system may be involved in the hepatoxicity of this chemical. Pretreatment of rats with phenobarbital, an inducer of MFO activity, can enhance carbon disulfide-induced hepatotoxicity (Bus 1985). Inhibition of the MFO system might potentially reduce carbon disulfide-mediated hepatoxicity. In support of this possibility, Bond and De Matteis (1969) reported that pretreatment of rats with SKF-525A, an inhibitor of cytochrome P-450-mediated metabolism, reduced the liver damage from carbon disulfide in phenobarbital-pretreated animals.

Two possible mechanisms for the neurotoxicity of carbon disulfide have been suggested. One mechanism involves the formation of dithiocarbamates. The inhibitory effect of carbon disulfide on the activity of the copper-requiring enzyme dopamine-β-hydroxylase was attributed to the formation of dithiocarbamates, which can complex copper (McKenna and DiStefano 1977b). Interference with the formation of this metabolite may be a potential strategy, albeit untested, to reduce neurotoxicity from carbon disulfide poisoning. An alternative mechanism postulated to explain the neurotoxic effect of carbon disulfide is the formation of a dithiocarbamate derivative, a form of vitamin B_6, of

pyridoxamine, with carbon disulfide (Vasak and Kopecky 1967). Since transaminases and amine oxidases require the pyridoxamine phosphate form of vitamin B_6 as a cofactor, it was further postulated that these enzymes would be inhibited in carbon disulfide poisoning. Although data are limited, supplementation of the diet with vitamin B_6 has been demonstrated to delay some of the neurotoxic effects of carbon disulfide (Teisinger 1974).

2.10 ADEQUACY OF THE DATABASE

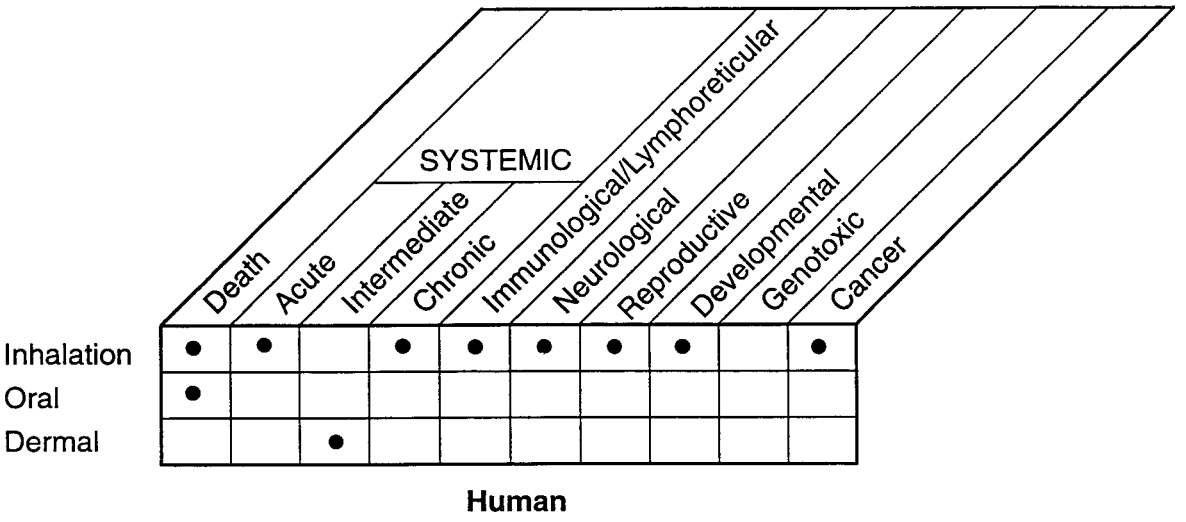
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon disulfide is available. Where adequate information 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 carbon disulfide.

