3. HEALTH EFFECTS

3.1 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 tetrachloride. 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.

3.2 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 (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of carbon tetrachloride are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

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

3.2.1 Inhalation Exposure

The highest NOAEL values and all LOAEL values from each reliable study for death, and respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, dermal, body weight, neurological, reproductive, and developmental effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.1 Death

In the past, when industrial and household use of carbon tetrachloride was still common, inhalation exposure to carbon tetrachloride resulted in a considerable number of deaths in humans (e.g., Forbes

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Human	15 min				250 M (1 alcoholic male died)	Norwood et al. 1950
2	Rat	8-10 hr				3000 (1/50)	Adams et al. 1952
3	Mouse	8 hr				9500 (LC50)	Svirbey et al. 1947
Systemic							
4	Human	Up to 3 hr	Hepatic		200 M (increased serum bilirubin)		Barnes and Jones 1967
			Renal		200 M (proteinuria)		
5	Human	15 min	Resp			250 M (edema)	Norwood et al. 1950
			Gastro		250 M (nausea)		
			Hepatic			250 M (severe central necrosis)	
			Renal			250 M (oliguria, nephrosis)	
6	Human	70-180 min	Cardio	50 M			Stewart et al. 1961
			Gastro	50 M			
			Hepatic	10 M	50 M (decreased serum iron)		
			Dermal	50 M			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
7	Rat	7 hr	Hepatic	50 M	100 M (fatty degeneration)		Adams et al. 1952
			Renal	100 M			
8	Rat	5-20 d 7 hr/d 5d/wk	Hepatic		10 M (fatty degeneration in 18 M treated 13 times over 17 d)		Adams et al. 1952
9	Rat	1d-15 wk 2d/wk 4hr/d	Hepatic			4800 M (necrosis, fibrosis, cirrhosis, mitogenic and anti-mitogenic activities)	Belyaev et al. 1992
10	Rat	4 d 6hr/d	Hepatic		50 M (steatosis, hydropic degeneration, necrosis, 2x increased alanine aminotransferase)		David et al 1981
11	Rat	2 wk 5d/wk 8hr/d or 11.5hr/d	Hepatic		100 (Fatty degeneration, increased serum sorbitol dehydrogenase)		Paustenbach et al. 1986b
			Renal	100			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
12	Rat	15 min	Hepatic		180 (increases 19x in alanine aminotransferase and 23% in relative liver weight)		Sakata et al. 1987
13	Rat	6-10 min/d 8 d	Hemato		325 (increased coagulation time)		Vazquez et al. 1990
Neurological							
14	Human	15 min			250 (dizziness)		Norwood et al. 1950
15	Human	70-180 min		50			Stewart et al. 1961
16	Rat (albino)	4 hr			611 M (30% inhibition of response to electrical stimulus)		Frantik et al. 1994
17	Rat	15 min				180 (coma)	Sakata et al. 1987
18	Mouse (H)	2 hr			1370 F (30% inhibition of response to electrical stimulus)		Frantik et al. 1994

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
19	Dog	2-10 hr					15000	(depression of central nervous system)	Von Oettingen et al. 1949
Developmental									
20	Rat	9 d Gd 6-15 7hr/d			330	(decreased fetal body weight and crown to rump length)			Schwetz et al. 1974
INTERMEDIATE EXPOSURE									
Death									
21	Monkey	6 wk 5d/wk 8hr/d					80	(1/3)	Prendergast et al. 1967
22	Rat	173-205 d 5d/wk 7hr/d					200	(9/15 male, 6/15 female)	Adams et al. 1952
23	Gn Pig	180-260d 5d/wk 7hr/d					100	(7/8 males, 4/8 females)	Adams et al. 1952
24	Gn Pig	6 wk 5d/wk 8hr/d					80	(3/15)	Prendergast et al. 1967
25	Gn Pig	90 d cont.					10	(3/15)	Prendergast et al. 1967
Systemic									
26	Human	8 hr/d intermit.	Gastro		20	(nausea)			Elkins 1942

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
27	Human	2 mo 8hr/d 5d/wk	Gastro		50 (dyspepsia, nausea)		Kazantzis and Bomford 1960
28	Monkey	232-277d 5d/wk 7hr/d	Resp	100			Adams et al. 1952
			Cardio	100			
			Gastro	100			
			Hemato	100			
			Musc/skel	100			
			Hepatic	50	100 (slight fatty degeneration)		
			Renal	100			
29	Monkey	10.5 mo 8hr/d 5d/wk	Cardio	200			Smyth et al. 1936
			Hemato	200			
			Hepatic		50 (fatty degeneration)		
			Renal		200 (cloudy swelling of cells in convoluted tubules and loop of Henle)		
30	Rat (Fischer- 344)	12 wk 6 hr/d 5 d/wk	Hepatic	20 M	100 M (increased serum ALT, SDH; hepatic necrosis)		DOE 1999

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
31	Rat	173-205 d 5d/wk 7hr/d	Resp	200					Adams et al. 1952
			Cardio	200					
			Hemato	200					
			Hepatic	5[b]	10	(hepatic fatty degeneration and incr liver wt)	50	(cirrhosis)	
			Renal	100	200	(degeneration of tubular epithelium, elevated blood urea nitrogen, and increased organ weight)			
32	Rat (Fischer- 344)	13 wk 6 hr/d 5 d/wk	Hemato	30	90	(decreased hemoglobin and hematocrit; increased spleen wt in F)			Japan Bioassay Research Center 1998
			Hepatic		10	(granulation, fatty change; increased liver wt)	270	(fibrosis, cirrhosis; incr liver wt, serum enzyme incr)	
			Renal		10 M	(increased kidney weight)	270	(protein casts in M and vacuolization of tubules in F)	
			Bd Wt	270 M	810 M	(decreased bd wt)			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
33	Rat	6 wk 5d/wk 8hr/d	Resp	80					Prendergast et al. 1967
			Cardio	80					
			Hemato	80					
			Hepatic				80	(fatty infiltration, cirrhosis)	
			Renal	80					
34	Rat	90 d cont.	Resp	10					Prendergast et al. 1967
			Cardio	10					
			Hemato	10					
			Hepatic	1	10	(fatty degeneration)			
			Renal	10					
35	Rat	10.5 mo 8hr/d 5d/wk	Cardio	400					Smyth et al. 1936
			Hemato	50	100	(hemolysis)			
			Hepatic	50			100	(cirrhosis)	
			Renal		50	(swelling of cells in the convoluted tubules and loop of Henle)			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
36	Mouse (B6C3F1)	12 wk 6 hr/d 5 d/wk	Hepatic	5 M	20 M (increased serum ALT, SDH; necrosis)		DOE 1999
37	Mouse	1d-15 wk 2d/wk 4hr/d	Hepatic			4800 (necrosis, fibrosis, cirrhosis, mitogenic and anti-mitogenic activities)	Belyaev et al. 1992
38	Mouse BDF1	13 wk 6 hr/d 5 d/wk	Hemato	90 F	270 F (decr erythrocyte and hemoglobin)		Japan Bioassay Research Center 1998
			Hepatic	10 F	10 M (cytological alterations)	30 (hepatic collapse; proliferative ducts in F)	
			Bd Wt	10 M	30 M		
39	Gn Pig	4-9 mo 5d/wk 7hr/d	Hepatic	5	10 (fatty degeneration)	25 (cirrhosis)	Adams et al. 1952
40	Gn Pig	90 d cont.	Resp	10			Prendergast et al. 1967
			Cardio	10			
			Hemato	10			
			Hepatic	1	10 (fatty degeneration)		
			Renal	10			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
41	Hamster (Golden Syrian)	12 wk 6 hr/d 5 d/wk	Hepatic	20 M	100 M (increased serum ALT and SDH; necrosis)		DOE 1999
Neurological							
42	Human	>3 mo 8hr/d 5d/wk				80 (narcosis)	Heimann and Ford 1941
43	Human	2 mo 8hr/d 5d/wk			40 (depression)		Kazantzis and Bomford 1960
44	Monkey	232-277d 5d/wk 7hr/d		100			Adams et al. 1952
45	Rat	10.5 mo 8hr/d 5d/wk				50 (sciatic and optic nerve injury)	Smyth et al. 1936
Reproductive							
46	Rat	10.5 mo 8hr/d 5d/wk		100		200 (decreased litters)	Smyth et al. 1936
CHRONIC EXPOSURE							
Death							
47	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk				125 (survival decreased by 86% in M and 97% in F)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
48	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk				25 (survival decreased by 29% in M and 61% in F)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
Systemic							
49	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk	Hemato	5 F	25 F (decreased hemoglobin, hematocrit)		Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
			Hepatic	5[c]		25 (increased liver wt, fibrosis, cirrhosis and deposition of ceroid; increased severity of fatty change and granulation)	
			Renal	5		25 (incr marked chronic nephropathy)	
			Bd Wt	5	25 (decreased bd wt gain)		

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
50	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk	Hemato	5	25 (increased extramedullary hematopoeisis in spleen)		Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
			Hepatic	5		25 F (thrombus, necrosis) 25 (increased liver wt, degeneration, cyst, deposit of ceroid, serum enzymes, cholesterol, bilirubin)	
			Renal	5 M	25 (decreased pH and ketone bodies; protein casts, M; increased occult blood and urobilinogen, F)		
			Bd Wt			25 (marked decreased bd wt gain)	

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
Cancer							
51	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk				125 (CEL: hepatocellular adenoma in 21/50 M and 40/50 F; hepatocellular carcinoma in 32/50 M and 15/50 F)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
52	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk				25 M (CEL: adrenal pheochromocytoma in 16/50 males)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
						125 F (CEL: adrenal pheochromocytoma in 22/49 females)	
						25 (CEL: hepatocellular adenoma in 27/50 males and 17/50 females; hepatocellular carcinoma in 42/50 males and 33/50 females.	

a The number corresponds to entries in Figure 3-1.

b Used to derive an intermediate-duration inhalation MRL of 0.03 ppm; the NOAEL was adjusted for intermittent exposure (7 hours/24 hours x 5 days/7 days). The duration-adjusted NOAEL of 0.9 ppm would be multiplied by the ratio of the rat and human blood:air partition coefficients (4.52/2.64) under EPA (1994) guidelines. However, as the ratio was greater than one, a default value of one was applied, resulting in a NOAEL-HEC of 0.9 ppm. The NOAEL-HEC was divided by an uncertainty factor of 30 (3 for extrapolation between animals and humans using a dosimetric adjustment and 10 for human variability).

c Used to derive a chronic-duration inhalation MRL of 0.03 ppm; the NOAEL was was adjusted for intermittent exposure (6 hours/24 hours x 5 days/7 days). The duration-adjusted NOAEL of 0.9 ppm would be multiplied by the ratio of the rat and human blood:air partition coefficients (4.52/2.64) under EPA (1994) guidelines. However, as the ratio was greater than one, a default value of one was applied, resulting in a NOAEL-HEC of 0.9 ppm. The NOAEL-HEC was divided by an uncertainty factor of 30 (3 for extrapolation between animals and humans using a dosimetric adjustment and 10 for human variability).

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Cardio = cardiovascular; CEL = cancer effect level; cont. = continuous; d = day(s); Derm = dermal; F = female; Gastro = gastrointestinal; gd = gestation day; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); incr = increased; intermit. = intermittent; LC50 = lethal concentration, 50% kill; LOAEL = Lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); musc/skel = musculoskeletal; NOAEL = no=observed-adverse-effect-level; ppm = parts per million; Resp = respiratory; wk = week(s).

Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation

Acute (≤14 days)

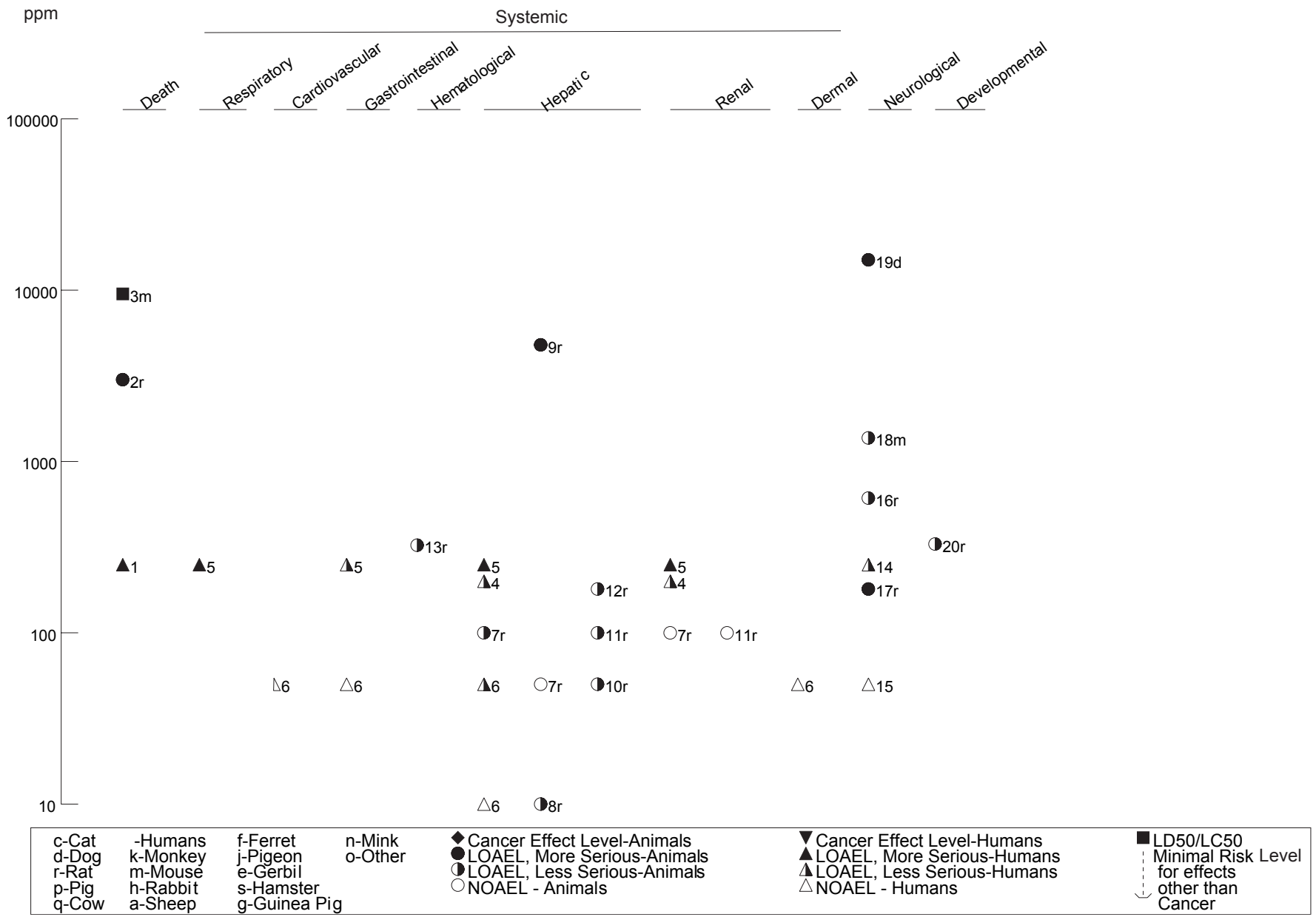


Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Intermediate (15-364 days)

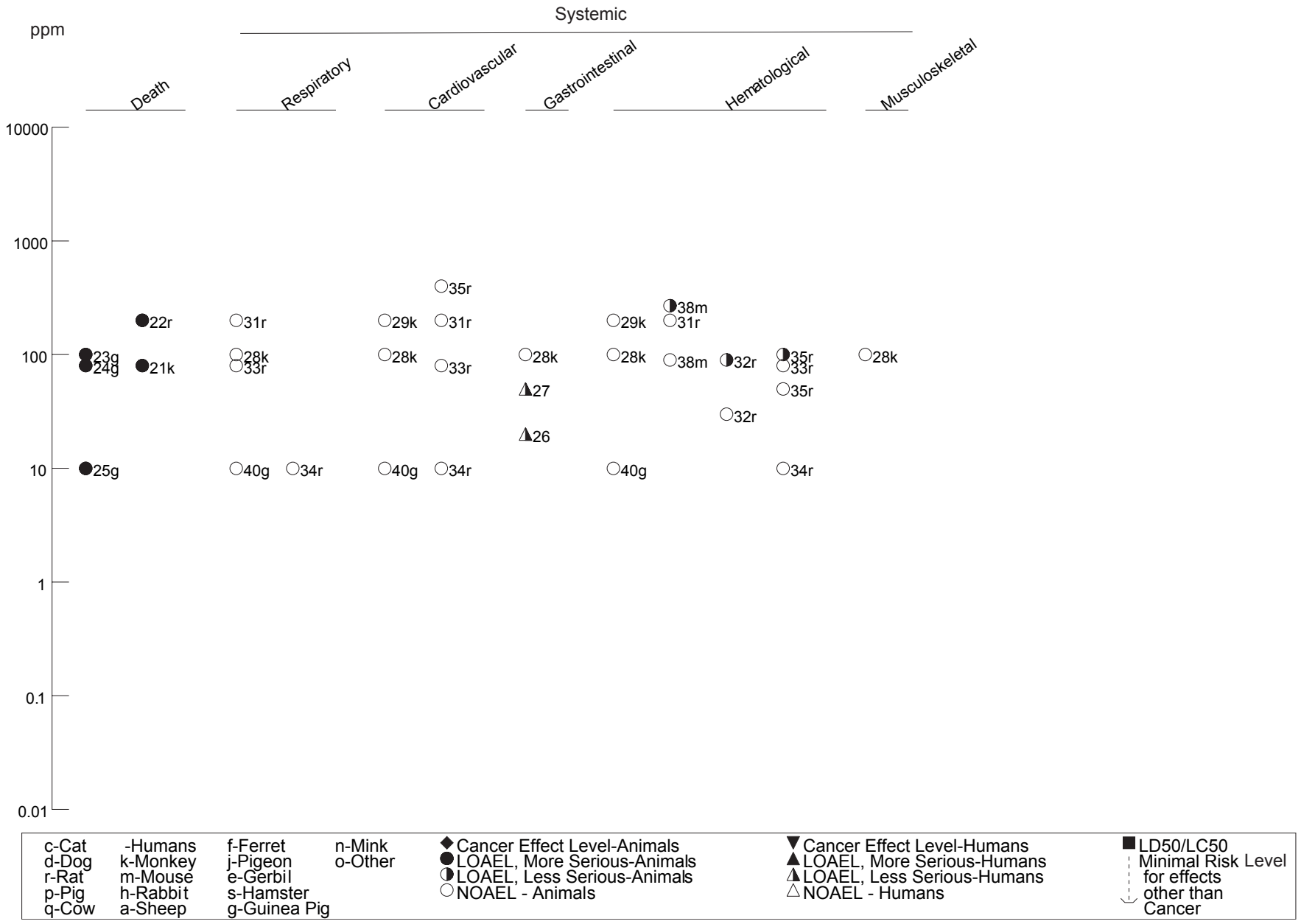
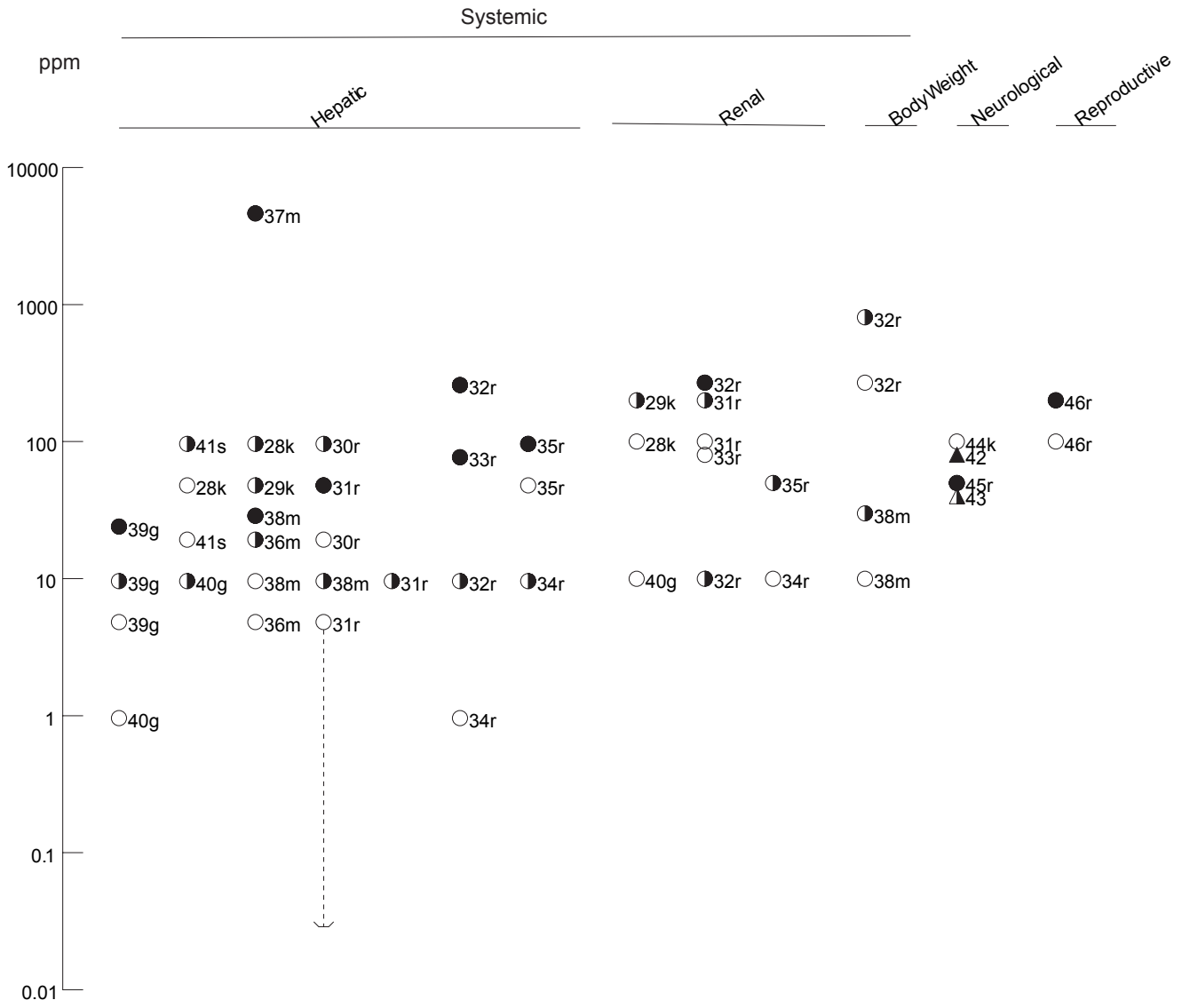


Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

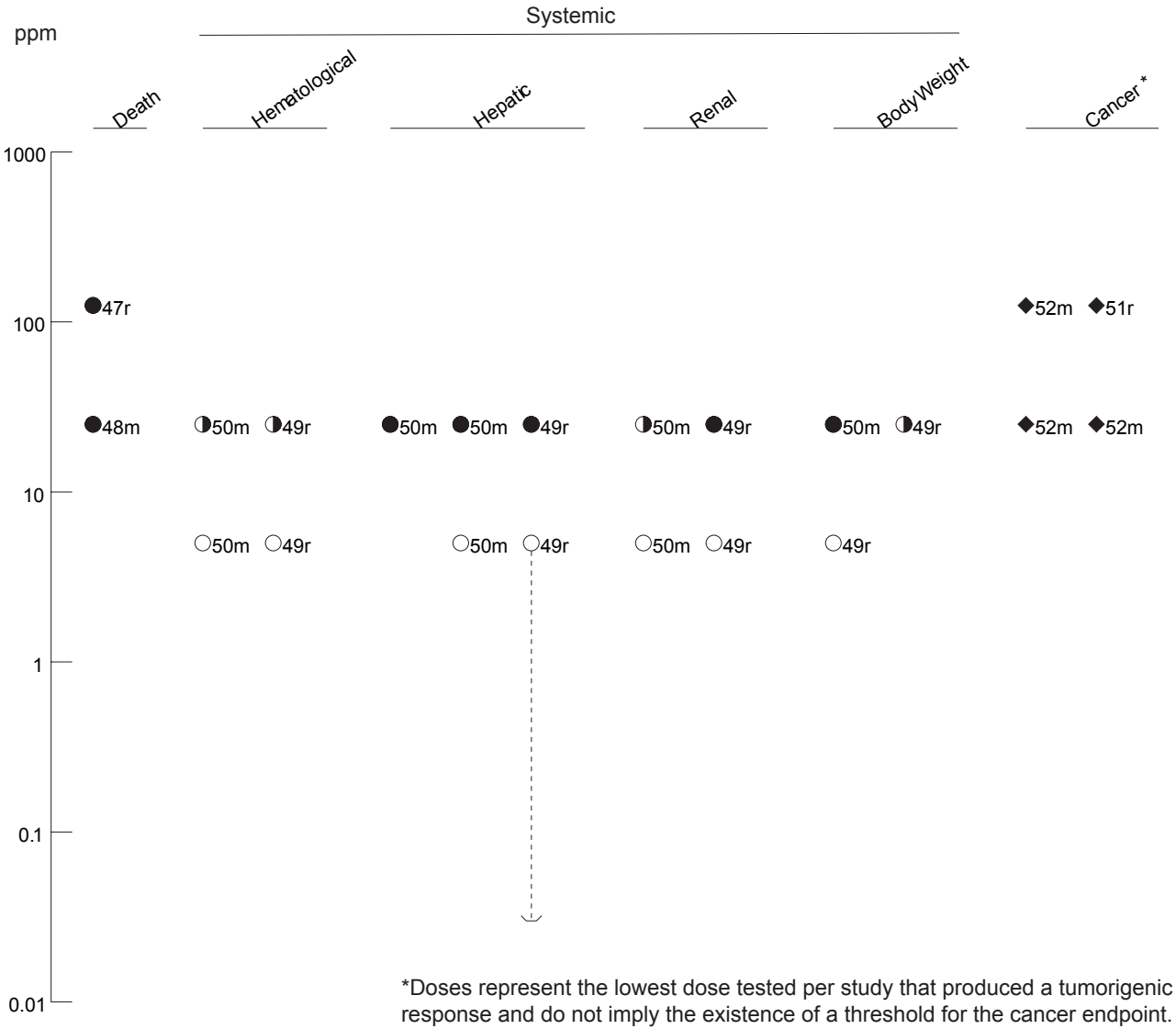
Intermediate (15-364 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◮ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

1944; Norwood et al. 1950; Umiker and Pearce 1953). However, quantitative estimates of the exposure levels that caused death are rare; furthermore, cleaning usages often involved concurrent dermal exposure. In one case involving inhalation of carbon tetrachloride by an alcoholic, the lethal exposure level was estimated at only 250 ppm for 15 minutes (Norwood et al. 1950). Other workers (nonalcoholics) were exposed at the same level for 4 hours with no significant clinical signs other than slight headache (Norwood et al. 1950). One of three naval officers who weekly misused a carbon tetrachloride fire-extinguishing fluid as a dry cleaning agent over 3 months died of heart failure that was secondary to nephrosis-induced pulmonary edema (Forbes 1944); the man who died was a heavy consumer of alcohol. The relative exposure by inhalation or through the skin was not known, but about 3 kg carbon tetrachloride was used during 3 months.

Lethal inhalation exposure levels in animals depend on exposure duration and species. In mice, the estimated LC_{50} for an 8-hour exposure is 9,500 ppm, with no deaths in 20 animals exposed to 6,300 ppm (Svirbely et al. 1947). In rats, exposure to 7,300 ppm caused no deaths after 1.5 hours, about 50% mortality by 4–6 hours, and 100% mortality by 8 hours (Adams et al. 1952). Exposure to 3,000 ppm for 8–10 hours caused death in 1 of 50 animals. Repeated exposure to 200 ppm 7 hours/day led to increased mortality in rats after approximately 190 days (Adams et al. 1952).

All LOAEL values from each reliable study for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal or endocrine effects in humans or animals after inhalation exposure to carbon tetrachloride. Studies have been conducted in both humans and animals to evaluate the respiratory, cardiovascular, hematological, hepatic, and renal effects of inhalation exposure to carbon tetrachloride. Gastrointestinal and dermal/ocular effects have been studied in humans but not in animals. These effects are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Pulmonary edema is a common finding in humans exposed to lethal levels of carbon tetrachloride in air. Thirteen fatal cases were reported following acute inhalation exposure in humans; exposure concentrations were not determined. Marked hemorrhagic congestion and edema were

observed in the lungs of all the victims who had been exposed for 1–6 hours (Umiker and Pearce 1953). However, these effects typically did not develop in lung until 8 days after exposure, and appeared to be secondary to severe renal injury rather than to a direct action of carbon tetrachloride on the lung. Lung appearance in the carbon tetrachloride victims was found comparable with that in cases of rapidly developing uremia occasioned by various causes of renal failure. Thus, the progressive uremia, electrolyte retention, and extracellular fluid build-up that accompanies renal failure is a likely principal cause of the observed pulmonary edema. In one fatal case, pulmonary edema secondary to renal malfunction developed following combined inhalation/dermal exposure over 3 months (Forbes 1944).

Lung injury is usually not as prominent an effect in animals exposed to carbon tetrachloride vapors as it is in humans. For example, lung injury was not observed in rats exposed to concentrations of 3,000–19,000 ppm for 7 hours, or in rats and monkeys exposed to 100 ppm for 7 hours/day, 5 days/week for 205 and 232 days, respectively (Adams et al. 1952). As it appears that lung injury is secondary to renal injury, then the absence of lung effects in animals may be because animals are also less susceptible to the renal injury produced by carbon tetrachloride than are humans.

Cardiovascular Effects. Most studies of humans exposed to carbon tetrachloride by inhalation have not detected significant evidence of cardiovascular injury, even at exposure levels sufficient to markedly injure the liver and/or kidney. Changes in blood pressure, heart rate, or right-sided cardiac dilation have sometimes, but not always, been observed (Ashe and Sailer 1942; Guild et al. 1958; Kittleson and Borden 1956; Stewart et al. 1961; Umiker and Pearce 1953), and are probably secondary either to fluid and electrolyte retention resulting from renal toxicity, or to central nervous system effects on the heart or blood vessels. Failure of the left side of the heart was the proximate cause of death in one naval officer who had a combined inhalation/dermal exposure over 3 months (Forbes 1944). The heart effect was secondary to the pulmonary edema that had developed from renal toxicity. Carbon tetrachloride also may have the potential to induce cardiac arrhythmias by sensitizing the heart to epinephrine, as has been reported for various halogenated hydrocarbon propellants (Reinhardt et al. 1971).

Similarly, except for what are likely secondary effects following acute lethal exposures, significant cardiovascular injury has not accompanied hepato- or nephrotoxic inhalation exposure to carbon tetrachloride in a variety of experimental animals (Adams et al. 1952; Prendergast et al. 1967; Smyth et al. 1936; von Oettingen et al. 1949).

Gastrointestinal Effects. One of the most common signs of exposure of humans to carbon tetrachloride is dyspepsia, with nausea, vomiting, and gastrointestinal pain (Forbes 1944; Stewart and Witts 1944). This is often one of the first clinical signs to become apparent following acute exposure (Guild et al. 1958; Norwood et al. 1950), but is also common in persons exposed for months to several years to concentrations as low as approximately 20 ppm (Elkins 1942; Smyth et al. 1936). Exposure levels of approximately 50 ppm do not cause significant dyspepsia if exposure is brief (Stewart et al. 1961), but may lead to nausea if exposure extends for several days (Kazantzis and Bomford 1960). Because inhalation exposure is unlikely to be directly irritating to the gastrointestinal tract, it is probable that these effects are secondary to effects on the autonomic nervous system (Stewart and Witts 1944).

Hematological Effects. Significant effects on the hematological system are not usually observed in humans exposed to carbon tetrachloride by inhalation (Heimann and Ford 1941; Norwood et al. 1950; Smyth et al. 1936). In some cases, moderate elevations in white cell counts are observed, perhaps in response to necrosis in the liver or kidneys. In a few cases, mild anemia is observed (Gray 1947), and may occasionally become severe (Straus 1954). A cross-sectional study of hepatic function in workers exposed <1–>5 years to carbon tetrachloride reported small (2.5–3.6%), statistically significant reductions in some hematological parameters compared to non-exposed workers, but without a dose response (Tomenson et al. 1995). At the low (<1 ppm) or medium (1–3 ppm) exposure levels, there were decreases in packed cell volume and in hemoglobin and erythrocyte count for the medium exposure group; no significant changes from controls were noted in the high exposure group (>4 ppm). The mechanism underlying anemia is not known, but it might be secondary to internal hemorrhaging as a result of decreased synthesis of clotting factors by the liver or a direct effect on bone marrow cells (Guild et al. 1958; Stevens and Forster 1953; Straus 1954). Since lipid peroxidation caused by carbon tetrachloride also affects calcium sequestration, clotting functions, which are regulated by calcium sequestration would be expected to be impaired, resulting in a tendency for internal hemorrhaging.

Similar observations have been obtained in inhalation studies in animals. Prothrombin time increased and there was lengthened activated partial thromboplastin time in rats exposed 22–40 times to 325 ppm carbon tetrachloride for 10 minutes/day, 5 days/week, indicating defective coagulation in both the extrinsic and intrinsic clotting pathways (Vazquez et al. 1990). No significant effects on hematology were detected in rats, monkeys, or guinea pigs exposed to concentrations of 10–200 ppm, 7 hours/day for periods of time up to 170 days (Adams et al. 1952; Prendergast et al. 1967). Rats exposed for 10 months to 100 ppm suffered some destruction of red blood cells, but this did not result in anemia (Smyth et al. 1936). No evidence of red blood cell hemolysis was observed at 50 ppm. Decreased hemoglobin and

hematocrit levels and increased spleen weight) at ≥90 ppm and reduced erythrocyte counts at 810 ppm were observed in male and female rats intermittently exposed to carbon tetrachloride vapor for 13 weeks (Japan Bioassay Research Center 1998); in similarly exposed mice at ≥270 ppm, reduced erythrocyte and hemoglobin counts were observed in females, and at 810 ppm, there were reductions in hematocrit in females and in hemoglobin counts in males. Significant reductions in hemoglobin and hematocrit values were observed in female rats exposed to ≥25 ppm carbon tetrachloride vapor for 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998). Splenic changes related to erythrocyte destruction included increased hemosiderin deposition in male rats at ≥5 ppm, a consequence of erythrocyte destruction observed in the 13-week studies, and increased extramedullary hematopoeisis in male and female mice at ≥25 ppm.

Hepatic Effects. Carbon tetrachloride has been known for many years to be a powerful hepatotoxic agent in humans and animals. The principal clinical signs of liver injury in humans who inhale carbon tetrachloride are swollen and tender liver, elevated levels of hepatic enzyme (aspartate aminotransferase) in the serum, elevated serum bilirubin levels and the appearance of jaundice, and decreased serum levels of proteins such as albumin and fibrinogen (Ashe and Sailer 1942; McGuire 1932; New et al. 1962; Norwood et al. 1950; Straus 1954). In cases of lethal acute or repeated exposures, autopsy generally reveals marked liver necrosis with pronounced steatosis (Forbes 1944; Jennings 1955; Markham 1967; Smetana 1939), and repeated or chronic exposure leads in some cases to fibrosis and/or cirrhosis (McDermott and Hardy 1963).

Quantitative information on the inhalation exposure levels that cause significant hepatic injury in humans is sparse. Liver necrosis was reported in one fatal case involving an alcoholic who was exposed to 250 ppm carbon tetrachloride for 15 minutes (Norwood et al. 1950). Humans exposed to concentrations of 50 ppm for 70 minutes or 10 ppm for 3 hours showed no measurable change in serum enzyme levels or urinary urobilinogen levels (Stewart et al. 1961). A slight decrease in serum iron levels occurred in two of four subjects exposed to 50 ppm for 1 hour, suggesting to the authors that minimal liver injury had occurred. However, all values were within or close to the normal range of serum iron concentrations, and there were no control subjects. Consequently, it is difficult to judge if the variations observed were treatment-related and whether they were of biological significance. No hepatic effects were observed in humans exposed to average concentrations of 80 ppm for 8 hours/day, 5 days/week for 3 months (Heimann and Ford 1941).

Occasional and slight elevations of serum bilirubin levels were seen in workers exposed for 8 hours/day for several months to many years to carbon tetrachloride concentrations ranging from 10 to 100 ppm, but no other clinical signs of injury were detected (Smyth et al. 1936). Similarly, workers exposed for up to 3 hours/day to carbon tetrachloride concentrations averaging about 200 ppm displayed small increases in serum enzyme levels and serum bilirubin levels, indicative of minimal liver damage (Barnes and Jones 1967). More recently, chronic occupational exposure of 35 male workers to <1 ppm (8 hours/day) of chlorinated solvents, primarily carbon tetrachloride and perchlorethylene, was not correlated with any significant changes in standard indicators of liver function (e.g., serum levels of protein, albumin, bilirubin, alanine and aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, and cholesterol) (Driscoll et al. 1992). However, when workers were segregated as to having relatively higher or lower exposure, higher exposure was correlated with significantly ($p<0.03–0.05$) lower fasting serum levels of three bile acids (chenodeoxycholate, taurocholate, and total deoxycholate). This effect was in the opposite direction to what might be expected based upon oral animal data and upon serum bile acid increases reported by the same authors for a companion worker population exposed to hexachloro-butadiene or trichloroethylene. Thus, these results should be viewed with caution, especially in view of the low exposure level to carbon tetrachloride and the variable concurrent exposure to several other solvents.

A cross sectional study of hepatic function (serum enzyme levels) was conducted on 135 workers occupationally exposed to carbon tetrachloride and 276 nonexposed controls who were employed in three plants in northern England (Tomenson et al. 1995). Workers were categorized according to their duration of employment (<1 year, 1–5 years, and >5 years), but the serum enzyme results were not presented by estimated duration of exposure because statistical analysis showed that duration of exposure had no significant effect. Exposures were estimated from historical personal monitoring data for each job category, and exposure groups were categorized as low (≤1 ppm), medium (1.1–3.9 ppm), or high (≥4.0–11.9 ppm). Alcohol consumption was equivalent among groups. A comparison of exposed workers and nonexposed controls found no significant difference in blood levels of alkaline phosphatase or gamma glutamyl transferase; however, when the exposed group was subdivided by level of exposure, these values were significantly elevated in the medium exposure group. None of the exposed subjects had hepatic disease that could be attributed to exposure to carbon tetrachloride.

In animals, the hepatic effects of inhalation exposure to carbon tetrachloride are much the same as in humans: elevated serum enzyme levels, steatosis, and centrilobular necrosis progressing to fibrosis. Statistically significant doubling of serum levels of mitochondrial glutamate dehydrogenase and sorbitol

dehydrogenase was observed in rats 24 hours after a 4-hour exposure at 530 ppm and of ALT and AST at 1,460 ppm (Brondeau et al. 1983). Following 4-hour exposure at 500–2,500 ppm, fasted rats showed a 2.0–2.5-fold higher level of serum enzymes indicative of hepatic injury than fed rats (Jaeger et al. 1975); the increases in serum enzyme levels were similar in fed and fasted rats at 5,000 ppm. In rats, hypoxia (10–12% oxygen) increased the severity of hepatic injury (serum enzyme levels) compared to normal air (21% oxygen) conditions during 2–3 hour exposures to carbon tetrachloride vapor (Shen et al. 1982; Siegers et al. 1985). In rats, exposure to concentrations of 10–100 ppm, 6–7 hours/day for approximately 2 weeks generally results in mild to moderate signs of liver injury (fatty degeneration), both after short-term (roughly 2 weeks) (Adams et al. 1952; David et al. 1981; Paustenbach et al. 1986a, 1986b). Four days of exposure at 50 ppm caused elevated serum alanine aminotransferase, altered hepatic glycogen distribution (preferential accumulation in the central and pericentral zones, rather than the uniform distribution observed in controls), steatosis, hydropic degeneration, and necrosis (David et al. 1981). Short-term exposure (15 minutes/day, 2 days/week for 8 weeks) caused fibrosis in rats exposed to 180 ppm (Sakata et al. 1987). A 4-hour exposure to 4,800 ppm or higher induced centrilobular necrosis within 24 hours (Belyaev et al. 1992; Magos et al. 1982). In rats exposed for 2 hours, there were significant reductions in hepatic CYP-450 levels at ≥100 ppm and significantly elevated serum enzymes (SDH 16-fold and ALT 2-fold) at 1,000 ppm (Sanzgiri et al. 1995); the results indicate an alteration of hepatic cell function at 100 ppm, but leakage from hepatocytes at 1,000 ppm. With continued biweekly exposures at 4,800 ppm for 4 hours/day, necrotic areas were largely replaced by hepatocellular proliferation after 2–3 weeks, and then fibrosis and eventually cirrhosis (Belyaev et al. 1992). Cirrhosis along with fatty degeneration was observed in rats exposed at 200 or 400 ppm (7 hours/day, 5 days/week for 2 weeks) (Adams et al. 1952). No acute MRL was established for inhalation exposure to carbon tetrachloride because the value calculated from the most acceptable data would be the same as or lower than the intermediate-duration MRL (see Section 2.3). The intermediate-duration inhalation MRL is expected to be protective for acute-duration inhalation exposures.

The pattern of hepatic injury following intermediate-duration exposure to carbon tetrachloride is similar to that seen in acute studies. Mild to moderate liver effects (increased liver weight, fatty degeneration) were observed following intermittent exposure (6–7 hours/day, 5 days/week) at 10 ppm for 12 weeks to 6 months (Adams et al. 1952; Bogers et al. 1987; DOE 1999; Japan Bioassay Research Center 1998; Smyth et al. 1936). Similar effects were observed in rats exposed continuously at 10 ppm for 6 weeks (Prendergast et al. 1967). In 12–13-week studies, increased serum enzyme levels (AST and SDH) and necrotic injury is apparent in mice intermittently exposed at 20 ppm (DOE 1999) and in rats exposed at 90 ppm or higher (Belyaev et al. 1992; Japan Bioassay Research Center 1998). Hepatic regeneration and

proliferation were evident within 2–3 weeks of treatment at 4,800 ppm (Belyaev et al. 1992). Fibrosis and cirrhosis developed in rats following intermittent (6–7 hours/day, 5 days/week) exposure at 50 ppm for 6 months (Adams et al. 1952) or at ≥90 ppm for 12–15 weeks (Belyaev et al. 1992; DOE 1999; Japan Bioassay Research Center 1998). Although hepatic histopathology was similar in rats and mice exposed for 13 weeks, only rats developed fibrosis and cirrhosis and only mice developed collapse of the liver (Japan Bioassay Research Center 1998). Guinea pigs appear to be somewhat more sensitive to carbon tetrachloride inhalation than rats (Prendergast et al. 1967; Smyth et al. 1936), and monkeys appear to be somewhat less sensitive than guinea pigs and rats (Adams et al. 1952; Prendergast et al. 1967). Another study found mice to be more susceptible to hepatic damage (necrosis) than rats or hamsters (DOE 1999). The basis of these species differences is likely related to differences in hepatic metabolism (see Section 3.4.3). Exposure to concentrations of 1–5 ppm, 6–7 hours/day, 5 days/week for periods up to 6 months or 1 ppm continuously for 6 weeks have not been observed to cause any significant changes in liver of rats, monkeys, or guinea pigs (Adams et al. 1952; Prendergast et al. 1967). The NOAEL of 5 ppm (Adams et al. 1952) was selected as the basis for an intermediate-duration MRL for inhalation exposure to carbon tetrachloride, as described in the footnote in Table 3-1.

Plummer et al. (1990) conducted experiments that suggest that the hepatotoxicity of carbon tetrachloride administered by inhalation is proportional to the concentration x time product (Haber's rule). Rats exposed for 4 weeks at equivalent time-weighted average concentrations—either continuously (24 hours/day, 7 days/week except for two 1.5-hour breaks 2 days/week) at 16 ppm or discontinuously (6 hours/day, 5 days/week) at 87 ppm—showed identical severity of liver histopathology.

In 2-year inhalation bioassays, concentration-related hepatic effects were observed in rats and in mice following intermittent exposure (6 hours/day, 5 days/week) to carbon tetrachloride vapor (Japan Bioassay Research Center 1998; Nagano et al. 1998). Alterations in some serum hepatic biomarkers were not statistically significant in rats exposed at 5 ppm. Statistically significant increases in liver weight and serum parameters (ALT, AST, LDH, leucine aminopeptidase and gamma-glutamyl transferase, total bilirubin) were observed at ≥25 ppm in rats and mice. Hepatic lesions at ≥25 ppm included basophilic, eosinophilic, clear and mixed cell foci, deposition of ceroid, fibrosis and cirrhosis, and increased severity of fatty change and granulation. In the parallel assay in mice, statistically significant decreases in some serum parameters at 5 ppm were not considered by the authors to be biologically significant because the control values for serum chemistry parameters in males were unusually high compared to available historical control values. Hepatic degeneration, thrombus, and deposition of ceroid were evident in both sexes, and hepatic necrosis was found in female mice treated at ≥25 ppm. The NOAEL of 5 ppm for

hepatic effects in rats (Japan Bioassay Research Center 1998) was selected as the basis for a chronic-duration inhalation MRL for carbon tetrachloride, as described in a footnote in Table 3-1.

Renal Effects. Nephritis and nephrosis are very common effects in humans following inhalation exposure to carbon tetrachloride (Jennings 1955; McGuire 1932; Norwood et al. 1950). The most obvious clinical signs, developing within hours to days after exposure, are oliguria or anuria with resulting edema. In some cases, this leads to generalized uremia, and is frequently accompanied by proteinuria, hemoglobinuria, and glucosuria (Forbes 1944; Guild et al. 1958; New et al. 1962; Smetana 1939; Umiker and Pearce 1953). In fatal cases, histological examination generally reveals relatively mild degeneration of the kidney (Ashe and Sailer 1942; Forbes 1944; Gray 1947; Jennings 1955; Norwood et al. 1950). The mechanism of the injury to the kidney is not known, but Sirota (1949) reported that back-diffusion of glomerular filtrate was important in the early stages of oliguria and decreased renal blood flow contributed in the later stages of oliguria following carbon tetrachloride inhalation in humans.

The exposure levels leading to renal damage in humans have not been well defined. An increased incidence of proteinuria was reported in workers exposed to vapor concentrations of around 200 ppm (Barnes and Jones 1967), while no change was observed in urinary properties following inhalation exposure to 50 ppm for 70 minutes or 10 ppm for 3 hours (Stewart et al. 1961).

Threshold concentrations for renal injury in animals exposed by inhalation to carbon tetrachloride are sometimes higher than those for hepatic effects. Animals appear to be less sensitive to renal injury than humans, possibly because of species differences in carbon tetrachloride metabolism by the kidney. No evidence of kidney damage was observed in rats, cats, monkeys, or guinea pigs exposed for 6–8 hours/day to concentrations of 10–200 ppm for periods of time from 1 to 90 days (Adams et al. 1952; Bogers et al. 1987; Prendergast et al. 1967). In a 13-week study, 10 ppm produced increased absolute and relative kidney weights in male rats; organ weight effects and vacuolization were observed in females at 90 ppm and hyaline degeneration of the glomerulus at 810 ppm (Japan Bioassay Research Center 1998). No renal effects were noted in similarly exposed mice. Slight renal swelling was noted in rats exposed to 50 ppm for 5–10.5 months for 7–8 hours/day, 5 days/week, and in monkeys exposed to 200 ppm for 10.5 months for 7–8 hours/day, 5 days/week (Adams et al. 1952; Smyth et al. 1936). Renal tubular degeneration was apparent following exposure at 200 ppm for 7 hours/day, 5 days/week for 6 months (Adams et al. 1952).

Chronic exposure (6 hours/day, 5 days/week for 2 years) to carbon tetrachloride vapor caused renal effects in rodents, with rats being more sensitive than mice (Japan Bioassay Research Center 1998). The most sensitive renal effect in rats was a dose-related enhancement of proteinuria (scores of 4+), having a significantly ($p \leq 0.01$) higher severity in males and females treated at 5 or 25 ppm (too few animals survived at 125 ppm for statistical analysis) compared to controls; however, as the severity in controls was so high (>90% of rats with scores of 3+ or 4+), the statistical difference is not biologically significant and this end point was not used as the basis for the chronic-duration inhalation MRL. Blood urea nitrogen levels and the severity and incidence of chronic progressive nephropathy were elevated at >25 ppm in both sexes. In the parallel study in mice, protein casts in the kidney were observed in male and female mice exposed at ≥25 ppm.

Dermal Effects. Very few reports mention any effect of carbon tetrachloride inhalation on the skin. Inhalation exposure to carbon tetrachloride for several days in the workplace caused a blotchy, macular rash in one man (but not in six others) (McGuire 1932). Similarly, a hemorrhagic rash occurred in a woman exposed to carbon tetrachloride vapors for several days in the workplace (Gordon 1944), and black and blue marks were seen in a patient exposed intermittently to carbon tetrachloride vapors for several years (Straus 1954). Because observations of dermal effects are so sporadic, it is difficult to judge whether these effects are related to carbon tetrachloride exposure, or are incidental. Conceivably, they may have been secondary to reduced synthesis of blood coagulation factors resulting from carbon tetrachloride-induced hepatotoxicity. No animal studies evaluated dermal effects following inhalation exposure.

Ocular Effects. Ocular effects following inhalation exposure to carbon tetrachloride are discussed under neurological effects.

Body Weight Effects. No human and very few animal reports mention the effect of carbon tetrachloride inhalation on body weight gain. In rodents intermittently exposed to carbon tetrachloride vapor for 6 hours/day, 5 days/week for 13 weeks, body weight gain was significantly reduced by 20% in male rats exposed at 810 ppm and by 8–15% in male mice exposed at ≥30 ppm, but not significantly in female rats or mice exposed at concentrations as high as 810 ppm (Japan Bioassay Research Center 1998). In the companion 2-year study, terminal body weights were depressed by 11% in male and female rats exposed at 25 ppm and by 22–45% at 125 ppm (Japan Bioassay Research Center 1998; Nagano et al. 1998); survival at 125 ppm was too low to determine statistical significance. In mice exposed at 25 ppm

for 2 years, body weight gain at termination showed a >30% reduction in males and a >20% reduction in females relative to controls (only single male and female mice survived in the 125 ppm groups).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to carbon tetrachloride.

3.2.1.4 Neurological Effects

Like many volatile halocarbons and other hydrocarbons, inhalation of carbon tetrachloride leads to rapid depression of the central nervous system. Because of its central nervous system depressant properties, carbon tetrachloride was used briefly as an anesthetic in humans, but its use was discontinued because it was less efficacious and more toxic than other anesthetics available (Hardin 1954; Stevens and Forster 1953). Depending on exposure levels, common signs of central nervous system effects include headache, giddiness, weakness, lethargy, and stupor (Cohen 1957; Stevens and Forster 1953; Stewart and Witts 1944). Effects on vision (restricted peripheral vision, amblyopia) have been observed in some cases (e.g., Forbes 1944; Johnstone 1948; Smyth et al. 1936; Wirtschafter 1933), but not in others (e.g., Stewart and Witts 1944). Sudden severe epileptiform seizure and coma occurred in a subsequently fatal case following weekly combined inhalation/dermal exposure over a period of 3 months (Forbes 1944). In several fatal cases, microscopic examination of brain tissue taken at autopsy revealed focal areas of fatty degeneration and necrosis, usually associated with congestion of cerebral blood vessels (Ashe and Sailer 1942; Cohen 1957; Stevens and Forster 1953).

Exposure levels leading to effects on the central nervous systems of humans are not precisely defined. No symptoms of lightheadedness or nausea were experienced by humans exposed to 50 ppm for 70 minutes or 10 ppm for 3 hours (Stewart et al. 1961), but nausea, headache, and giddiness were found to be common symptoms in workers exposed to carbon tetrachloride for 8 hours/day at concentrations of 20–125 ppm (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960). Dizziness has also been reported in humans following short-term exposure (15 minutes) at a higher concentration (250 ppm) (Norwood et al. 1950). This suggests that the threshold for central nervous system effects in humans is, as a conservative estimate, probably in the range of 20–50 ppm for an 8-hour workday.

Central nervous system depression is also observed in animals exposed to carbon tetrachloride vapors. Rats reportedly became inactive within 15 minutes after exposure to a concentration of 180 ppm (Sakata et al. 1987), although when compared with other studies, this concentration appears too low to be capable of inducing such an effect. Drowsiness or stupor occurred in rats exposed for 0.1–8.0 hours to 4,600 ppm, with ataxia and unconsciousness at 12,000 ppm, and death (from respiratory failure) at 19,000 ppm (Adams et al. 1952). Similarly, dogs exposed for 2–10 hours to 15,000 ppm experienced profound depression of the autonomic system, as evidenced by decreases in respiration, reflex activity, body temperature, heart rate, and blood pressure (the latter due to marked vasodilation) (von Oettingen et al. 1949). Exposure of rats, monkeys, or guinea pigs to concentrations of carbon tetrachloride up to 400 ppm, 8 hours/day, 5 days/week for over 10 months did not cause any observable effects on activity, alertness, or appetite, indicating that this level did not cause obvious central nervous system depression in animals (Smyth et al. 1936). However, histological examination of sciatic and optic nerves revealed degenerative changes in a number of animals exposed to 200–400 ppm, and in a few animals (rats) after exposure to levels as low as 50 ppm under the same exposure schedule. The changes were apparently not severe enough to impair movement or vision. Exposure to ≥5 ppm carbon tetrachloride vapor for 6 hours/day, 5 days/week for 2 years resulted in decreased absolute brain weights in male, but not female, rats, however, this effect was attributed to the overall depression in body weight, since brain weights relative to body weight were increased (Japan Bioassay Research Center 1998). Furthermore, no histopathology was detected in the brain in either sex at any concentration.

The highest NOAEL values and all LOAEL values for each reliable study for neurotoxicity in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

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

In rats that inhaled carbon tetrachloride vapors for three generations, there was a decrease in fertility in animals exposed to concentrations of 200 ppm or higher for 8 hours/day, 5 days/week for 10.5 months (Smyth et al. 1936). Since both sexes were exposed, it was not possible to determine if this was due to effects on males, females, or both. Moderate to marked degeneration of testicular germinal epithelium has been seen in rats exposed repeatedly (7 hours/day, 5 days/week) to 200 ppm or higher for 192 days (Adams et al. 1952).

Deposition of ceroid was observed in the ovaries of mice that were exposed to 125 ppm of carbon tetrachloride vapor, 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998). At 25 ppm, absolute and relative testicular weights were elevated in male mice.

All LOAEL values for each reliable study for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located on developmental effects in humans after known inhalation exposure to carbon tetrachloride. A questionnaire-based study of 3,418 pregnant women in West Germany found no association between probable occupational exposure to carbon tetrachloride (as estimated from a job exposure matrix) and the birth of infants who were small for their gestational age (Seidler et al. 1999).

In rats, inhalation exposure to 330 or 1,000 ppm for 7 hours/day on gestational days 6–15 caused reduced feed intake by dams, maternal weight loss, and clear maternal hepatotoxicity (elevated serum ALT, relative liver weight), but no effect on conception, number of implants, or number of resorptions (Schwetz et al. 1974). There were no gross anomalies, but dose-related statistically significant reductions in fetal body weight and crown-rump length were observed. The incidence of delayed ossification of sternebrae was significantly increased at 1,000 ppm. The lowest exposure level in this study is a LOAEL for both maternal and fetal toxicity.

All LOAEL values for each reliable study for developmental toxicity in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Two case reports of liver cancer in humans suggested that previous exposure to carbon tetrachloride vapors might have contributed to the development of the cancer (Tracey and Sherlock 1968; Johnstone 1948). In the first case, a 66-year-old male died of hepatocellular carcinoma 7 years after acute intoxication with carbon tetrachloride sufficient to cause jaundice as well as vomiting and diarrhea (Tracey and Sherlock 1968). As the tumor was too extensive to make a diagnosis of cirrhosis, the report

could not rule out the man's history of moderate alcohol consumption as contributory . In the second case, a 30-year-old female died of "liver cancer" after 2–3 years of occupational exposure to carbon tetrachloride that was sufficient to produce signs of central nervous system depression. The evidence of these studies is much too weak to establish a cause-and-effect relationship between exposure to carbon tetrachloride and hepatic cancer in humans.

A number of epidemiological studies have been conducted to evaluate the association of risk of increased mortality from particular types of cancer and occupational exposure to carbon tetrachloride. Both positive and negative associations have been reported, varying with the target organ. IARC (1999) has noted that few of these studies had definitive evidence of exposure to carbon tetrachloride—generally, few positive cases were identified among exposed individuals—and that extensive exposure to other possible carcinogenic chemicals could not be excluded, and in fact, was likely. Thus, the positive associations discussed below are considered suggestive, but are not conclusive.

An analysis of cancer mortality and solvent exposure among a cohort of 6,678 active and retired male workers in the rubber industry found a significant association between age-adjusted exposure to carbon tetrachloride and lymphosarcoma (odds ratio [OR] 4.2, $p<0.05$; based on six cases) and lymphatic leukemia (OR 15.3, $p<0.001$; based on eight exposed cases) (Checkoway et al. 1984; Wilcosky et al. 1984). A retrospective cohort mortality study of 14,457 workers employed at an aircraft maintenance facility for at least 1 year during 1952–1956 included 6,737 workers who had ever been exposed to carbon tetrachloride (Blair et al. 1998; Spirtas et al. 1991). In the first study, standard mortality ratios (SMRs) for selected causes of death were calculated for workers exposed to solvents compared to the Utah death rates (Spirtas et al. 1991). A statistically significant association was found in women for deaths from non-Hodgkin's lymphoma and exposure to carbon tetrachloride (SMR 325, 95% confidence interval [CI] 119–708); excess SMRs in men for non-Hodgkin's lymphoma and in both sexes for multiple myeloma following carbon tetrachloride exposure were not statistically significant. In the follow-up study, a Poisson regression analysis was performed on cancer incidence data to evaluate the risk from exposure to carbon tetrachloride (Blair et al. 1998). Among men and women, the relative risk (RR) of mortality from non-Hodgkin's lymphoma or non-Hodgkin's lymphoma following exposure to carbon tetrachloride was not significantly increased. A study of causes of death in a cohort of 5,365 workers exposed to dry-cleaning solvents in St. Louis, Missouri, found statistically significant excesses in deaths from all cancers, esophageal cancer, and cervical cancer compared to the general U.S. population (Blair et al. 1990). The risk of esophageal cancer showed a statistically significant association with estimated cumulative exposure to dry cleaning solvents (SMR = 2.8 for the highest cumulative exposure group), but

not with level or duration of exposure. The most heavily exposed workers showed a 4-fold increased risk for cancers of the lymphatic and hematopoietic system, but the number of deaths (five) was small.

No positive association was found between likely occupational exposure to carbon tetrachloride and the risk of increased mortality from pancreatic cancer among residents of 24 states (Kernan et al. 1999), astrocytic brain tumors in workers in the petrochemical industry in three states (Heineman et al. 1994), rectal cancer in Montreal (Dumas et al. 2000), renal carcinoma in Minnesota (Dosemici et al. 1999), lung cancer among men working in a chemical plant in Texas (Bond et al. 1986), or respiratory system tumors in workers in the rubber industry (Checkoway et al. 1984; Wilcosky et al. 1984). No significantly elevated risk of cancer was detected in a cohort of 4,772 Finnish laboratory workers who were exposed to chemicals including carbon tetrachloride, but the duration of exposure was too short (<16 years) to detect cancers with longer latency periods (Kauppinen et al. 2003). A case-control study estimating the occupational exposures to some industrial chemicals among women in 24 states found a weak association between probable high-intensity exposure to carbon tetrachloride and an increased risk (OR ratio 1.21, 95% CI: 1.1–1.3) of breast cancer in Caucasian women (Cantor et al. 1995). However, a retrospective cohort study of workers in an aircraft maintenance facility found no association in woman for exposure to carbon tetrachloride and increased mortality from breast cancer (Blair et al. 1998).