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

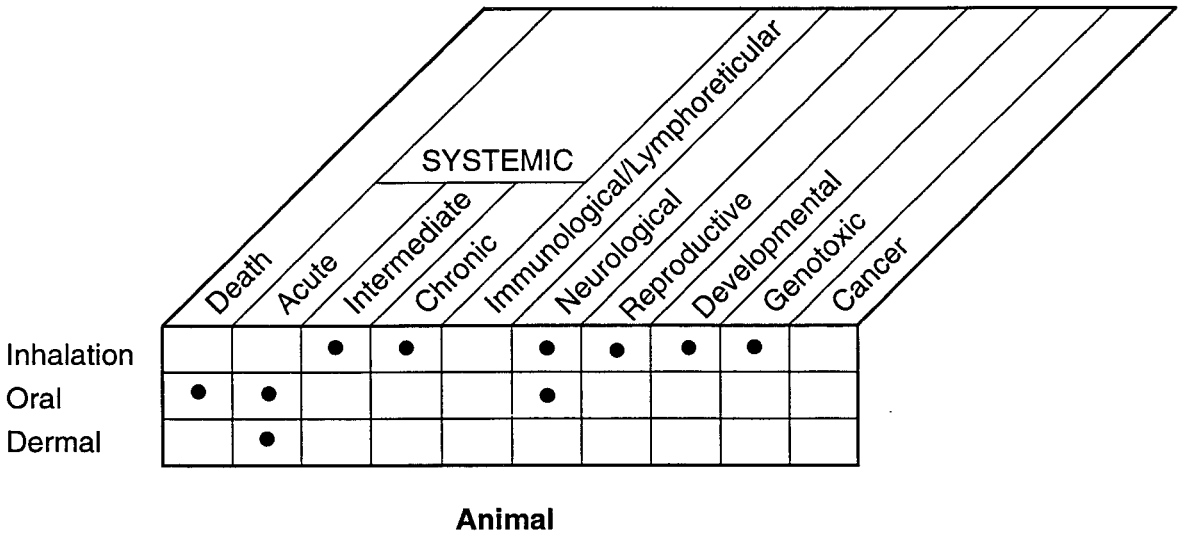
2.10.1 Existing Information on Health Effects of Carbon Disulfide

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

FIGURE 2-4. Existing Information on Health Effects of Carbon Disulfide



Human



Animal

● Existing Studies

There are human data on inhalation exposure of carbon disulfide that provide information on acute and chronic systemic effects. There are also data on immunologic, neurologic, developmental, and reproductive effects. There are some limited data on the carcinogenic potential of carbon disulfide, but these are preliminary and confounded by multiple exposure problems. There are no oral exposure data from humans and only limited information on dermal exposures. The dermal data address the occupational hazard of blister formation following accidental exposure.

Animal data on inhalation exposure cover primarily intermediate systemic, neurological, developmental, and reproductive effects. One chronic study is available that examines systemic effects in rats. Oral data are limited to lethality data and information on acute systemic and neurological effects in mice and rats. Dermal data consist of some information regarding blister formation in humans and rabbits following dermal exposure.

2.10.2 Identification of Data Needs

Acute-Duration Exposure. The data on acute inhalation exposure in humans are limited. Transient respiratory difficulties were reported in some individuals following an accident in transporting carbon disulfide, but the actual exposures could not be determined (Spyker et al. 1982). Adverse effects including deaths were reported in a community in India following an accidental release of large amounts of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon plant (Kamat 1994). There are sufficient animal data to identify the nervous system (Liang et al. 1983; Magos and Jarvis 1970; Magos et al. 1974; Tarkowski et al. 1980), the cardiovascular system (Chandra et al. 1972; Tarkowski and Sobczak 1971), and the liver (Freundt et al. 1974a; Magos and Butler 1972; Magos et al. 1973) as target organs for acute inhalation exposure. Lethality (Gibson and Roberts 1972), decreased respiratory rate (Tarkowski and Sobczak 1971), and increased post-implantation loss (PAI 1991) have also been reported in acute studies. The acute studies have been done primarily in rodents; additional studies on other species, for example, primates, would be useful for generalizing to expected health effects in humans. No acute-duration inhalation MRL was derived since potential MRL values would not have been as protective of human health as the chronic-duration MRL. In addition, there is greater confidence in the chronic-duration MRL since it is based on human data.