Chronic exposure to carbon tetrachloride vapor induced tumors in rats and mice (Japan Bioassay Research Center 1998; Nagano et al. 1998). Following intermittent exposure for 2 years (6 hours/day, 5 days/week), significant increases in the incidences of hepatocellular adenoma and carcinoma were observed in male and female rats exposed at 125 ppm (22.3 ppm, duration adjusted) and in mice exposed at ≥25 ppm (4.5 ppm, duration adjusted). Adrenal pheochromocytomas were also induced in male mice exposed at ≥25 ppm and female mice at 125 ppm.

The carcinogenicity of carbon tetrachloride is currently undergoing reassessment by the EPA under the IRIS program, with the final report scheduled for 2006. As chronic inhalation data were not available for the earlier assessment, the EPA extrapolated oral dose-response data on liver tumor risk to yield estimates of the carcinogenic risk from inhalation exposure to carbon tetrachloride (EPA 1984). Based on the assumption that a 70-kg person breathes 20 m^3/day of air and that 40% of inhaled carbon tetrachloride is absorbed, the calculated upper-bound unit risk (the upper 95% confidence limit on the excess cancer risk associated with lifetime exposure to carbon tetrachloride at a concentration of 1 μg/m^3) is $1.5x10^{-5}$. Based on this, the concentration of carbon tetrachloride in air corresponding to excess cancer risk levels of 10^{-4},

10^{-5}, 10^{-6}, and 10^{-7} are 0.001, 0.0001, 0.00001, and 0.000001 ppm, respectively. Because these are upper-bound estimates, the true risk could be lower. These values are displayed in Figure 3-1.

3.2.2 Oral Exposure

The highest NOAEL values and all LOAEL values from each reliable study for death and respiratory, cardiovascular, gastrointestinal, hematolgical, musculoskeletal, hepatic, renal, immunological/ lymphoreticular, neurological, reproductive, developmental, and cancer effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.1 Death

Ingestion of concentrated solutions of carbon tetrachloride can cause death in humans within hours to days. The principal clinical signs observed in fatal cases include gastrointestinal irritation, central nervous system depression, and cardiovascular disturbances, with death usually resulting from severe injury to kidney and/or liver (Guild et al. 1958; reviewed in von Oettingen 1964).

There is considerable variation in the doses that have been found to cause lethality, with alcohol ingestion leading to markedly increased risk. Twelve fatalities were reported following oral exposure (Umiker and Pearce 1953). In most cases, about 50–150 mL had been ingested, but one case involved only 5.3 mL (about 121 mg/kg). A review of some of the earlier literature found that ingestion of 14–20 mL (320–450 mg/kg) was fatal in the majority of cases (von Oettingen 1964). In other cases, ingestion of 2.5–15 mL (60–340 mg/kg) as a treatment for hookworm produced death in only a very small number of people out of hundreds of thousands treated, although doses as low as 1.5 mL (40 mg/kg) caused death in a few cases (Lamson et al. 1928). Two fatal cases have been reported in humans dosed with approximately 70 mg/kg (Phelps and Hu 1924).

A single dose oral LD_{50} value of approximately 13,000 mg/kg was reported for mice, and 14 daily doses of 625 mg/kg were lethal for 6 of 20 exposed male mice (Hayes et al. 1986). In rats fed carbon tetrachloride in stock diets or protein-free diets, LD_{50} values of 10,200 or 23,400 mg/kg, respectively were reported (McLean and McLean 1966). The authors attributed the difference in sensitivity in animals in this study to protein depletion, which has reportedly afforded protection against carbon tetrachloride toxicity. This may result from protein depletion-induced reduction in cytochrome P-450 synthesis, with a

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
ACUTE EXPOSURE								
Death								
1	Human	Once (C)				40	(lowest quantifiable dose producing death out of 6 cases)	Lamson et al. 1928
2	Human	Once				70	(death in 2/2)	Phelps and Hu 1924
3	Human	Once				120 M	(lowest quantifiable dose producing death out of 12 cases)	Umiker and Pearce 1953
4	Rat	Once (G)				10200	(LD50)	McLean and McLean 1966
5	Rat (Sprague-Dawley)	Once (G)				7500	(LD50)	Pound et al. 1973
6	Rat (Sprague-Dawley)	1 d 1-2x/d (GO)				8000	(death in 17/20)	Thakore and Mehendale 1991
7	Mouse	Once (G)				13000	(LD50)	Hayes et al. 1986
8	Mouse	14 d (G)				625 M	(death in 6/20 males)	Hayes et al. 1986

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
9	Cat	Once (G)					400	(death in 25/36)	Chandler and Chopra 1926
Systemic									
10	Human	Once	Cardio		2500	(sinus bradycardia and arrhythmia, auricoventricular nodal rhythm, auricular fibrillation)			Conaway and Hoven 1946
			Renal		2500	(increased blood urea nitrogen)			
11	Human	Once (W)	Hepatic		110	(degeneration of hepatocytes)			Docherty and Burgess 1922
			Renal		180	(swelling of proximal convoluted tubules)			
12	Human	Once (W)	Hepatic		90	(slight fatty inflitration)			Docherty and Nicholls 1923
			Renal	90					
13	Human	Once	Renal				2700	(acute tubular necrosis, increased blood urea nitrogen, anuria, proteinuria)	Guild et al. 1958

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
14	Human	Once	Hepatic			670 (severe necrosis; fatty deposits)	MacMahon and Weiss 1929
			Renal			670 (mild proteinuria, elevated blood urea nitrogen; kidneys swollen, fatty degeneration)	
15	Human	Once	Gastro		100 (nausea)		Ruprah et al. 1985
16	Human	1-6 d	Resp			120 (substantial hemorrhagic edema of the lung)	Umiker and Pearce 1953
17	Rat (Fischer- 344)	8-10 d 1x/d (GO)	Hepatic			280 (centrilobular necrosis, increased alkaline phosphatase and 5-nucleotidase)	Blair et al. 1991

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
18	Rat (Sprague-Dawley)	Once (G)	Hepatic	40 M	80 M (slight vacuolization of some centrilobular hepatocytes)	160 M (increased centrilobular vacuolization with significant necrosis, substantially elevated ornithine carbamyl transferase, sorbitol deydrogenase and alanine aminotranferase)	Bruckner et al. 1986
			Renal	160 M			
19	Rat (Sprague-Dawley)	11 d 9 doses (G)	Hepatic		20 M (limited centrilobular vacuolization, moderately elevated sorbitol deydrogenase, alanine aminotranferase, ornithine carbamyl transferase)	80 M (increased centrilobular vacuolization with some limited necrosis, greatly elevated ornithine carbamyl transferase, sorbitol deydrogenase, alanine aminotranferase)	Bruckner et al. 1986
			Renal	160 M			
20	Rat (Sprague-Dawley)	Once (GW)	Hepatic		10 (increased alanine aminotransferase, sorbitol dehydrogenase, ornithine carbamyl transferase; hepatic centrilobular vacuolization)		Kim et al 1990b
21	Rat	Once (F)	Hepatic		20 M (cytoplasmic vacuolization of hepatocytes)		Korsrud et al. 1972

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
22	Rat	Once (G)	Hepatic		80 M (decreased P-450)	1600 M (centrilobular necrosis)	Matsubara et al. 1983
23	Rat (Fischer- 344)	10 d 1x/d (GO)	Hepatic		5 M[b] (slight vacuolation)		Smialowicz et al. 1991
			Renal	40 M			
24	Rat (Fischer- 344)	once (GW)	Hepatic			80 M (necrosis; increased serum alanine aminotransferase, sorbitol dehydrogenase)	Steup et al. 1993
25	Rat (Sprague-Dawley)	Once (G)	Renal		4000 M (mitochondrial swelling in cells of proximal tubules)		Striker et al. 1968

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
26	Rat (Sprague-Dawley)	1 d 1-2x/d (GO)	Hepatic				480 M	(necrosis, vacuolation; elevated serum levels of aspartate transaminase, alanine transaminase, sorbitol dehydrogenase, decreased liver microsomal cytochrome P-450, aminopyrine demethylase, aniline hydroxylase)	Thakore and Mehendale 1991
27	Rat	Once (GO)	Hepatic	800	1600 3200	(elevated urinary taurine) (lipid vacuoles, 96 hours post-treatment)	3200	(48 hours post-treatment: necrosis lipid vacuolation, inflammation, elevated serum taurine, elevated serum alanine and aspartate amino-transferases, reduced liver taurine)	Waterfield et al. 1991
28	Mouse	Once (G)	Hepatic	10	40	(necrosis)			Eschenbrenner and Miller 1946
29	Mouse (B6C3F1)	14 d 1x/d (GO)	Hepatic		50 F	(increased relative organ weight and SGPT)			Guo et al. 2000
			Bd Wt	1000 F					

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
30	Mouse (CD-1)	14 d (G)	Hemato		625 (decreased fibrinogen and lymphocyte levels)		Hayes et al. 1986
			Hepatic		625 (increased liver weight, elevated lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase)		
			Renal	2500			
31	Dog	Once (G)	Hepatic		3200 (centrilobular necrosis)		Chandler and Chopra 1926
			Renal		3200 (fatty degeneration)		
32	Dog	Once (G)	Hepatic	160	400 (centrilobular necrosis)		Gardner et al. 1925
			Renal		6400 (fatty accumulation in cortical tubules)		
Immuno/ Lymphoret							
33	Rat	10 d 1x/d (GO)		160			Smialowicz et al. 1991

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
34	Mouse (BALB/c)	7 d 1x/d (GO)			500 F (suppressed T-cell activity)		Delaney et al. 1994
35	Mouse (B6C3F1)	14 d 1x/d (GO)			50 F (decreased: IgM antibody-forming cell activity per spleen and host resistance to Listeria monocytogenes)		Guo et al. 2000
Neurological							
36	Human	Once (C)		70			Hall 1921
37	Human	Once (C)		120	300 (drowsiness)		Leach 1922
38	Human	Once				4800 (narcosis)	Stevens and Forster 1953
Developmental							
39	Rat (Fischer- 344)	Gd 6-15 1x/d (GO)		25 F		50 F (total litter resorption in 5/12)	Narotsky et al. 1997a
40	Rat (Fischer- 344)	Gd 6-15 1x/d (G)		25 F	50 F (maternal piloerection and reduced body wt gain)	50 F (total litter resorption in 2/14)	Narotsky et al. 1997a

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
41	Rat	2-3 d (G)				1400	(total litter resorption in 11/29)	Wilson 1954
42	Mouse (B6D2F1)	Gd 1-5 or GD 6-10 or Gd 11-1 1x/d (GO)		826 F				Hamlin et al. 1993
INTERMEDIATE EXPOSURE								
Death								
43	Rat (Sprague-Dawley)	13 wk 5 d/wk (GO)				25 M	(10% mortality)	Koporec et al. 1995
44	Rat (Sprague-Dawley)	13 wk 5 d/wk (GW)				25 M	(25% mortality)	Koporec et al. 1995
Systemic								
45	Rat	12 wk (GO)	Hepatic			20	(increased serum enzymes; necrosis; cirrhosis)	Allis et al. 1990

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
46	Rat	12 wk 5d/wk 1x/d (G)	Hepatic	1[c]	10 (substantially elevated sorbitol dehydrogenase, mild centrilobular vacuolization)	33 (substantially elevated sorbitol dehydrogenase, ornithine carbamyl transferase, alanine aminotransferase, cirrhosis)	Bruckner et al. 1986
			Renal	33			
			Bd Wt	10	33 (Bd wt gain reduced by 16%)		
47	Rat (Sprague-Dawley)	13 wk 5 d/wk (GO)	Hepatic		25 M (increases 2x in serum ALT and 10x in SDH; minimal-to-slight vacuolzation, minimal fibrosis)	100 M (necrosis, hyperplasia)	Koporec et al. 1995
			Bd Wt	25 M		100 M (Bd wt decreased 25%)	
48	Rat (Sprague-Dawley)	13 wk 5 d/wk (GW)	Hepatic		25 M (increases 2 x in serum ALT and 10 x in SDH; vacuolation, fibrosis)	100 M (necrosis, hyperplasia)	Koporec et al. 1995
			Bd Wt	25 M		100 M (Bd wt decr 25%)	
49	Mouse	90 d 5d/wk (G)	Hepatic	1.2	12 (elevated alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase; mild necrosis)		Condie et al. 1986

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
50	Mouse	120 d (G)	Hepatic	80			Eschenbrenner and Miller 1946
51	Mouse	90 d (G)	Hemato	1200			Hayes et al. 1986
			Hepatic		12 (centrilobular necrosis, elevated lactate dehydrogenase, alanine aminotransferase, asparte aminotransferase, and alkaline phosphatase)		
			Renal	1200			
52	Dog (Beagle)	28 d 1 x/d (C)	Hepatic		797 (incr serum ALT and OCT; vacuolization, single-cell necrosis)		Litchfield and Gartland 1974
53	Dog (Beagle)	8 wk 1 x/d (C)	Hepatic	32 F			Litchfield and Gartland 1974
Neurological							
54	Rat	1x/wk 10 wk (G)			290 M (increased serotonin synthesis)		Bengtsson et al. 1987
Cancer							
55	Mouse	120 d (G)				20 (CEL: hepatoma)	Eschenbrenner and Miller 1946

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
56	Hamster	30 wk 1x/wk (GO)				120 (CEL: hepatoma)	Della Porta et al. 1961
CHRONIC EXPOSURE							
Death							
57	Rat (Osborne-Mendel)	78 wk 5d/wk (G)				94 M (survival at 110 wks decr by 46%)	NCI 1976
58	Mouse	78 wk 5d/wk (G)				1250 (survival decr by 80%)	NCI 1976
Systemic							
59	Rat (Osborne-Mendel)	78 wk 5d/wk (G)	Hepatic			47 M (cirrhosis, bile duct proliferation, fatty accumulatioin)	NCI 1976
Cancer							
60	Rat	78 wk 5d/wk (G)				47 M (CEL: hepatocellular carcinomas)	NCI 1976
61	Mouse (B6C3F1)	78 wk 5d/wk (G)				1250 (CEL: 100% with hepatic carcinoma)	NCI 1976

a The number corresponds to entries in Figure 3-2.

b Used to derive an acute-duration oral MRL of 0.02 mg/kg/day; the minimal LOAEL was divided by an uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

c Used to derive an intermediate-duration oral MRL of 0.007 mg/kg/day; the NOAEL was first adjusted for intermittent exposure (5 days/7 days) and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

(C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = female; (F) = feed; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; (GW) = gavage in water; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mg/kg/day = milligrams per kilograms per day; NOAEL = no-observed-adverse-effect level; Resp = respiratory; (W) = water; wk = week(s); x = time(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral

Acute (≤14 days)

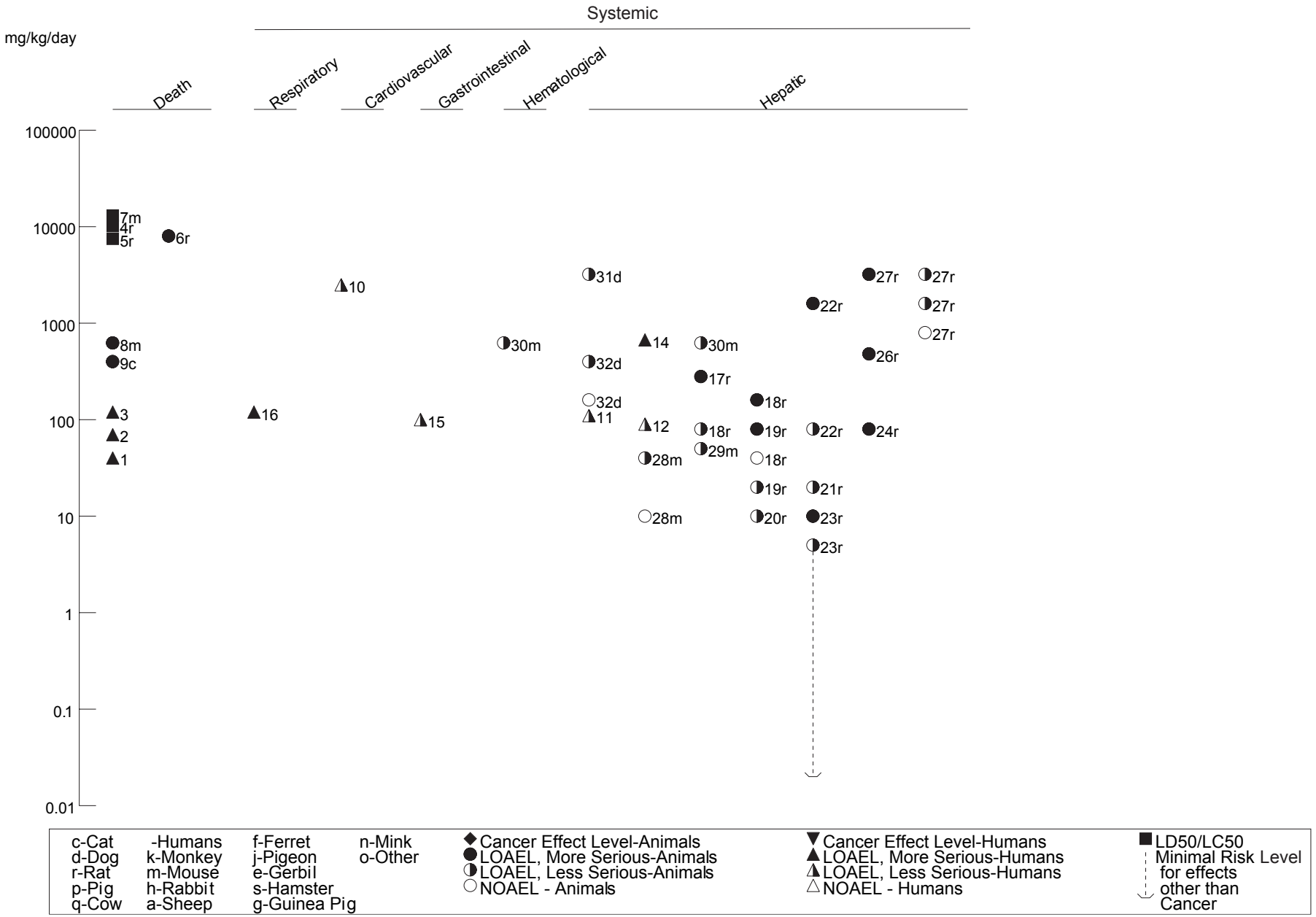


Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Acute (≤14 days)

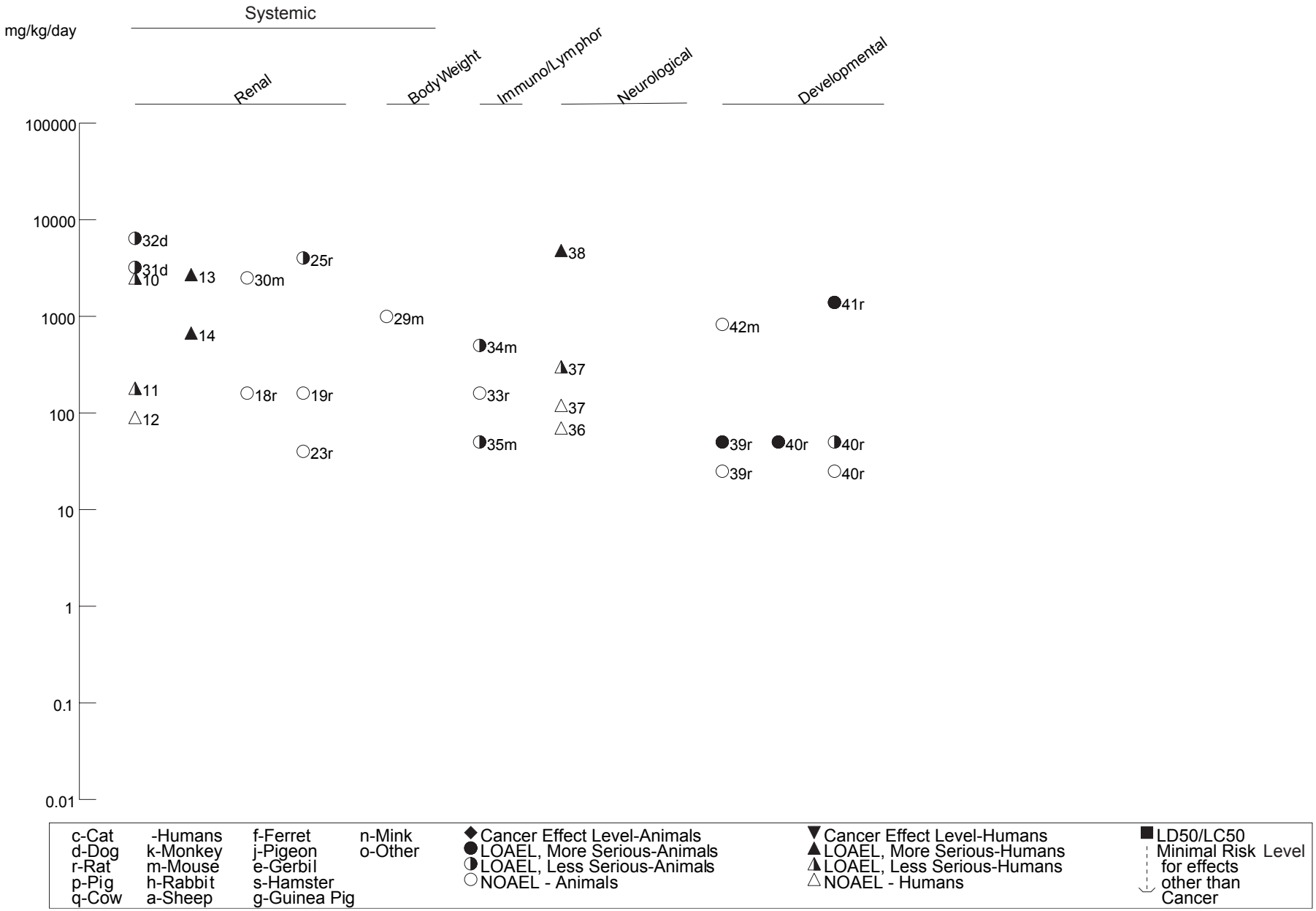
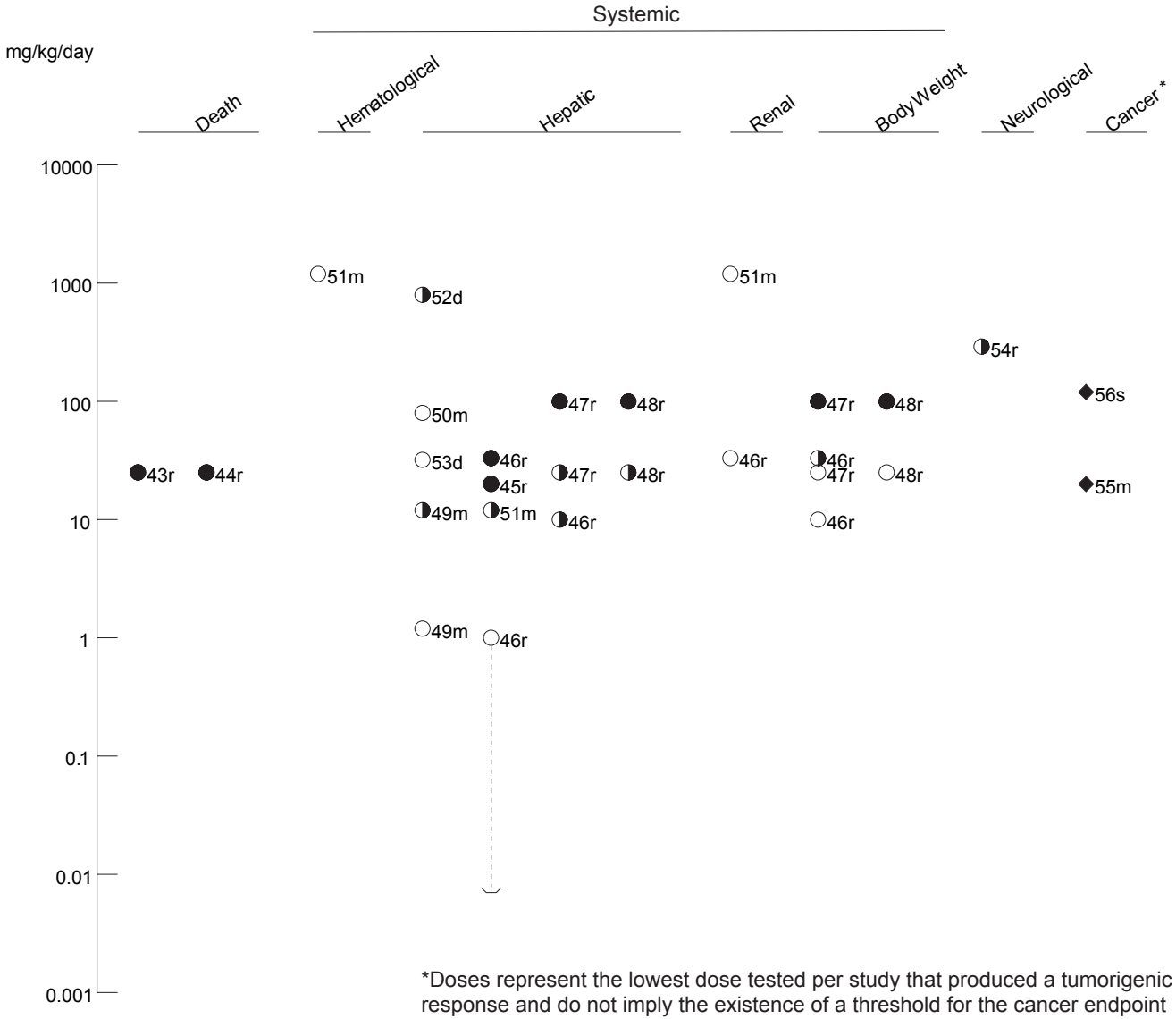


Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Intermediate (15-364 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint

c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

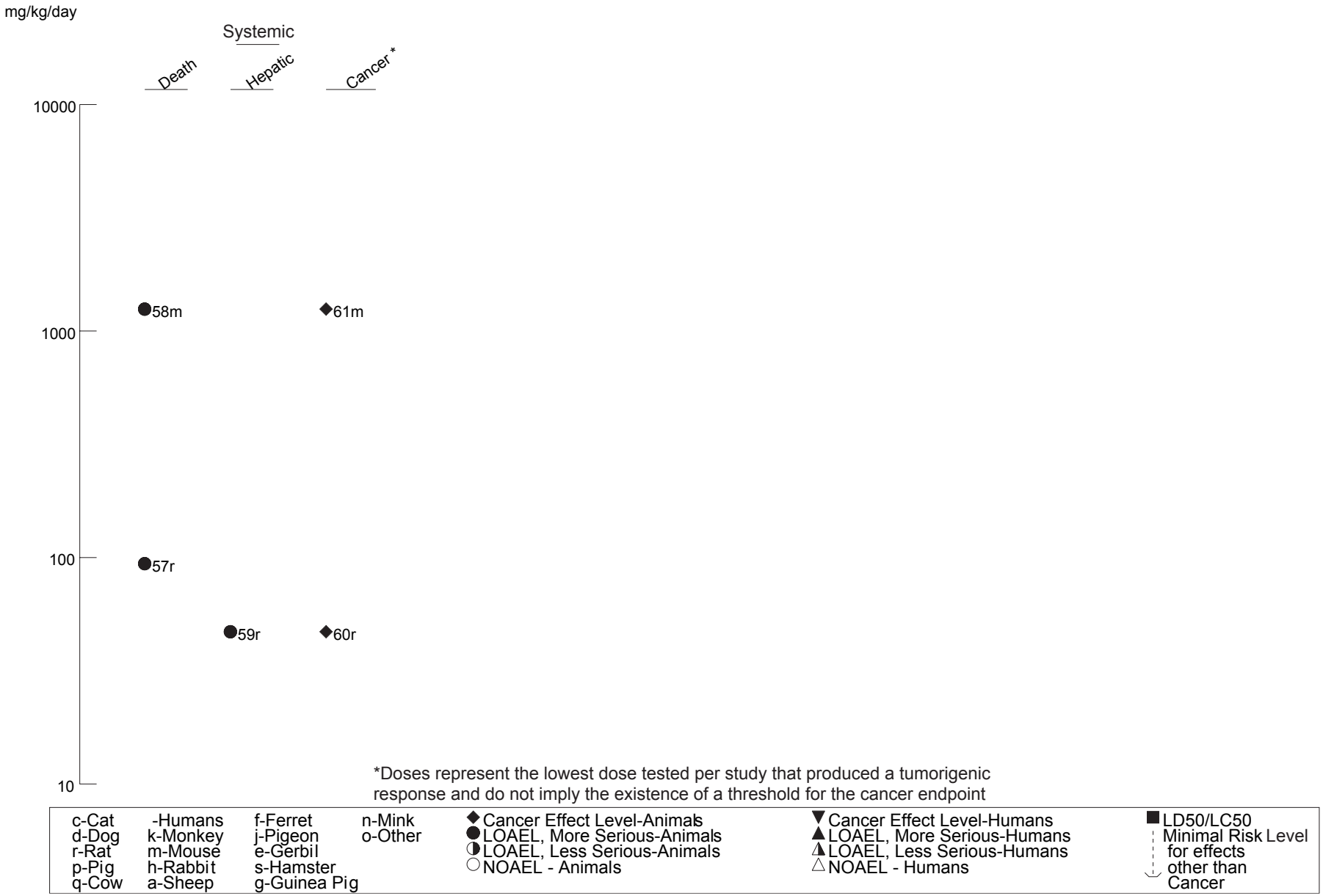
◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Chronic (≥365 days)



consequent diminished metabolic activation of carbon tetrachloride to toxic metabolites. In other studies using rats, an LD_{50} value of approximately 7,500 mg/kg was reported (Pound et al. 1973), while 17/20 animals were killed within 14 days of a single oral gavage exposure to 8,000 mg/kg (Thakore and Mehendale 1991). Doses as low as 400 mg/kg have resulted in the death of cats (Chandler and Chopra 1926).

One study reported a vehicle effect on mortality in rats repeatedly dosed on weekdays with carbon tetrachloride for 13 weeks (Koporec et al. 1995). When the vehicle was corn oil, mortality was 45% at the high dose of 100 mg/kg/day (average daily dose of 17.8 mg/kg/day) and 10% at the low dose of 25 mg/kg/day (average daily dose of 17.8 mg/kg/day); there were no deaths in the control group. When administration was as an aqueous emulsion in 1% Emulphor, the respective mortalities were 75 and 25%. As hepatic effects were the same for the equivalent dose groups, it is not known whether effects on other organ systems (not evaluated) were associated with the vehicle effects on mortality.

In rats exposed to ≥47 mg/kg of carbon tetrachloride 5 days/week for 78 weeks and observed for an additional period, survival at 110 weeks was reduced by 46% compared to controls because of hepatotoxicity (NCI 1976a, 1976b). In the same study, survival in mice treated at doses of ≥1,250 mg/kg was reduced by ≥80% compared to controls on account of hepatic carcinogenicity.

All LOAEL values for each reliable study for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding endocrine, dermal, ocular, or metabolic effects in humans or animals after oral exposure to carbon tetrachloride. Studies have been conducted in humans and animals to evaluate the respiratory, cardiovascular, hematological, or hepatic effects. Gastrointestinal and renal effects have been evaluated in humans, and musculoskeletal effects have been noted in animals. These effects are discussed below. The highest NOAEL values and all LOAEL values for each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. A number of human fatalities have been reported following ingestion of carbon tetrachloride (Umiker and Pearce 1953). Edema and hemorrhage of the lung were common autopsy

findings. Injury to the lung usually did not become apparent until 8 days or longer after poisoning, and the effects on the lung were essentially the same as observed in cases of uremia due to other causes. This suggests that the late-developing edema and hemorrhagic injury to lung is secondary to severe kidney injury.

In animals, acute oral exposure to doses of 4,000 mg/kg has been observed to cause respiratory edema, atelectasis, and hemorrhage (Gould and Smuckler 1971). This is accompanied by marked disruption of subcellular structure in most pulmonary cell types, including granular pneumocytes, capillary endothelial cells, and Clara cells (Boyd et al. 1980; Gould and Smuckler 1971; Hollinger 1982). It has been shown that Clara cells were most severely injured because they are the most active in metabolic activation of carbon tetrachloride. Injury to capillary endothelial cells is dose-dependent, with increased release of cellular enzymes occurring at doses as low as 160 mg/kg (Hollinger 1982). No studies of respiratory effects following longer-term oral exposure were located.

Cardiovascular Effects. Effects of carbon tetrachloride ingestion on the cardiovascular system have not been the subject of extensive investigation. Most studies in humans have not detected significant gross or histopathological changes in heart tissue at dose levels that cause marked hepatic and renal damage (Leach 1922; MacMahon and Weiss 1929). Electrocardiographic changes (sinus arrhythmia, QRS complex splintering, elevated S-T_4 and P-R intervals) suggestive of myocardial injury were seen in a man who ingested several mouthfuls of carbon tetrachloride, but these appeared to be fully reversible (Conaway and Hoven 1946).

The few animal studies located appear to be in general agreement with the human findings (Gardner et al. 1925; Korsrud et al. 1972). Effects of carbon tetrachloride ingestion on blood pressure are sometimes observed, but these are likely secondary to effects on the central nervous system, or to effects on fluid and electrolyte balance following renal injury.

Gastrointestinal Effects. Humans who ingest oral doses in excess of 30 or 40 mL (680–910 mg/kg) frequently experience nausea, vomiting, and abdominal pain (Hardin 1954; New et al. 1962; Smetana 1939; Umiker and Pearce 1953; von Oettingen 1964). Nausea has been reported after an oral dose of as little as 100 mg/kg (Ruprah et al. 1985). These effects could be the direct result of irritation of the gastrointestinal tract caused by the high dose or secondary to effects on the central nervous system. Oral doses of 3–5 mL (70–110 mg/kg) were widely used in the past for the treatment of hookworms with only mild gastrointestinal distress (Hall 1921; Leach 1922).

No studies were located regarding gastrointestinal effects in animals after oral exposure to carbon tetrachloride.

Hematological Effects. Oral exposure to carbon tetrachloride has not been reported to have substantial direct hematological effects in humans or animals. Focal hemorrhagic lesions and mild anemia are sometimes observed in humans who have ingested carbon tetrachloride (Guild et al. 1958; Stewart et al. 1963), but this is likely due to decreased hepatic synthesis and/or secretion of clotting factors.

Only one study was identified that examined the hematological effects of carbon tetrachloride in animals. Intermediate oral exposure of mice to carbon tetrachloride did not result in any consistently significant hematological changes (Hayes et al. 1986).

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

Only a single animal oral study was located that described effects on skeletal muscles, but as it employed co-treatment with phenobarbital to enhance the development of hepatotoxicity (inflammation, necrosis and fibrosis), it is omitted from the LSE table. Male rats were exposed once per week by gavage to carbon tetrachloride doses of approximately 260–1,300 mg/kg/day, for either 3 or 10 weeks (Weber et al. 1992). Histological examination of various muscle tissues revealed no evidence of necrosis or inflammation, a finding supported by normal plasma levels of albumin, creatinine, creatinine phosphokinase, and urea nitrogen. However, muscle atrophy was observed that was apparently selective for fast glycolytic fibers, but not fast or slow oxidative fibers. This was shown to result from increased protein catabolism, and not from decreased protein synthesis. Although the mechanisms are not clearly understood, this muscle effect may be secondary to induced hepatic damage. This conclusion was partially inferred from the observed complete lack of myocyte necrosis, the fiber selectivity of the effect, the absence of enhanced catabolism in muscle exposed directly *in vitro* to 10-fold higher concentrations of carbon tetrachloride, the elimination of disuse atrophy as a factor, and the correlation of this effect with only liver inflammation and necrosis, not cirrhosis (a condition which has been associated in humans with a negative nitrogen balance).

Hepatic Effects. Ingestion of carbon tetrachloride can lead to marked hepatotoxicity. In most reports involving humans, exposure has involved ingestion of several mouthfuls or more (probably 500 mg/kg or higher). Typical clinical signs of hepatic damage in such patients include a swollen liver, along with elevated serum levels of hepatic enzymes and decreased serum levels of liver-synthesized proteins (e.g., albumin, fibrinogen). In cases of death (usually occurring within 1–15 days), typical histological findings include fat accumulation, hepatic degeneration, and moderate to severe centrilobular necrosis; hepatitis was also diagnosed (Ashe and Sailer 1942; Jennings 1955; MacMahon and Weiss 1929; Umiker and Pearce 1953).

Single oral doses of 3–5 mL (70–110 mg/kg) were widely used in the past for treatment of hookworm, and ingestion of this dose resulted in clinical signs of liver injury in only a small number of cases (Hardin 1954; Lamson et al. 1928). Single doses of 4–8 mL (90–180 mg/kg) were found to result in fat accumulation in liver in several individuals (Docherty and Burgess 1922; Docherty and Nicholls 1923), and doses of only 1 mL (child) and 3 mL (adult) (approximately 80 mg/kg) have resulted in hepatic necrosis and death in a few cases (Phelps and Hu 1924). These results are indicative of differential susceptibility to carbon tetrachloride in humans. Certain confounding variables (age) may have been contributing factors to lethality at lower dose levels. One of the two cases involved a 5-year-old child, while the second report involved an adult; however, factors that may have increased susceptibility to the compound in this case could not be determined (Phelps and Hu 1924). No studies were located regarding the effects of longer-term or chronic oral exposure in humans to carbon tetrachloride.

The hepatotoxic effects of carbon tetrachloride have been widely studied in animals. Indeed, carbon tetrachloride is used as a model chemical in many laboratory investigations of the basic mechanism of action of hepatotoxic chemicals. Oral exposure to carbon tetrachloride has been observed to result in a wide spectrum of adverse effects on the liver, the most prominent of which are destruction of the smooth and rough endoplasmic reticulum and its associated enzyme activities (Reynolds and Yee 1968), inhibition of protein synthesis (Lutz and Shires 1978), impaired secretion of triglycerides with resultant fat accumulation (Recknagel and Ghoshal 1966; Recknagel and Glende 1973; Waterfield et al. 1991), centrilobular necrosis (Blair et al. 1991; Reynolds and Yee 1968; Waterfield et al. 1991), and eventually fibrosis and cirrhosis (Allis et al. 1990; Bruckner et al. 1986;). Hepatic injury, as indicated by regenerative proliferation following carbon tetrachloride ingestion, initially affects the peri-portal zone and spreads in a dose-dependent fashion to the perivenous-to-midlobular zones over time (Lee et al. 1998). In rats treated with 7,970 mg/kg, peak increases were observed in serum levels of ALT at 24 hours and AST at 48 hours and increased activity of DNA-synthesizing enzymes (thymidine kinase and

thymidylate synthetase) was observed at 48–72 hours (Nakata et al. 1975). These results reflect the initial leakage of enzymes from damaged hepatocytes followed by cell regeneration. Similar observations have been seen in dosed mice, in which hepatic necrosis was observed during the first 24 hours after dosing and hepatic mitotic activity was noted by 48 hours (Eschenbrenner and Miller 1946).