Three case reports cited in Gosselin et al. (1984) indicate that half an ounce (concentration not specified) caused death following ingestion. There are no other human data on acute-duration oral exposure to carbon disulfide. There are sufficient animal data to identify the cardiovascular system (Hoffman and Klapperstück 1990) and the liver (Jones-Price et al. 1984b; Masuda et al. 1986) as targets for acute-duration oral exposure to carbon disulfide. Adverse effects including decreased body weight gain (Jones-Price et al. 1984b), hind-limb paralysis (Jones-Price et al. 1984b), decreased norepinephrine levels (Kanada et al. 1994), and developmental effects have also been reported. An acute-duration oral MRL of 0.01 mg/kg/day was derived based on dose-dependent decreases in the activities of liver microsomal drug-metabolizing enzymes in mice from the study by Masuda et al. 1986.

There are no human data on acute-duration dermal exposure to carbon disulfide. There is one acute study showing blister formation in rabbit ears when carbon disulfide was applied to the skin (Hueper 1936). Given that there is a possibility of dermal exposures in occupational settings as well as from contaminated water supplies near hazardous waste sites, additional data on dermal exposures would be useful. No pharmacokinetic data exist that show that dermal exposures to carbon disulfide result in patterns of distribution similar to those seen with inhalation or oral exposures. These kinds of data would be useful for projecting the similarities of target organs across routes as people near hazardous waste sites may be exposed by all three routes.

Intermediate-Duration Exposure. There are no human data on intermediate-duration inhalation exposures. Most occupational exposures are considered to be chronic-duration inhalation exposures, and there are no oral data for humans. The only dermal data for humans concern blisters on the fingers of rayon workers following 6 weeks of exposure (Hueper 1936).

There are animal data that identify the nervous system (Jirmanova and Lukas 1984; Juntunen et al. 1977; Liang et al. 1983; Merigan et al. 1985, 1988), the cardiovascular system (Antov et al. 1985), and the liver (Jarvisalo et al. 1977a; Tsuyoshi 1959; Wronska-Nofer 1972, 1973) as targets for intermediate-duration inhalation exposure to carbon disulfide. NOAELs and LOAELs exist for these exposures. Again, most of these data come from rodent studies, although some work has been done in monkeys (Merigan et al. 1988) and dogs (Lewey et al. 1941). The toxic effects are generally the same across species. There is some evidence that the reproductive (Tepe and Zenick 1984; Zenick et al. 1984) and developmental systems (Danielsson et al. 1984; Lehotzky et al. 1985; Tabacova and

Balabaeva 1980b; Tabacova et al. 1983; Yaroslavskii 1969) are also targets for intermediate-duration inhalation exposures. Lethality (Toxicogenics 1983c), decreased body weight (Rebert and Becker 1986), and hematologic (Toxicogenics 1983c) and musculoskeletal (Szendzikowski et al. 1974) effects have also been reported in intermediate-duration inhalation studies. No intermediate-duration MRL was derived since potential values would not have been as protective of human health as the chronic duration MRL. There are very few data on intermediate-duration oral exposures (Pilarska et al. 1973) and no data on intermediate-duration dermal exposures. Decreased body weight has been observed in rats following oral exposure to carbon disulfide for an intermediate-duration period (Hoffman and Klapperstück 1990). No intermediate-duration oral MRLs were derived because of a lack of reliable LOAELs and a lack of definitive target organs. Additional information on inhalation, oral, and dermal exposures would be useful for assessing the health risks to humans living near hazardous waste sites including identification of target organs.