Although the occurrence of these effects has been confirmed in a very large number of studies, only a few investigations have focused on the dose-dependency of hepatic injury. After a single oral dose of 1,600 mg/kg to rats, urinary taurine levels were significantly increased ($p<0.01–0.05$) within 24 hours and liver weight was reduced (Waterfield et al. 1991). During the first 48 hours after a higher dose (3,200 mg/kg), first liver, then serum, and finally urinary levels of taurine were elevated. Similar effects, as well as reduced hepatic microsome levels of cytochrome P-450, aminopyrine demethylase, and aniline hydroxylase, were observed in rats after a single oral dose of 480 mg/kg/day (Thakore and Mehendale 1991). These effects were much more severe after 8,000 mg/kg, a dose found to be lethal within 14 days for most animals. Additionally, the liver evidenced necrosis, lipid vacuolation, and inflammation, and serum alanine and aspartate amino transferase levels were elevated. Strain differences in sensitivity have been identified (Magos et al. 1982). Twenty hours after a single dose, slight centrilobular damage was observed at 600 mg/kg in Fisher rats, but at 1,200 mg/kg in Porton-Wistar rats; the dose required to elevate serum ALT 10-fold over background was 610 mg/kg for Fisher rats, but 2,000 mg/kg for Porton-Wistar rats. These results were consistent with the higher background levels of serum ALT in Fisher rats, twice that of Porton-Wistar rats (Magos et al. 1982); this study is omitted from the LSE table because exact group sizes were not reported. Single oral doses of only 40–80 mg/kg have also been observed to produce liver injury in rats and mice (Bruckner et al. 1986; Eschenbrenner and Miller 1946; Steup et al. 1993). In rats receiving 80 mg/kg, elevated serum ALT and SDH were observed as early as 3 hours after administration and histopathology (glycogen depletion and focal necrosis) by 6 hours (Steup et al. 1993); recovery was largely complete by 72 hours. When exposures are continued for 10–11 days, doses of 5–40 mg/kg/day produced mild signs of liver change, while 80 mg/kg/day caused clear hepatic injury (Bruckner et al. 1986; Smialowicz et al. 1991). The LOAEL of 5 mg/kg/day from the study by Smialowicz et al. (1991) has been employed to calculate an acute-duration oral MRL of 0.02 mg/kg/day, as described in the footnote on Table 3-2. At this dose, the earliest sign (vacuolar degeneration) of hepatocyte toxicity was just detectable. The severity of this hepatocellular injury with accompanying necrosis increased in a dose-related manner from 10 to 40 mg/kg/day.

The incidence and severity of hepatic effects were dose-related in intermediate-duration oral exposure studies in animals. In rodents ingesting carbon tetrachloride 5 days/week for 12–13 weeks, no hepatic

effects were detected at doses of 1–1.2 mg/kg (Bruckner et al. 1986; Condie et al. 1986). At 10 mg/kg, mild centrilobular vacuolization and elevated serum levels of sorbitol dehydrogenase were observed in rats (Bruckner et al. 1986). Significant elevation in some serum enzymes (ALT, AST, lactate dehydrogenase) and mild necrosis were seen in mice at doses of 12 mg/kg and higher (Condie et al. 1986; Hayes et al. 1986). More extensive hepatic lesions (fatty accumulation, fibrosis, cirrhosis, necrosis) were noted in rats at doses of 20–33 mg/kg (Allis et al. 1990; Bruckner et al. 1986; Koporec et al. 1995). At 100 mg/kg/day, hepatic effects in rats also included cytomegaly and various types of hyperplasia (Koporec et al. 1995). The vehicle used to administer carbon tetrachloride modifies the observed effect levels. The incidence and severity of hepatic lesions was increased when carbon tetrachloride was administered in an aqueous emulsion (in 1% Emulphor) compared to administration in corn oil (Koporec et al. 1995). Dogs appear to be less vulnerable to the hepatic effects of carbon tetrachloride than rodents. In female dogs given 32 mg/kg/day for 8 weeks, there was no liver histopathology and no significant increase in serum enzymes (Litchfield and Gartland 1974). Male and female dogs that received 797 mg/kg/day for 4 weeks exhibited liver histopathology (centrilobular fatty vacuolization sometimes with single cell necrosis); the severity of hepatic lesions in individual dogs was correlated with the level of increases in serum enzyme levels (ALT and ornithine carbamyl transferase). Based on the NOAEL of 1 mg/kg in rats that was reported by Bruckner et al. (1986), an intermediate oral MRL of 0.007 mg/kg/day was calculated as described in the footnote in Table 3-2.

A no-effect level for hepatic effects has not been determined in chronic-duration oral exposure studies in animals. Alumot et al. (1976) reported no significant effects on serum enzyme levels or hepatic fat content of rats exposed to nominal doses of approximately 11–14 mg/kg/day for 2 years. However, the doses in this study cannot be reliably estimated because of uncertainty regarding the method of exposure; diets were fumigated with carbon tetrachloride vapor for 2 days prior to use and the degree of loss from evaporation is not known. Although the gradual intake from the diet would be expected to result in less hepatic toxicity than the same daily amount delivered as a single oral bolus (see, for example, Bruckner et al. 1986 discussed above), this study does not provide definitive information on threshold levels for oral exposure to carbon tetrachloride. The only other chronic-duration oral information comes from chronic gavage bioassays by the NCI (1976a, 1976b) on chloroform and trichloroethylene in which carbon tetrachloride was employed as a positive control for hepatic carcinogenicity. Rats treated with doses as low as 47 mg/kg, 5 days/week for 78 weeks exhibited severe hepatotoxicity, the most prominant effects being portal cirrhosis in 58%, bile duct proliferation in 62%, and fatty accumulation in 58% of treated animals; fibrosis, necrosis, and regenerating nodules were observed less frequently. The main hepatic effect in mice was carcinogenicity, discussed in Section 3.2.2.7. No chronic-duration oral MRL was

derived for carbon tetrachloride because the lowest tested doses in well-conducted bioassays caused severe hepatic toxicity. Under ATSDR guidance, MRLs are not derived on dose levels causing severe effects.

Renal Effects. Nephritis is a common finding in fatal cases of carbon tetrachloride ingestion in humans (Umiker and Pearce 1953), and renal failure may contribute to death in many cases (Gosselin et al. 1976; von Oettingen 1964). Typically, clinical signs of renal dysfunction (oliguria or anuria, albuminuria, proteinuria, elevated blood urea nitrogen edema, hypertension) tend to develop within 1–6 days after exposure, somewhat later than the appearance of hepatic injury (Conaway and Hoven 1946; Guild et al. 1958; Kluwe 1981; MacMahon and Weiss 1929; Smetana 1939; Umiker and Pearce 1953). In nonfatal cases, renal function usually returns to normal within several weeks (Guild et al. 1958; Kluwe 1981; Smetana 1939). Histological changes in the kidney are observed primarily in the proximal tubular epithelium, where cells become swollen and granular, with moderate to severe necrosis (Docherty and Burgess 1922; Guild et al. 1958; MacMahon and Weiss 1929; Smetana 1939).

Studies in animals confirm that the kidney is a target tissue for carbon tetrachloride, although in rodents, the kidney is much less sensitive than the liver to carbon tetrachloride. Doses of 4,000 mg/kg resulted in swollen and pale kidneys in rats within 2 days, with morphological changes present primarily in proximal tubular epithelial cells. All histological and functional signs of renal injury were fully reversible within 5 days (Striker et al. 1968). Fatty degeneration of the kidney has been observed in dogs after a single dose of 3,200 mg/kg (Chandler and Chopra 1926) and swelling of the convoluted tubules after 6,400 mg/kg (Gardner et al. 1925). Exposure of rats to 160 mg/kg/day for about 10 days did not induce adverse renal effects (Bruckner et al. 1986; Smialowicz et al. 1991), nor did 12 weeks exposure to 33 mg/kg/day, 5 days/week (Bruckner et al. 1986). Only marginal indication of kidney injury was detected in mice exposed to doses of 2,500 mg/kg/day for 14 days or 1,200 mg/kg/day for 90 days (Hayes et al. 1986). It should be recalled that these doses result in marked hepatotoxicity.

Body Weight Effects. Reduced body weight gain has been observed in rats orally dosed with carbon tetrachloride 5 days/week for 90 days. Terminal body weights were 16% lower than controls in rats dosed with 33 mg/kg (Bruckner et al. 1986) and 25% lower in rats dosed with 100 mg/kg (Koporec et al. 1995).

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to carbon tetrachloride.

Studies in rodents have shown significant suppression of immune function following exposure to carbon tetrachloride. Exposure of female mice to carbon tetrachloride at 500 mg/kg/day for 7 consecutive days suppressed the T-cell-dependent humoral responses to sheep red blood cells (SRBC) (Delaney et al. 1994). The effect was mediated by an increase in serum levels of transforming growth factor beta-1 (TGF-beta-1), which occurred 24–48 hours after exposure in single-dose experiments (at 250–500 mg/kg, but not 50 mg/kg). Exposure of rats to carbon tetrachloride (up to 160 mg/kg/day for 10 days) by gavage did not alter the primary antibody response to SRBC, lymphoproliferative responses to mitogen or mixed leukocytes, natural killer cell activity, or cytotoxic T-lymphocyte responses; also, spleen and thymus weights were comparable to controls (Smialowicz et al. 1991). In female mice that were given daily gavage doses between 50 and 500 mg/kg/day for 14 days (sufficient for hepatotoxicity), the T-cell-dependent humoral response to SRBC was suppressed at ≥50 mg/kg/day, serum anti-SRBC IgM titers were reduced at 100 mg/kg/day, and the absolute number and percentage of $CD4^+CD8^-$ T-cells per spleen was reduced at 500 mg/kg/day (Guo et al. 2000). Exposure had no effect on the mixed leukocyte response to allogenic spleen cells, or the activities of cytotoxic T-lymphocytes or natural killer (NK) cells. In this study, exposure to carbon tetrachloride also decreased host resistance to *Streptococcus pneumoniae* and *Listeria monocytogenes*, with the effective dose dependent on the magnitude of the challenge. In rats exposed twice weekly for 4–12 weeks to 3,688 mg/kg/day, there was histologic evidence of hemorrhage, hemosiderin deposition, and lymphocyte depletion in the pancreaticoduodenal lymph node (Doi et al. 1991), an effect that may be secondary to induced hepatic damage.

The highest NOAEL values and all LOAEL values for each reliable study of immunological and lymphoreticular effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Ingestion of carbon tetrachloride frequently results in marked depression of the central nervous system. Neurological signs in humans include headache, vertigo, weakness, blurred vision, lethargy, and coma, sometimes accompanied by tremor and parasthesias. Mental confusion and disorientation tend to appear

later. These symptoms have been reported in people who ingested single oral doses of carbon tetrachloride ranging from 5 to 473 mL (approximately 114–10,800 mg/kg) (Cohen 1957; Leach 1922; Stevens and Forster 1953; Stewart et al. 1963). The onset of initial effects is very rapid, and is likely the result of direct narcotic action on the central nervous system, similar to other anesthetic halocarbons. Recovery from the depressant effects generally appears to be complete (Stevens and Forster 1953; Stewart et al. 1963), although in some fatal cases, histological examination of the brain has revealed patchy pontine necrosis, demyelination, and Purkinje cell damage, with widespread hemorrhagic infarcts (Cohen 1957). Single oral doses of 70 or 120 mg/kg have been reported to be without significant neurological effect (Hall 1921; Leach 1922).

Only one animal study was located that specifically reported neurological effects other than those that typically attend acute high-dose exposure (e.g., lethargy, coma, related cardiac effects of arrhythmia, and blood pressure changes). When rats pretreated with phenobarbitol received weekly doses of carbon tetrachloride for 10 weeks (initially 289 mg/kg/day, increasing to a maximum of approximately 1,600 mg/kg/day according to body weight gain), a condition of diffuse micronodular liver cirrhosis was induced (Bengtsson et al. 1987). This was accompanied by significantly increased synthesis of the neurotransmitter serotonin in all six areas of the brain that were monitored. Serotonin levels were not, however, reliably correlated with any abnormal open-field behavior, which was used as an indicator of the possible portal-systemic encephalopathy that may accompany liver failure.

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

3.2.2.5 Reproductive Effects

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

Rats (males and females) ingested carbon tetrachloride in their food for 5–6 weeks (Alumot et al. 1976). No effects were noted on most reproductive parameters monitored (percent conception, percent with litters, mean litter size, mean body weight of offspring at birth and at weaning). An increase in neonatal mortality was observed in the low dose group (about 6 mg/kg/day), but not in the high dose group (about 15 mg/kg/day). The authors concluded that this response was not treatment related, and that these doses of carbon tetrachloride had no adverse effect on reproduction.

The highest NOAEL values for reproductive effects in rats after chronic exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

An epidemiological study was conducted using birth outcome and drinking water exposure databases from a four-county area in northern New Jersey (Bove et al. 1992a, 1992b, 1995). The cross-sectional study of data from 75 out of 146 towns spanned the period 1985–1988 and evaluated the entire study population of 80,938 singleton live births and 599 singleton fetal deaths. Estimated carbon tetrachloride concentrations in the drinking water of >1 ppb were associated with the following adverse developmental outcomes (odds ratio, 95% confidence interval, significance): full-term birth weight <2,500 g (2.26, 1.41–3.6, $p<0.001$), small for gestational age (1.35, 1.03–1.8, $p<0.03$), and neural tube defects (5.39, 1.31–22.2, $p<0.025$). However, the weight/size effects were also associated with trihalomethanes that were present in the drinking water at larger concentrations than carbon tetrachloride and the neural tube defects were based on a total of two cases in the group exposed to carbon tetrachloride. Methodological limitations of the study may have resulted in chance, missed, or under- or overestimated associations. As acknowledged by the authors, inhalation and/or dermal exposure through bathing and showering could be at least as significant as the oral exposure. Although these studies suggest a causative role for carbon tetrachloride in the generation of certain adverse developmental outcomes, issues that could beneficially be addressed in the future include better-defined exposure levels (these levels appear to be rather low for a causative agent) and the potential for such effects to be the result of complex mixture exposure.

A case-control study of selected congenital malformations and maternal residential proximity to NPL sites in California between 1989 and 1991 did not find an increased risk of conotruncal heart defects or oral cleft defects associated with sites containing carbon tetrachloride (Croen et al. 1997).

No teratogenic effects (morphological anomalies) were reported in rats following maternal oral exposure to carbon tetrachloride, but total resorption of fetuses was reported at maternally toxic doses. Doses of 1,400 mg/kg/day during gestation caused marked maternal toxicity in rats, and total resorption of fetuses in some animals, but no adverse effects in surviving litters (Wilson 1954). In rats treated with carbon tetrachloride by gavage in corn oil or an aqueous vehicle (Emulphor EL-620) on gestational days 6–15, no maternal or developmental toxicity occurred at a dose of 25 mg/kg/day (Narotsky et al. 1997a). Total loss of some litters and clinical signs of toxicity (piloerection and reduced body weight gain) occurred in

dams treated with ≥50 mg/kg/day. Effects were slightly more severe when the vehicle was corn oil (5/12 litters resorbed) than when an aqueous vehicle was used (2/24 litters resorbed).

Temporal variations during gestation in sensitivity to carbon tetrachloride were reported in rats. When pregnant rats were given a single dose of 150 mg/kg carbon tetrachloride on gestational day 6, 7, 8, 10, or 12, the incidences of full litter loss ranged between 36 and 72% during gestation days 6–10 (maximal day 8) and 0% on day 12 compared to 4% for the controls (Narotsky et al. 1997b). The authors concluded that gestational days 6–10 represented a critical period of vulnerability to carbon tetrachloride in rats. Dams later found to have had full litter resorption exhibited bloody vaginal discharges within 24 hours of dosing. No additional developmental toxicity was reported in surviving litters. Offspring were not evaluated for possible neurobehavioral deficits.

Mice appear to be less vulnerable than rats to carbon tetrachloride. When pregnant mice were given oral doses as high as 826 mg/kg/day on five consecutive days (the preimplantation period [gestation days 1–5], or organogenesis periods [gestation days 6–10 or 11–15]), there were no overt signs of maternal toxicity and no adverse effects on survival, growth or development during the fetal and postnatal stages (Hamlin et al. 1993).

The highest NOAEL value for developmental effects in rats after acute exposure is recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding carcinogenic effects in humans after oral exposure to carbon tetrachloride.

Studies in animals (rats, hamsters, and several strains of mice) provide convincing evidence that ingestion of carbon tetrachloride increases the risk of liver cancer (Andervont 1958; Della Porta et al. 1961; Edwards 1941; Edwards and Dalton 1942; Edwards et al. 1942; Eschenbrenner and Miller 1944, 1946; NCI 1976a). In general, carbon tetrachloride-induced liver tumors were either hepatomas or hepatocellular carcinomas that appeared after exposure periods of only 10–30 weeks (Edwards 1941; Eschenbrenner and Miller 1944; NCI 1976a). For example, daily oral doses as low as 20 mg/kg produced hepatic tumors in mice exposed for 120 days (Eschenbrenner and Miller 1946). In most cases, the incidence of hepatic tumors was very high (75–100%) in exposed animals. In each of these studies, the

carbon tetrachloride was administered daily by single bolus gavage. As noted in the discussion of oral hepatic effects, such a dosing regimen may exacerbate cancer effects relative to those that might be observed under conditions of food or drinking water exposure. Based on these studies, both IARC (1987) and EPA (IRIS 1993) have concluded there is sufficient evidence that carbon tetrachloride is carcinogenic in experimental animals, and that it is possibly or probably carcinogenic in humans.

The EPA (1984) reviewed the available information on the carcinogenic effects of carbon tetrachloride following oral exposure, and concluded that the studies by Della Porta et al. (1961) in hamsters, Edwards et al. (1942) in mice, and NCI (1976a, 1976b, 1977; Weisburger 1977) in rats and mice had adequate dose-response data to allow quantitative estimation of the unit cancer risk (the excess risk of cancer associated with lifetime ingestion of water containing 1 µg/L, assuming intake of 2 L/day by a 70-kg person). Since each study was judged to have some limitations, no one study was selected as the basis for the risk calculation. Rather, calculations were performed for all four data sets, and the geometric mean of these estimates was taken to be the most appropriate value. These calculations are summarized in Table 3-3. Because of the uncertainty in the data and in the calculations, the EPA identified the geometric mean of the upper 95% confidence limit ($3.7x10^{-6}$) as the preferred estimate of unit cancer risk.

Based on this value, the upper-bound lifetime risk from ingestion of 1 µg/kg/day of carbon tetrachloride is $1.3x10^{-4}$, and the daily intake levels associated with lifetime risks of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} are 0.77, 0.077, 0.0077, and 0.00077 µg/kg/day, respectively.

Because these are based on upper-bound estimates, the true risk could be lower. These values, along with doses of carbon tetrachloride that have been observed to cause cancer in animals, are presented in Figure 3-2.

3.2.3 Dermal Exposure

The highest NOAEL values and all LOAEL values from each reliable study for death and hepatic and dermal effects in each species and duration category are recorded in Table 3-4.

Table 3-3. Summary of Carcinogenic Unit Risk Calculations for Oral Exposure to Carbon Tetrachloride[a]

		Unit cancer risk[b]	
Reference	Species	Best estimate	Upper 95% confidence limit
Della Porta et al. (1961)	Hamster	2.5×10^{-5}	3.4×10^{-5}
Edwards et al. (1942)	Mouse	7.1×10^{-6}	9.4×10^{-6}
NCI (1976)	Mouse	1.4×10^{-6}	1.8×10^{-6}
NCI (1976)	Rat	1.9×10^{-7}	3.1×10^{-7}
	Geometric Mean	2.5×10^{-6}	3.7×10^{-6}

[a]Source: EPA 1984
[b]The estimated probability of cancer in a 70-kg person ingesting 2 L/day of water containing 1 µg/L of carbon tetrachloride for a lifetime

Table 3-4 Levels of Significant Exposure to Carbon Tetrachloride - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form
				Less Serious		Serious		
ACUTE EXPOSURE								
Death								
Gn Pig	Once 24 hr					15000 mg/kg	(LD50, 24 hours)	Roudabush et al. 1965
Gn Pig	Once contact for 5 d					260 mg/cm²	(5/20)	Wahlberg and Boman 1979
Rabbit	Once 24 hr					15000 mg/kg	(LD50, 24 hours)	Roudabush et al. 1965
Systemic								
Gn Pig	Once 15 min-16 hr	Hepatic		513 mg/cm²	(hydropic changes, slight necrosis)			Kronevi et al. 1979
		Dermal		513 mg/cm²	(karyopynosis, spongiosis, perinuclear edema)			
Gn Pig	Once 24 hr	Dermal		120 mg/kg/day	(primary irritation)			Roudabush et al. 1965
Rabbit	Once 24 hr	Dermal		120 mg/kg/day	(primary irritation)			Roudabush et al. 1965

cm = centimeters; d = day(s); Derm = dermal; Gn pig = Guinea pig; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; mg/kg/day = milligrams per kilograms per day; NOAEL = no-observed-adverse-effect level

3.2.3.1 Death

One of three naval officers who weekly misused a carbon tetrachloride fire-extinguishing fluid as a dry cleaning agent over 3 months died of heart failure that was secondary to nephrosis-induced pulmonary edema (Forbes 1944); the man who died was a heavy consumer of alcohol. It is likely that the individual was exposed by inhalation as well as dermally, but the actual intakes were not known; 4 pints of the fluid (equivalent to 3 kg carbon tetrachloride) were used during the time period.

3.2.3.2 Systemic Effects

No studies were located regarding hematological, musculoskeletal, endocrine, or ocular effects after dermal exposure of humans or animals to carbon tetrachloride. Respiratory, cardiovascular, gastrointestinal, hepatic, renal, ocular, and dermal effects were reported in humans. Hepatic and dermal effects were also seen in animals. These effects are discussed below. The LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-4.

Respiratory Effects. Pulmonary edema that developed in one fatal case of combined weekly inhalation/dermal exposure over 3 months appeared to be secondary to renal malfunction (Forbes 1944).

Cardiovascular Effects. Failure of the left side of the heart was the proximate cause of death in one case repeatedly exposed dermally and by inhalation over 3 months (Forbes 1944). The heart effect developed secondary to nephrosis-induced pulmonary edema.

Gastrointestinal Effects. There are case reports of three humans who experienced gastrointestinal symptoms, including nausea and vomiting, after dermal application of carbon tetrachloride-based lotion (Perez et al. 1987). No quantitative estimate of the amount of carbon tetrachloride applied or absorbed was provided. Three individuals who were repeatedly exposed dermally and by inhalation over 3 months experiences anorexia and vomiting (Forbes 1944).

No studies were located regarding gastrointestinal effects in animals after dermal exposure to carbon tetrachloride.

Hepatic Effects. Liver injury, characterized by an elevated serum enzyme (alanine aminotransferase level), was described in case reports of three humans after dermal application of carbon tetrachloride (Perez et al. 1987). In the absence of quantitative estimates of the amount of carbon tetrachloride applied or absorbed, NOAEL and LOAEL values cannot be determined. Severe centrilobular necrosis was found at autopsy following the death of one of three naval officers who were exposed by inhalation and dermally over 3 months (Forbes 1944); none of the exposed men exhibited jaundice. Actual exposure levels could not be determined.

Hydropic changes and isolated necrotic areas were reported in the liver of guinea pigs 16 hours after dermal contact with 513 mg/cm^2 of carbon tetrachloride (1.0 mL placed in a sealed enclosure covering a sealed enclosure covering a 3.1 cm^2 area of clipped skin) (Kronevi et al. 1979).

Renal Effects. Acute renal failure, as evident by anuria and azoturia, was reported in three case reports of humans after dermal application of carbon tetrachloride-based lotion (Perez et al. 1987). The usefulness of this finding is limited by the lack of data concerning the amount of carbon tetrachloride applied or absorbed. Albuminuria and uremia developed in three naval officers exposed weekly by inhalation and dermally to an unknown amount of carbon tetrachloride over 3 months (Forbes 1944). The one fatality, an alcoholic, showed signs of albuminous degeneration of the convoluted tubules and Bowman's capsule and granular casts in the distal convoluted tubules.

No studies were located regarding renal effects in animals after dermal exposure to carbon tetrachloride.

Dermal Effects. In humans, direct dermal contact with undiluted carbon tetrachloride causes a mild burning sensation with mild erythema (Stewart and Dodd 1964). Some individuals appear to be hypersensitive, developing marked swelling, itching, and blisters following dermal contact (Taylor 1925).

Similar effects of dermal contact with carbon tetrachloride have been described in animals. A dose of 124 mg/cm^2 carbon tetrachloride produced moderate primary irritation within 24 hours when applied occluded to the intact or abraded skin of rabbits or guinea pigs, with irritation scores of 2.2–4.1 on skin (Roudabush et al. 1965). Direct dermal contact of guinea pigs with liquid carbon tetrachloride (occluded; 513 mg/cm^2) caused degenerative changes in epidermal cells and marked intercellular edema or spongiosis (Kronevi et al. 1979). These effects became apparent within 15 minutes, and progressed in severity over the course of several hours. These effects require direct dermal contact because similar effects on the skin are not observed following inhalation or oral exposure.

Ocular Effects. Double-vision was reported in an individual exposed dermally and by inhalatioin from weekly use of carbon tetrachloride fire-extinguishing fluid as a dry-cleaning agent over a period of 3 months (Forbes 1944).

No data were located for ocular effects in animals exposed dermally.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after dermal exposure to carbon tetrachloride

3.2.3.4 Neurological Effects

A case of polyneuritis was reported in a man who had repeated dermal contact 8 hours/day with carbon tetrachloride using it as a degreasing agent (Farrell and Senseman 1944). Sudden severe epileptiform seizure and coma occurred in a subsequently fatal case following weekly combined inhalation/dermal exposure (Forbes 1944). The individual, an alcoholic, was one of three men who misused a carbon tetrachloride fire-extinguishing fluid as a dry-cleaning agent over a period of 3 months (Forbes 1944).

No studies were located regarding neurological effects in animals after dermal exposure to carbon tetrachloride.

3.2.3.5 Reproductive Effects

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

3.2.3.6 Developmental Effects

No studies were located exclusively regarding developmental effects in humans or animals after dermal exposure to carbon tetrachloride. However, note the epidemiological studies discussed in Section 3.2.2.6,

which almost certainly involved significant dermal and inhalation exposures in addition to the emphasized oral exposure.

3.2.3.7 Cancer

No studies were located regarding carcinogenic effects in humans or animals following dermal exposure to carbon tetrachloride.

3.3 GENOTOXICITY

The genotoxic potential of carbon tetrachloride has been evaluated *in vivo* (Table 3-5) and *in vitro* (Table 3-6).

Inhalation Exposure. No studies were located on genetic effects in humans or animals after inhalation exposure to carbon tetrachloride.

Oral Exposure. No studies were located regarding genetic effects in humans after oral exposure to carbon tetrachloride.

Results of *in vivo* tests in animals orally exposed to carbon tetrachloride suggest that genotoxic effects only occur at doses high enough to cause hepatic toxicity. No sex-linked recessive mutations were induced in *Drosophila melanogaster* exposed dietarily (Foureman et al. 1994). In oral gavage studies, there were no increases in the frequencies of chromosomal aberration, sister chromatid exchange, or micronucleus formation in the liver of rats or in the frequency of micronucleus formation in bone marrow of mice (Sawada et al. 1991; Suzuki et al. 1997). Some studies reported negative results in the liver for unscheduled DNA synthesis in rats (Mirsalis and Butterworth 1980; Mirsalis et al. 1982). However, authors of one study attributed increased DNA synthesis in rats exposed at a high dose not to unscheduled DNA synthesis *per se*, but to the increased tissue regeneration following hepatic necrosis (Craddock and

Table 3-5. Genotoxicity of Carbon Tetrachloride *In Vivo*

Species (test system)	End point	Results	Reference
Oral route:			
Drosophila melanogaster[a]	Sex-linked recessive mutation	–	Foureman et al. 1994
Rat hepatocytes[b]	Chromosomal aberrations	–	Sawada et al. 1991
Rat hepatocytes[b]	Sister chromatid exchange	–	Sawada et al. 1991
Rat hepatocytes[b]	Micronuclei	–	Sawada et al. 1991
Rat hepatocytes[c]	Unscheduled DNA synthesis	–	Mirsalis and Butterworth 1980
Rat hepatocytes[d]	Unscheduled DNA synthesis	–	Mirsalis et al. 1982
Rat hepatocytes[e]	Unscheduled DNA synthesis	–	Craddock and Henderson 1978
Rat hepatocytes[f]	Unscheduled DNA synthesis	[+]	Craddock and Henderson 1978
Rat hepatocytes[g]	DNA damage	–	Bermudez et al. 1982
Rat liver[h]	DNA adducts (lipid peroxidation)	+	Chaudhary et al. 1994
Rat liver (partially hepatectomized)[i]	Caffeine elutable (single-stranded) DNA	–	Stewart 1981
Hamster liver and kidney[j]	DNA adducts (lipid peroxidation)	+	Wang and Liehr 1995
Mouse liver, stomach, kidney, bladder, lung, brain, bone marrow[k]	DNA damage (comet assay) after 3 hours	–	Sasaki et al. 1998
Mouse stomach, kidney, bladder, lung, brain, bone marrow[l]	DNA damage (comet assay) after 24 hours	–	Sasaki et al. 1998
Mouse liver[m]	DNA damage (comet assay) after 24 hours	[+]	Sasaki et al. 1998
Mouse liver[n]	DNA damage (alkaline elution) after 4 hours	–	Schwartz et al. 1979
Mouse bone marrow[o]	Micronuclei after 24–72 hours	–	Suzuki et al. 1997
Intraperitoneal injection:			
D. melanogaster[p]	Sex-linked recessive mutation	–	Foureman et al. 1994
Rat forestomach, liver, lung, colon, kidney[q]	DNA adducts (lipid peroxidation)	[+]	Wacker et al. 2001
Rat liver[r]	DNA adducts (lipid peroxidation)	[+]	Chung et al. 2001
Rat liver[s]	Covalent binding to DNA	+	Castro et al. 1989
Hamster liver[s]	Covalent binding to DNA	+	Castro et al. 1989
Mouse liver[s]	Covalent binding to DNA	+	Castro et al. 1989
Mouse peripheral reticulocytes[t]	Micronuclei	–	Suzuki et al. 1997
Mouse bone marrow[u]	Micronuclei after 24 or 48 hours	–	Crebelli et al. 1999

Table 3-5. Genotoxicity of Carbon Tetrachloride *In Vivo*

Species (test system)	End point	Results	Reference
Oral route:			
Rat liver[v]	DNA damage (reduced viscosity) after 2 hours	–	Brambilla et al. 1983
Rat liver[w]	DNA damage (alkaline elution) after 4 hours	–	Barbin et al. 1983

[a]Males exposed to dietary concentration of 25,000 ppm for 72 hours prior to mating with untreated females.
[b]Male F344 rats exposed to 1,600 mg/kg carbon tetrachloride by oral gavage in corn oil 4–72 hours prior to sacrifice.
[c]Male F344 rats exposed to 100 mg/kg carbon tetrachloride by oral gavage in corn oil.
[d]Male F344 rats exposed to 400 mg/kg carbon tetrachloride by oral gavage in corn oil.
[e]Female Wistar rats exposed to 4,000 mg/kg carbon tetrachloride by oral gavage in liquid paraffin; injected with hydroxyurea to stop *de novo* DNA synthesis and tritiated thymidine two hours after dosing.
[f]Female Wistar rats exposed to 4,000 mg/kg carbon tetrachloride by oral gavage in liquid paraffin; injected with hydroxyurea to stop *de novo* DNA synthesis and tritiated thymidine 17 hours after dosing. Increase in DNA synthesis associated with increased tissue regeneration, but no unscheduled DNA synthesis.
[g]Male Fischer 344 rats exposed to 400 mg/kg carbon tetrachloride by oral gavage in corn oil; nuclei of hepatocytes isolated 2, 12, or 24 hours after dosing were analyzed by alkaline elution.
[h]Sprague-Dawley rats (sex not specified) exposed to 0.1 mg/kg carbon tetrachloride by oral gavage 4 days before sacrifice.
[i]Female Wistar rats, 3 weeks after partial hepatectomy and treatment with tritiated thymidine, exposed to 200–800 mg/kg carbon tetrachloride by oral gavage in corn oil 4 or 24 hours before sacrifice.
[j]Female Syrian golden hamsters exposed to 160 or 1,600 mg/kg carbon tetrachloride by oral gavage in corn oil 4 days prior to sacrifice.
[k]Male CD-1 mice, exposed to 2,000 mg/kg carbon tetrachloride by oral gavage in corn oil.
[l]Male CD-1 mice, exposed to 500, 1,000, or 2,000 mg/kg carbon tetrachloride by oral gavage in corn oil.
[m]Male CD-1 mice, exposed to hepatotoxic doses of 1,000 or 2,000 mg/kg carbon tetrachloride by oral gavage in corn oil; no effect observed at 500 mg/kg.
[n]Male and female NMRI mice exposed to 4,000 mg/kg carbon tetrachloride by oral gavage in corn oil 4 hours before sacrifice.
[o]Male BDF_1 mice exposed to 500, 1,000, or 2,000 mg/kg carbon tetrachloride by oral gavage in olive oil 24 hours before sacrifice.
[p]Males injected with 0.7% NaCl containing 2,000 ppm carbon tetrachloride in ethanol 24 hours before mating.
[q]Female F344 rats injected with 500 mg/kg carbon tetrachloride 4–24 hours before sacrifice.
[r]Male F344 rats injected with 3,200 mg/kg carbon tetrachloride in olive oil.
[s]Animals injected with 770 mg/kg radiolabeled carbon tetrachloride 6 hours before sacrifice.
[t]Male BDF_1 mice injected with 2,000 or 3,000 mg/kg carbon tetrachloride 24–72 hours before sacrifice.
[u]Male or female CD-1 mice injected with 1,500 or 3,000 mg/kg carbon tetrachloride 24–48 hours before sacrifice.
[v]Male Sprague-Dawley rats injected with 200 mg/kg carbon tetrachloride.
[w]Male BD-V1 rats injected with 4,000 mg/kg carbon tetrachloride.

– = negative result; + = positive result; [+] = hepatotoxicity was evident; DNA = deoxyribonucleic acid

Table 3-6. Genotoxicity of Carbon Tetrachloride *In Vitro*

Test system Species (concentration)[a]	End point	Results With activation	Results Without activation	Reference
Prokaryotic organisms:				
Escherichia coli K-12 343/113 (15.4 mg/mL)	Differential DNA repair	–	–	Hellmer and Bolcsfoldi 1992
E. coli WP2, WP67, CM871; liquid micromethod, sealed (12.5 µg)	Differential DNA repair	+	+	De Flora et al. 1984
E. coli WP2, WP67, CM871; 2-hour preincubation, sealed	Differential DNA repair	–	+	De Flora et al. 1984
E. coli WP2, WP67, CM871; spot test	Differential DNA repair	NT	–	De Flora et al. 1984
E. coli PQ37 (1540 µg/mL)	SOS induction (DNA repair)	–	–	Brams et al. 1987
E. coli WP2*/pKM101; WPuvrA/pKM101* (1,000 ppm; enclosed gas atmosphere 24 hours)	Reversion frequency	+	+	Araki et al. 2004
E. coli WPuvrA/pKM101 (10,000 ppm; enclosed gas atmosphere 24 hours)	Reversion frequency	+/–	+/–	Araki et al. 2004
Salmonella typhimurium TA98 (10,000 ppm; enclosed gas atmosphere 24 hours)	Reversion frequency	–	+/–	Araki et al. 2004
S. typhimurium TA100, TA1535, TA1537 (50,000 ppm)	Reversion frequency	–	–	Araki et al. 2004
S. typhimurium TA100, TA1535 (10 mg/plate)	Reversion frequency	–	–	McCann et al. 1975
S. typhimurium TA1535, TA1538 (1,230 µg/mL)	Reversion frequency	–	No data	Uehleke et al. 1977
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reversion frequency	No data	–	Simmon et al. 1977
S. typhimurium TA1535, TA98, TA100) (2,830 µg/plate)	Reversion frequency	–	–	Barber et al. 1981
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 (10 mg/plate)	Reversion frequency	–	–	De Flora 1981, De Flora et al.1984
S. typhimurium TA1537, TA100 (2,450 µg/plate)	Reversion frequency	+[b]	+[b]	Varma et al. 1988
S. typhimurium TA98 (2,450 µg/plate)	Reversion frequency	NT	+[b]	Varma et al. 1988
S. typhimurium TA1535 (2,450 µg/plate)	Reversion frequency	NT	–	Varma et al.1988
S. typhimurium TA1535/ pSK1002 (5.3 mg/plate)	SOS induction (*umu* expression)	–	–	Nakamura et al. 1987

Table 3-6. Genotoxicity of Carbon Tetrachloride *In Vitro*

Test system Species (concentration)[a]	End point	Results With activation	Results Without activation	Reference
S. typhimurium BA13 and BAL13; sealed (1,230 µg/plate)	Forward mutation (Ara^R test)	–	–	Roldan-Arjona et al. 1991
Eukaryotic organisms:				
Aspergillus nidulans P1 (diploid) (0.5%)	Somatic segregation, crossovers, non-disjunction frequency	NT	+ (CT)	Gualandi 1984
A. nidulans P1 (diploid) (0.0275%)	Somatic segregation	NT	+ (CT)	Benigni et al. 1993
A. nidulans 35 (haploid); (growth mediated) (0.5%)	Forward mutation (su meth G1)	NT	+ (CT)	Gualandi 1984
A. nidulans 35 (haploid); plate incorporation (0.5%)	Forward mutation (su meth G1)	NT	–	Gualandi 1984
Saccharomyces cerevisiae D7 (5230 µg/mL)	Frequency of convert-ants recombinants, revertants	No data	+	Callen et al. 1980
S. cerevisiae RS112 (diploid) (4 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+ (CT)	Schiestl et al. 1989; Brennan and Schiestl 1998
S. cerevisiae RS112 (diploid) (4 mg/mL)	Interchromosomal recombinant (ADE^+)	NT	–	Schiestl et al. 1989
S. cerevisiae RS112 (diploid) (4 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+/– (see text)	Galli and Schiestl 1998
S. cerevisiae RS112 (diploid); arrested in G1 (5 mg/mL)	Interchromosomal recombinant (ADE^+)	NT	+ (CT)	Galli and Schiestl 1996
S. cerevisiae RS112 (diploid); arrested in G1 (8 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+ (CT)	Galli and Schiestl 1996
S. cerevisiae AGY3 (arrested in G2) (8 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+ (CT)	Galli and Schiestl 1995
S. cerevisiae D61.M (6.4 mg/mL)	Aneuploidy	NT	–	Whittaker et al. 1989
Mammalian cells:				
Rat liver cell line (RL_1) (0.02 µg/mL)	Chromatid gaps, deletions or aberrations	No data	–	Dean and Hodson-Walker 1979
Rat hepatocytes (Wistar) (154 µg/mL)	DNA strand breaks, adducts	NT	+	Beddowes et al. 2003
Rat hepatocytes (460 µg/mL)	DNA damage	NT	+(CT)	Sina et al. 1983
Rat hepatocytes (CD) (154 µg/mL)	Unscheduled DNA synthesis	NT	–	Selden et al. 1994
Human/peripheral lymphocytes (48 µg/mL)	Sister chromatid exchange	–	–	Garry et al. 1990
Human/peripheral lymphocytes (76 µg/mL)	Chromosomal aberration	–	–	Garry et al. 1990
Human/ peripheral lymphocytes (1,540 µg/mL)	Micronuclei	+[c]	+[c]	Tafazoli et al. 1998

Table 3-6. Genotoxicity of Carbon Tetrachloride *In Vitro*

Test system Species (concentration)[a]	End point	Results With activation	Results Without activation	Reference
Human/ peripheral lymphocytes (3,080 µg/mL)	DNA damage (comet assay)	–	–	Tafazoli et al. 1998
Human/peripheral lymphocytes (16 mg/mL)	Unscheduled DNA synthesis	+/– (CT)	–	Perocco and Prodi 1981
Human lymphoblastoid cells AHH-1 (1,540 µg/mL)	Micronucleus formation	NT	–	Doherty et al. 1996
Human lymphoblastoid cells h2E1, MCL-5 (308 µg/mL)	Micronucleus formation	NT	+	Doherty et al. 1996
Lamb *(Ovis aries)*/peripheral lymphocytes (16 µg/mL)	Chromosomal aberration	NT	–	Sivikova et al. 2001
Lamb *(Ovis aries)*/peripheral lymphocytes (4 µg/mL)	Micronucleus formation	+	+	Sivikova et al. 2001
Chinese hamster ovary cells (1,490 µg/mL)	Sister chromatid exchange	–	–	Loveday et al. 1990
Chinese hamster ovary cells (3,000 µg/mL)	Chromosomal aberration	–	–	Loveday et al. 1990
Chinese hamster ovary cells (8 mg/mL)	Chromosomal aberration at anaphase	NT	+	Coutino 1979
Chinese hamster V79 cells (246 µg/mL)	Aneuploidy	NT	+	Onfelt 1987
Chinese hamster V79 cells	Spindle disturbances (c-mitosis)	NT	+ (CT)	Onfelt 1987
Syrian hamster embryo cells (3 µg/mL)	Clonal transformation	NT	+/-	Amacher and Zelljadt 1983
Calf thymus DNA (308 µg/mL)	Covalent binding	+	NT	DiRenzo et al. 1982

[a]Concentrations are the highest tested in negative studies and the lowest tested in negative studies.
[b]Effect not dose-related but cytotoxicity not evaluated.
[c]Effect not dose-related, seen only in cells from one of two subjects.