Chronic-Duration Exposure and Cancer. There are data on chronic occupational exposures that identify the nervous system (Aaserud et al. 1988; Cassitto et al. 1978; Foa et al. 1976; Johnson et al. 1983; Peters et al. 1988; Putz-Anderson et al. 1983; Seppalainen and Tolonen 1974; Seppalainen et al. 1972; UK/IHSE 1981; Vasilescu 1976), the cardiovascular system (Cirla et al. 1972; Franco et al. 1982; Hernberg et al. 1970, 1971, 1973, 1976; Lieben et al. 1974; MacMahon and Monson 1988; Nurminen et al. 1982; Swaen et al. 1994; Tiller et al. 1968; Tolonen et al. l979), the liver (El-Sobkey et al. 1979; Mack et al. 1974; Rubin and Arieff 1945; Sidorowicz et al. 1980), and the eye (DeLaey et al. 1980; DeRouck et al. 1986; Raitta and Tolonen 1975; Raitta et al. 1974; Szymankova 1968) as primary targets for inhalation exposure to carbon disulfide. There are no data on chronic human oral or dermal exposures. Most of the occupational studies have limitations concerning the exposure measurements and concomitant exposures; some are limited by the methods used to assess the health effect end points. However, the Johnson et al. (1983) study, which evaluates nerve conduction velocity in workers exposed by inhalation to carbon disulfide, has been found acceptable for the derivation of a chronic-duration MRL. There is only one chronic inhalation study in rabbits (Cohen et al. 1959) and no chronic oral or dermal animal studies. Additional data concerning the effects of chronic low-level exposure to carbon disulfide following the inhalation, oral, and dermal routes would be useful to establish a dose-effect relationship for the major health effects, and to identify target organs for oral and dermal exposures. Long-term animal studies in several species, such as mice, rats, and monkeys, that investigate several dose points could be used to determine the neurotoxic mechanism of action.

There are no data suggesting an increased risk of cancer from exposure to carbon disulfide. Epidemiological data have been presented that indicate that there could be an association between carbon disulfide exposure and lymphocytic leukemia, but these studies had several problems with multiple comparisons, poor exposure variables, multichemical exposures, and no supporting animal or human data (Arp et al. 1983; Checkoway et al. 1984; Wilcosky et al. 1984). In addition, the lymphocytic leukemia may be associated with benzene impurity. A large population sample with better exposure data covering a minimum of 20 years would be helpful for addressing the repeatability of these findings. There are no studies regarding cancer in humans following oral or dermal exposure. In addition, there are no studies regarding cancer in animals via inhalation, oral, or dermal routes of exposure. A chronic animal bioassay would provide important information on effect and possibly on mechanism.

Genotoxicity. There are no human genotoxicity data for any route of exposure. Measurements of chromosomal aberrations or DNA adduct formation done on workers exposed via inhalation would be useful for assessing the genotoxic potential of carbon disulfide. Data obtained in this way, or from animals exposed *in vivo*, may provide evidence of some mechanism for the observed reproductive effects and, if correlated with exposures, could offer a potential biomarker of effect. This would provide some way of monitoring populations around hazardous waste sites. One *in vivo* animal study of the genotoxicity (chromosomal aberrations in the bone marrow of rats) of carbon disulfide reported negative results (NIOSH 1980). Bacterial mutagenicity assays (*Salmonella typhimurium* and *Escherichia coli)* have also generally been negative (Donner et al. 1981; Hadenstedt et al. 1979). Additional bacterial assays would not be useful at this time.

Reproductive Toxicity. There are human data that indicate that chronic inhalation exposure to carbon disulfide can affect the reproductive system in both males and females. In males, sperm morphology, hormone levels, and libido have been altered by occupational exposure to carbon disulfide (Lancranjan 1972; NIOSH 1983; Schrag and Dixon 1985; Vanhoorne et al. 1994; Wagar et al. 1981; Wyrobek 1983). In human females, menstrual irregularities have been associated with inhalation exposure to carbon disulfide, although more serious effects such as increased miscarriage and reduced fertility have not been universally noted (Cai and Bao 1981; Heinrichs 1983; Hemminki and Niemi 1982; Wang and Zhao 1987; Zhou et al. 1988; Zielhuis et al. 1984). There are no human data on the reproductive effects of oral or dermal exposure to carbon disulfide. Data on rats support the reproductive effects seen in humans after inhalation exposure only (Tepe and Zenick 1984; Zenick

et al. 1984). Additional reproductive studies on other species, such as mice, rabbits, dogs, and monkeys, would be useful to determine the dose-effect relationship between exposure and reproductive end points. It would also be useful to investigate reproductive organ pathology in a 90-day study of toxicity via inhalation, oral, and dermal exposures. There are no oral or dermal reproductive studies in animals.

Developmental Toxicity. There are no convincing human data that support an increased rate of congenital malformations in children born to mothers exposed by any route to carbon disulfide (Bao et al. 1991). Limitations of the Bao et al. (1991) study included the lack of exposure analyses and dose response assessments. There are data on rodents that suggest an increased fetotoxicity following inhalation exposure to carbon disulfide (Tabacova and Balabaeva 1980b; Yaroslavskii 1969). In addition, neurobehavioral effects have been reported in the offspring of exposed animal mothers (Lehotzky et al. 1985; Tabacova et al. 1983). Additional data from species other than rodents, for example, monkeys, would be useful for verifying that developmental effects are a result of exposure to this chemical. There are no animal data that provide information on the developmental effects of either oral or dermal exposure. There is evidence that carbon disulfide can cross the placenta and is distributed to the fetal brain, blood, liver, and eyes (Danielsson et al. 1984). Well-designed studies via inhalation, oral, and dermal exposures show that a dose-response relationship would be useful for determining the dose at which developmental effects could be expected to occur.

Immunotoxicity. There are no data that suggest that the immune system is a target for carbon disulfide exposure for any route or in any species. However, the results of one study indicated that the β-lipoprotein isolated from carbon disulfide exposed-workers (presumably exposed via inhalation) is antigenically identical to lipoproteins isolated from healthy nonexposed controls (Bobnis et al. 1976). There are no studies regarding immunological/lymphoreticular effects in humans following oral or dermal exposures. Also, there are no studies concerning immunological/lymphoreticular effects in animals following inhalation, oral, or dermal exposures. A 90-day study that investigates immune parameters should include routine immune function parameters (e.g., macrophage activity, T-cell activity, mitogen response, cell-mediated immune response) and immunopathology. This would be useful information for determining if there could be an immune system effect that has been overlooked.

Neurotoxicity. It is clear in both humans and animals that the nervous system is the primary target organ for carbon disulfide exposure for the inhalation route. There are behavioral (e.g., depression, decreased performance and memory), histopathological (e.g., polyneuropathy), and neurophysiological (e.g., decreased nerve conduction velocity) data in humans (Aaserud et al. 1988; Cassitto et al. 1978; Cirla et al. 1972; Foa et al. 1976; Johnson et al. 1983; Lancranjan 1972; Peters et al. 1986a, 1986b, 1988; Putz-Anderson et al. 1983; Seppalainen and Tolonen 1974; Seppalainen et al. 1972; Vasilescu 1976). The results of inhalation toxicity studies in animals have revealed neurophysiological (e.g., decreased nerve conduction velocity), neurochemical (e.g., altered noradrenalin and dopamine levels), and neurobehavioral (e.g., hind-limb paresis) (Frentik et al. 1994; Magos and Jarvis 1970; Tarkowski and Sobczak 1971; Wilmarth et al. 1993). A chronic-duration inhalation MRL was calculated on the basis of reduced motor nerve conduction velocity in humans (Johnson et al. 1983). There are no human data pertaining to neurological effects following oral or dermal exposures. There are limited animal data on neurotoxic effects (hind-limb paralysis) by the oral route (Jones-Price et al. 1984a, 1984b), and none for the dermal route (Dietzmann and Laass 1977). Studies investigating neurotoxic effects in animals following oral or dermal exposure would be useful for determining thresholds and dose-response relationships for neurotoxic effects.

Epidemiological and Human Dosimetry Studies. There are many epidemiological studies that address the effects of inhalation exposure to carbon disulfide. These studies include both occupational- and community-based cohorts and have investigated neurological (Aaserud et al. 1988; Cassitto et al. 1978; Cirla et al. 1972; Foa et al. 1976; Johnson et al. 1983; Peters et al. 1988; Putz-Anderson et al. 1983; Seppalainen and Tolonen 1974; Seppalainen et al. 1972), cardiovascular (El-Sobkey et al. 1979; Franco et al. 1982; Hernberg et al. 1970, 1971, 1973, 1976; Lieben et al. 1974; MacMahon and Monson 1988; Nurminen and Hernberg 1985; Nurminen et al. 1982; Rubin and Arieff 1945; Sidorowicz et al. 1980; Sugimoto et al. 1978; Tiller et al. 1968; Tolonen et al. 1975, 1979; UK/HSE 1981), and reproductive (Cai and Bao 1981; Heinrichs 1983; Hemminki and Niemi 1982; Lancranjan 1972; NIOSH 1983; Wagar et al. 1981; Wang and Zhao 1987; Zhou et al. 1988) effects. These studies have found positive associations between carbon disulfide exposure and adverse health effects. These findings are not universal, however. The epidemiological studies have the limitation of poor exposure measurements that are not individualized to the study participants. In addition, the exposures postulated to have occurred in these cohorts cover a wide range of levels, making extrapolations from one study to another difficult; many of these studies also documented concomitant exposures to other chemicals, most notably hydrogen sulfide. A principle components

exposure index has been developed for simultaneous exposures to hydrogen sulfide and carbon disulfide by Vanhoorne et al. (1995). Nonetheless, they provide data on human health effects, and the weight of the evidence is that the environment where carbon disulfide is used exerts a range of effects at different exposure levels. Clearly, occupational workers, as well as communities around hazardous waste sites or point-emission sources, are at risk for exposure to levels of carbon disulfide that have been associated with adverse health effects. The biggest drawback in the existing studies is the lack of the ability to establish a dose relationship between exposure and effect. More precise measurements of exposure, control of exposure to other chemicals, and long-term follow-up of occupational cohorts may lead to a better understanding of the dose-effect of carbon disulfide. Monitoring of populations around hazardous waste sites where carbon disulfide is known to be present would be useful, providing specific biomarkers of exposure could be identified.

Biomarkers of Exposure and Effect

Exposure. Biomarkers of exposure for carbon disulfide include levels of carbon disulfide in exhaled breath (Philips 1992), blood (WHO 1979), and urine (Djuric 1967; McKee et al. 1943) and carbon disulfide thiometabolites in the urine (Beauchamp et al. 1983; Campbell et al. 1985). These measurements can indicate whether acute-, intermediate-, or chronic-duration exposure to carbon disulfide has occurred. The presence of carbon disulfide in various biological media is the most specific biomarker of exposure. However, few studies have been able to demonstrate a straightforward correlation between the above parameters and actual carbon disulfide exposure. There are two possible explanations for this observation. The first one is that too many variables tend to confound the measurements of carbon disulfide in the blood, breath, and urine of exposed individuals, rendering these biomarkers somewhat unreliable for quantitative evaluation. The second possibility is that the limitations of the protocols and analytical methods employed for measuring carbon disulfide in biological media have largely precluded acquiring data related to ascertaining the reliability of these biomarkers. Therefore, with the development of new and more sensitive methods, blood, breath, and urine levels of carbon disulfide may become even more useful biomarkers of exposure. More quantitative, correlative studies are necessary in order to assess this possibility.

At the present time, the biomarkers that correlate best with exposure are metabolite levels in the urine (Baselt 1980; Beauchamp et al. 1983; Campbell et al. 1985; Lieben 1974i; WHO 1986). The iodine-azide and the TTCA tests, which measure the presence of urinary carbon disulfide metabolites, have

been shown to correlate well with actual exposure. However, the iodine-azide test is nonspecific (Dox et al. 1992). TTCA is produced in humans after exposure to Antabuse and in rats after exposure to Captan (Cox et al. 1992). Moreover, other investigations are necessary in order to determine whether the interaction of carbon disulfide with other substances (such as hydrogen sulfide, drugs, carbon tetrachloride, malathion, and alcohol), disease states, and variations in diet and in individual metabolism, as well as other factors, could confound the results of the iodine-azide test and the TTCA test for carbon disulfide exposure. Baseline urine, breath, and blood samples are necessary to correct for non-workplace exposures For exposures around hazardous waste sites, the influence of workplace exposures must also be corrected for in this manner

Effect. Few specific biomarkers of effect have been identified for carbon disulfide. However, many biological parameters have been tentatively linked with carbon disulfide's effects on certain enzyme systems, on the liver and the kidneys, and on the nervous, cardiovascular, reproductive, and endocrine systems (Baselt 1980; Beauchamp et al. 1983; Cai and Bao 1981; Campbell et al. 1985; Djuric 1967; El-Sobkey et al. 1979; Heinrichs 1983; Hernberg et al. 1971; Lancranjan 1972; Lewey et al. 1941; Lieben 1974; McKee et al. 1943; Prerovska and Drdkova 1967; Toyama and Sakurai 1967; Valentine et al. 1993; Wang and Zhao 1987; WHO 1979, 1981, 1986; Zhou et al. 1988). No studies attempting to quantitatively correlate these parameters with carbon disulfide effects are available. CT-scan (computerized tomography) and electromyographical parameters as well as measurements of nerve conduction velocity may serve as indicators of nervous system effects (Beauchamp et al. 1983; WHO 1981). High serum lipid content (in particular cholesterol) may be linked with cardiovascular effects (El-Sobkey et al. 1979; Lewey et al. 1941; Prerovska and Drdkova 1967; Toyama and Sakurai 1967). Reduction in steroid hormone synthesis may indicate early reproductive system effects (Cai and Bao 1981; Heinrichs 1983; Lancranjan 1972; Wang and Zhou 1987; Zhou et al. 1988). Also higher plasma creatinine levels and elevated blood sugar could signal renal dysfunction due to carbon disulfide exposure (Hernberg et al 1971; Lewey et al. 1941). Changes in various enzyme systems (for example, lowered succinic-oxidase activity) may prove useful as biomarkers of effect (McKee et al. 1943). The effect of carbon disulfide on membrane spectrin of nerve and blood membranes should be investigated further (Valentine et al. 1993). However, changes in these biological parameters are not specific to carbon disulfide exposure, and future studies are needed to determine the extent to which they quantitate the results of carbon disulfide exposure. In addition, these studies should evaluate whether such biological parameters may serve as reliable and accurate biomarkers of carbon disulfide exposure. However, since many different things can cause these effects, biomarkers may never be

specific for exposure to carbon disulfide. Nervous system effects are well documented for inhalation exposures but not for oral or dermal exposures.

Absorption, Distribution, Metabolism, and Excretion. There are human and animal data that address the absorption, distribution, metabolism, and excretion of carbon disulfide following inhalation exposure (Abramova 1966; Bond and DeMatteis 1969; Brieger 1967; Cohen et al. 1958, 1959; Dalvi and Neal 1978; Dalvi et al. 1974, 1975; DeMatteis 1974; DeMatteis and Seawright 1973; Djerassi and Lumbroso 1968; Djuric 1967; Freundt et al. 1975; McKee et al. 1943; McKenna and Distefano 1977a, 1977b; Okayama et al. 1988; Pergal et al. 1972a, 1972b; Santodonato et al. 1985; Snyderwine and Hunter 1987; Soucek 1957; Strittmatter et al. 1950; Teisinger 1974; Van Doorn et al. 1981a, 1981b; Vasak and Kopecky 1967). Data indicate rapid and extensive absorption of inhaled carbon disulfide, distribution through the body, and primary excretion by exhalation. Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate that in turn can either degrade to sulfur and carbonyl sulfide or hydrolize to sulfur and monothiocarbamate. Biotransformation of carbon disulfide in humans exposed by the inhalation route causes TTCA, OTCA, and thiourea to be excreted in the urine, and carbonyl sulfide and carbon dioxide in the breath. The data that exist for humans are largely supported by animal studies (rabbits and dogs) for this route. However, there are very few animal and human data regarding the pharmacokinetics of carbon disulfide following oral or dermal exposure, making an assessment of relative rates very difficult (Cohen et al. 1958; DeMatteis and Seawright 1973; Dutkiewicz and Baranowska 1967). The limited data indicate that a range fraction of orally administered carbon disulfide is absorbed by rats. Carbon disulfide is appreciably absorbed via the dermal route in rabbits. Animal data suggest that there are two major pathways. Steady-state phenomena do play a role in the retention and excretion of carbon disulfide, with less exposed individuals retaining more of the chemical than chronically exposed individuals (Beauchamp et al. 1983). Additional information regarding the pharmacokinetics of carbon disulfide following oral and dermal exposure would be useful. The use of TTCA as a relatively selective biological marker of exposure to carbon disulfide is now well accepted (ACGIH 1994). Since the critical TTCA concentration is tied to the TLV-TWA for inhalation, more research is required to ensure that the TLV-TWA is truly protective.

Comparative Toxicokinetics. Both human and animal data indicate that the target organs for carbon disulfide are similar across species (Cohen et al. 1958; DeMatteis and Seawright 1973; Dutkiewicz and Baranowska 1967; Freundt et al. 1975; McKee et al. 1943; Soucek 1957; Teisinger

and Soucek 1949; Toyama and Kusano 1953). There are no studies that directly compare the toxicokinetics across species. Most of the animal studies on toxicity end points have used high doses. The studies in rats, mice, and rabbits have generally been consistent in their conclusions regarding the pharmacokinetics of carbon disulfide. Data from species other than rodents would also be useful for determining the species most comparable to humans, so that animal toxicity data can be better evaluated. No striking differences between the results of rodent studies and those from human studies were noted except that sulfate excretion is far more important in animals than in humans except in the latter for exposure to high doses of carbon disulfide (Strittmatter et al. 1950). Additional information on the comparative pharmacokinetics following exposure from the oral and dermal routes would be useful, as most of the data currently available are from inhalation studies. The volatility of carbon disulfide may well affect kinetic parameters measured in dermal exposures, and metabolic parameters following oral exposures could differ from those following inhalation exposure.

Methods for Reducing Toxic Effects. Reduction of absorption of carbon disulfide may result from treatment with activated carbon and with sodium bicarbonate solutions (Dreisbach and Robertson 1987; Stutz and Janusz 1988). Some of the treatment methods currently available for use in carbon disulfide exposure, such as activated charcoal, gastric lavage, and induced emesis, support the survival of the exposed individual. Other treatments, such as reversal of central nervous system depression by administration of caffeine and sodium benzoate or providing excess zinc or copper salts, may provide only temporary relief of symptoms (Morgan 1982). Additional information on the ultimate mechanism of carbon disulfide toxicity is needed before insights may be gained regarding treatment of exposure victims.

2.10.3 On-going Studies

No studies of carbon disulfide were reported in the Federal Research in Progress database (FEDRIP 1995).

A 13-week inhalation study in rats sponsored by the National Toxicology Program (NTP) has been completed, but the results were not published as of March 1996. The NTP has also reported that an inhalation carcinogenicity study in rats has been planned, but no details on this study are available. Preliminary reports on NTP-funded genotoxicity studies indicate positive results in a sister chromatid

exchange assay (*in vitro*) and negative results in both a chromosome aberration assay (*in vitro*) and in two *Salmonella* tests. No further data on these studies were available.

The following studies, reported in Federal Research in Progress (FEDRIP 1989), have not been published as of this date.

K. Boekelheide (Brown University) is studying the mechanism involved in testicular injury in rats from exposure to carbon disulfide and other industrial and environmental toxicants.

B. Gold (Rutgers University Medical and Dental School) is conducting studies on the neurotoxicity of carbon disulfide in rats.

R. Rubin (Johns Hopkins University) is evaluating the interaction of alcohols and solvents on the toxicity and kinetics of carbon disulfide in rats, mice, and hamsters.

D.W. Lynch (NOSH) is studying the adverse effects of carbon disulfide on the cardiovascular system in rats.