– = negative result; + = positive result; +/– = weak positive; CT = increase with cytotoxicity; NT = not tested; plate = plate incorporation assay; sealed = assay vessel sealed to prevent evaporation

Henderson 1978). Single high doses of carbon tetrachloride resulted in DNA breakage in the liver (but not other tissues) that was detectable electrophoretically (comet assay) in mice, but only 24 hours after dosing (Sasaki et al. 1998). No increase in DNA breakage was detected in mice assayed only 3 or 4 hours after receiving a high dose (Sasaki et al. 1998; Schwarz et al. 1979) or in rats exposed at a lower dose (Bermudez et al. 1982). Stewart (1981) found no increase in the generation of DNA repair intermediates (with accessible single-strand regions) in the livers of partially hepatectomized female Wistar rats given doses insufficient to cause overt hepatic necrosis 24 hours after exposure. The results of these studies suggest that DNA breakage resulting from oral exposure to carbon tetrachloride is related to hepatic cytotoxicity.

Some genotoxicity of carbon tetrachloride following oral exposure is related to the lipid peroxidation activity of its metabolites. Increases in endogenous lipid peroxidation adducts of DNA, such as malondialdehyde deoxyguanosine, were noted in the liver and kidney of hamsters and the liver of rats dosed with carbon tetrachloride (Chaudhary et al. 1994; Wang and Liehr 1995).

Dermal Exposure. No studies were located regarding genotoxic effects in humans or animals after dermal exposure to carbon tetrachloride.

Other Routes of Exposure In Vivo. Results of genotoxicity assays in which carbon tetrachloride was administered by intraperitoneal injection were similar to results from oral gavage assays. Carbon tetrachloride caused no increase in frequencies of sex-linked recessive mutations in *D. melanogaster* (Foureman et al. 1994) or micronucleus formation in mice (Crebelli et al. 1999; Suzuki et al. 1997). Hepatic DNA damage was not detectable electrophoretically in rats assayed 2–4 hours after exposure (Barbin et al. 1983; Brambilla et al. 1983).

Two kinds of DNA adducts related to carbon tetrachloride metabolism have been detected in injection studies. Covalent binding of metabolites of radiolabeled carbon tetrachloride was detected in the hepatic DNA of rats, hamsters, and mice 6 hours after injection (Castro et al. 1989); the level of binding to DNA was similar in the three species, whereas binding to nuclear proteins was 3 times higher in mice and hamsters than in rats. In other rat studies, carbon tetrachloride significantly increased the level of an endogenous lipid peroxidation product, *trans*-4-hydroxy-2-nonenal, and its deoxyguanosine adduct, 1,N^2-propanodeoxyguanosine (Chung et al. 2000; Wacker et al. 2001). The affected tissues included the liver and forestomach, and to a lesser degree the lung, colon, and kidney (Chung et al. 2000; Wacker et al. 2001). Hepatic increases in adduct formation were about 2-fold following a dose of 500 mg/kg and ~37-

fold following a dose of 3,200 mg/kg (Chung et al. 2000). *In vivo* genotoxicity studies are summarized in Table 3-5.

In Vitro. The genotoxicity of carbon tetrachloride has been studied in prokaryotic and eukaryotic cells *in vitro* (Table 3-6).

The majority of mutagenicity assays for bacteria exposed to carbon tetrachloride have been negative with or without metabolic activation, but volatilization of the chemical in standard plate incorporation methods using unsealed plates may have contributed to some negative findings. In *Salmonella typhimurium*, negative results were reported for a preincubation forward mutation assay in strains BA13 and BAL13 using sealed plates (Roldan-Arioina et al. 1991), for an SOS induction assay in strain TA1535/pSK1002 (Nakamura et al. 1987), and for reverse mutation assays in several strains (Araki et al. 2004; Barber et al. 1981; De Flora 1981, De Flora et al. 1984; McCann et al. 1975; Simmon et al. 1977; Uehleke et al. 1977; Varma et al. 1988). Increases in reverse mutation frequency were observed in plate incorporation assays for strains TA1537 and TA100 with or without activation and TA98 without activation, but the responses were not dose-related and cytotoxicity was not examined (Varma et al. 1988). Weak positive results for mutagenicity were also reported for strain TA98 exposed to 10,000 ppm carbon tetrachloride vapor in an enclosed system (Araki et al. 2004). In the same gas-phase system, weakly increased reversion frequencies with or without metabolic activation were reported for *Escherichia coli* strain WPuvrA/pKM101 and stronger increases for strain WP2/pKM101, which is repair-proficient. Negative results were reported for an SOS induction assay in *E. coli* strain PQ37 (Brams et al. 1987) and a differential DNA repair assays in strains K-12 343/113 (Hellmer and Bolcsfoldi 1992). Induction of differential DNA repair was observed in strains WP2, WP67, and CM871 when assays were conducted in sealed vessels, but not when conducted as spot tests (De Flora et al. 1984).

Assays for the genotoxicity (somatic segregation, non-disjunction frequency, forward mutation) of carbon tetrachloride in the mold *Aspergillus nidulans* were negative or positive only with cytotoxicity (Benigni et al. 1993; Gualandi 1984). Carbon tetrachloride did not induce aneuploidy in the yeast *Saccharomyces cerevisiae* strain D61.M (Whittaker et al. 1989). Similarly, cytotoxicity was generally observed at concentrations at which positive or weak positive results were reported for genotoxicity (interchromosomal recombination or reversion) in *S. cerevisiae* (Brennan and Schiestl 1998; Callen et al. 1980; Galli and Schiestl 1996). Galli and Schiestl (1998) observed that carbon tetrachloride induced intrachromosomal recombination in dividing cells or cells arrested in G1 or G2 phase, but not cells in S-phase. These authors suggested that chemical-induced cytotoxicity prematurely pushed G1 cells into

S-phase, indicating that genotoxicity might result from the failure to completely repair DNA before replication, resulting in DNA strand breaks.

In vitro genotoxicity assays in mammalian cells treated with carbon tetrachloride gave results consistent with *in vivo* bioassays. Carbon tetrachloride yielded weak positive results (1 out of 2,003 clones counted) in a clonal transformation assay in hamster embryo cells exposed without activation (Amacher and Zelljadt 1983). No increase in unscheduled DNA synthesis was observed in rat hepatocytes or human peripheral lymphocytes treated without metabolic activation, although weak positive results were observed in human lymphocytes treated with activation at cytotoxic concentrations (Perocco and Prodi 1981; Selden et al. 1994). Negative results in standard chromosomal aberration assays were reported for exposed rat hepatocytes, human peripheral lymphocytes, lamb lymphocytes, and Chinese hamster ovary cells (Dean and Hodson Walker 1979; Garry et al. 1990; Loveday et al. 1990; Sivikova et al. 2001). However, one study that examined Chinese hamster ovary cells for chromosomal aberrations at anaphase rather than metaphase reported 6-fold increases in lag chromosomes (indicative of centromere or microtubule malfunction) and multipolar spindles (Coutino 1979). Aneuploidy was induced in treated Chinese hamster V9 lung cells, but a 10% increase in spindle aberrations (c-mitosis) only occurred at a concentration at which 50% cytotoxicity was observed (Onfelt 1987). The frequency of sister chromatid exchanges was not elevated in human peripheral lymphocytes or Chinese hamster ovary cells treated with or without metabolic activation (Garry et al. 1990; Loveday et al. 1990). Micronucleus formation was induced in treated peripheral lymphocytes taken from one of two human donors (Tafazoli et al. 1998). In micronucleus assays in cultured human lymphoblastoid cells, negative results were reported for a cell line (AHH-1) with a low native level of CYP1A1 activity, but positive results for derivative cell lines expressing cDNA for one or more human microsoal enzymes (CYP2E1 in line h2E1 and CYP 1A2, 2A6, 3A4, and 2E1 and epoxide hydrolase in line MCL-5) (Doherty et al. 1996). These results demonstrate that biotransformation of carbon tetrachloride is needed for micronucleus induction. Increases in single-strand DNA breaks were observed in rat hepatocytes exposed to cytotoxic concentrations of carbon tetrachloride, but not in exposed human lymphocytes (Beddowes et al. 2003; Sina et al. 1983; Tafazoli et al. 1998)

As reported for *in vivo* studies, DNA adducts have been detected in mammalian cells following exposure to carbon tetrachloride *in vitro*. Covalent binding of radiolabeled carbon tetrachloride to DNA and protein was detected in hepatic nuclear preparations from male Sprague-Dawley rats, Syrian Golden hamsters, and C3H mice (Castro et al. 1989). When NADPH was added to the reaction mixtures, the level of covalent binding to DNA was enhanced for hamsters and mice, but not rats. Direct covalent

binding of carbon tetrachloride to calf thymus DNA was detected following microsomal bioactivation (DiRenzo et al. 1983); the degree of binding was increased 2.2-fold when the DNA samples were pre-treated with pronase, suggesting that reactive metabolites of carbon tetrachloride react with both DNA and protein. Carbon tetrachloride treatment of rat hepatocytes or hepatic microsomes also increased the frequency of DNA adducts generated as by-products of lipid peroxidation (Beddowes et al. 2003; Castro et al. 1997). In treated hepatocytes, there were statistically significant increases (compared to background levels) in malondialdehyde deoxyguanosine adducts (Beddowes et al. 2003). Increases in 8-oxodeoxy-guanosine adducts were observed at the threshold of, and concomitant with, cytotoxicity. A biochemical study using DNA bases and liver microsomes from male Sprague-Dawley rats demonstrated that the bioactivation of carbon tetrachloride resulted in the formation of adducts to guanine (2,6-diamino-4-hydroxy-5-formamidopyrimidine), cytosine (5-hydroxycytosine), and thymidine (5-hydroxy-methyluracil), but not to adenine (Castro et al. 1997). The authors attributed formation of these adducts to reactive metabolites (trichloromethyl or trichloromethylperoxyl free radicals) or to reactive aldehydes, such as malondialdehyde, which are generated by lipid peroxidation. *In vitro* genotoxicity studies are summarized in Table 3-6.

3.4 TOXICOKINETICS

Carbon tetrachloride is absorbed readily from the gastrointestinal and respiratory tracts, and more slowly through the skin. It is distributed to all major organs, with highest concentrations in the fat, liver, bone marrow, adrenals, blood, brain, spinal cord, and kidney (Bergman 1983; Dambrauskas and Cornish 1970; McCollister et al. 1951; Paustenbach et al. 1986a, 1986b). Once carbon tetrachloride is absorbed, it is metabolized by cytochrome P-450 enzymes, with the production of the trichloromethyl radical (Lai et al. 1979; Poyer et al. 1978). Aerobically, metabolism of the trichloromethyl radical can eventually form phosgene (Shah et al. 1979). Anaerobically, the radical can undergo reactions to form chloroform (Glende et al. 1976; Uehleke et al. 1973), hexachloroethane (Fowler 1969; Uehleke et al. 1973), or carbon monoxide (Wolf et al. 1977), as well as bind directly to lipids, proteins, and deoxyribonucleic acid (DNA) (Rao and Recknagel 1969). Carbon tetrachloride is excreted primarily in exhaled air (initial elimination half-life of 1–3 hours) and in the feces, while relatively minimal amounts are excreted in the urine (McCollister et al. 1951; Paustenbach et al. 1986a; Stewart and Dodd 1964; Stewart et al. 1961, 1963, 1965; Young and Mehendale 1989).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Although there are many cases of human overexposure to carbon tetrachloride vapor, there are few quantitative studies of pulmonary absorption of carbon tetrachloride in humans. Based on the difference in carbon tetrachloride concentration in inhaled and exhaled air, absorption across the lung was estimate to be about 60% in humans (Lehmann and Schmidt-Kehl 1936). Monkeys exposed to 50 ppm absorbed an average of 30.4% of the total amount of carbon tetrachloride inhaled, at an average absorption rate of 0.022 mg carbon tetrachloride/kg/minute (McCollister et al. 1951). The concentration of carbon tetrachloride in the blood increased steadily in monkeys, but did not reach a steady-state within 344 minutes of exposure. In rats exposed to 100 or 1,000 ppm for 2 hours, the total absorbed dose of carbon tetrachloride was 17.5 or 179 mg/kg of body weight, respectively (Sanzgiri et al. 1995). (These results were used to establish dose levels for parallel oral-route studies described in Section 3.4.1.2.) Carbon tetrachloride was rapidly absorbed from the lungs as indicated by the near peak levels that were measured in arterial blood at the earliest timepoint (5 minutes). A near steady-state was achieved within 10 or 15 minutes and was maintained for the duration of the 2-hour exposures. In rats, mice, and hamsters exposed to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, the initial body burdens of carbon tetrachloride equivalents (CE) immediately following exposure were 12.1, 1.97, and 3.65 µmol, respectively (Benson et al. 2001).

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral exposure to carbon tetrachloride. It would be anticipated, however, that carbon tetrachloride is well absorbed from the gastrointestinal tract of humans, since carbon tetrachloride is readily absorbed from the gastrointestinal tract of animals (see below), and there are many accounts of human poisonings resulting from ingestion of carbon tetrachloride (e.g., Ashe and Sailer 1942; Conway and Hoven 1946; Gosselin et al. 1976; Guild et al. 1958; Kluwe 1981; Lamson et al. 1928; Phelps and Hu 1924; Ruprah et al. 1985; Stewart et al. 1963; Umiker and Pearce 1953; von Oettingen 1964).

Results from several animal studies indicate that carbon tetrachloride is rapidly and extensively absorbed from the gastrointestinal tract. Typically, 80–85% of an oral dose may be recovered in expired air, indicating that gastrointestinal absorption is at least 85% (Marchand et al. 1970; Paul and Rubinstein

1963). The time course of absorption depends on exposure conditions, with peak blood levels occurring as early as 3–6 minutes after dosing (Kim et al. 1990a). While oral absorption from water or other aqueous vehicles is very rapid and extensive, when carbon tetrachloride is administered using corn oil as the vehicle, absorption is slowed and diminished (Gillespie et al. 1990; Kim et al. 1990a). Similar findings were reported by Withey et al. (1983) for several other halogenated hydrocarbons. The absorption rate and, therefore, peak blood levels will be inversely proportional to the volume of corn oil employed in oral dosing.

Sanzgiri et al. (1995) compared pharmacokinetics of carbon tetrachloride administered to fasted rats as a single bolus by gavage or by infusion over 2 hours. The doses, 17.5 and 179 mg/kg, were established by uptake measured in a 2-hour inhalation experiment (see Section 3.4.1.1). Carbon tetrachloride was rapidly absorbed in the gastrointestinal tract. Peak arterial blood concentrations were reached within 15 minutes of bolus administration and then declined, whereas infusion caused a steady increase over the 2-hour period. The peak concentrations were higher for the bolus group than for the infusion group.

3.4.1.3 Dermal Exposure

Carbon tetrachloride is significantly absorbed through the skin of humans, though less readily than from the lung or gastrointestinal tract. When volunteers immersed their thumbs in undiluted carbon tetrachloride for 30 minutes, carbon tetrachloride was detected in the alveolar air of each subject within 10 minutes, indicating relatively rapid percutaneous absorption (Stewart and Dodd 1964). The alveolar concentration of carbon tetrachloride rose steadily thereafter and peaked by about 30 minutes postexposure. The authors estimated that immersion of both hands in liquid carbon tetrachloride for 30 minutes would yield an exposure equivalent to breathing 100–500 ppm for 30 minutes. The investigators noted that the amount of carbon tetrachloride that can penetrate human skin appeared to be related to the method of application, the duration and area of skin exposure, and the type of skin exposed.

Studies in animals confirm that liquid carbon tetrachloride is absorbed through the skin (Jakobson et al. 1982; Morgan et al. 1991; Tsuruta 1975). The rate of uptake is high enough (54 nmol/min/cm^2 in mice) that absorbed doses may be comparable to the doses absorbed from relatively high levels of carbon tetrachloride in air (Tsuruta 1975). Uptake kinetics are linear only for a short time (about 30 minutes), after which blood levels tend to decrease (Jakobson et al. 1982; Morgan et al. 1991). This is probably due to local vasoconstriction in the exposed skin area. During the course of a 24-hour exposure (2 mL/3.1 cm^2 skin), rats absorbed 27% (0.54 mL) of the applied neat solution, whereas >99% of the

carbon tetrachloride in 110–648 µg/mL aqueous solutions (approximately one-third to completely saturated) was absorbed (Morgan et al. 1991). Rather broad peak blood concentrations of approximately 8–70 ng/mL were observed 2–8 hours into the exposure period. In monkeys, the dermal absorption of radioactive carbon tetrachloride vapor at concentrations of 485 or 1,150 ppm over a period of 240 or 270 minutes, respectively, was negligible, as measured in samples of blood and expired air (McCollister et al. 1951).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in humans after inhalation exposure to carbon tetrachloride.

Inhalation studies in monkeys (McCollister et al. 1951), rats (Benson et al. 2001; Dambrauskas and Cornish 1970; Paustenbach et al. 1986a, 1986b; Sanzgiri et al. 1997), and hamsters and mice (Benson et al. 2001) reveal that the highest carbon tetrachloride concentrations occur in fat, and in organs or tissues with high fat content such as bone marrow, liver, brain, and kidney. In rats exposed to 1,000 ppm for 2 hours (receiving a dose of 179 mg/kg), the maximal concentration of carbon tetrachloride was reached within 30 minutes (the earliest timepoint) in the liver, kidney, lung, brain, heart, muscle, spleen, and gastrointestinal tract, and by 240 minutes in fat (Sanzgiri et al. 1997). The maximal concentration of carbon tetrachloride (µg/g tissue) achieved in the kidney was 1.25 times higher than the liver. The area under the tissue concentration versus time curve (AUC) for the first 30 minutes of exposure was 322, 409, 460, and 710 µg per minute/mL, respectively, for the liver, kidney, brain, and fat. The half-life of clearance from different organs (evaluated over 24 hours) ranged from 204 minutes for the kidney, 249 minutes for the liver to 665 minutes for fat. Through the use of a low temperature whole-body autoradiographic technique, Bergman (1983) observed a particularly high uptake of ^{14}C-carbon tetrachloride into the white matter of brain, spinal cord, and spinal nerves in mice exposed by inhalation. Considerably lower levels were found in the kidney, lung, spleen, muscle, and blood.

Immediately following exposure to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, the proportion of the initial body burden as carbon tetrachloride equivalents (CE) present in the major tissues was 30% for rats and hamsters and 40% for mice (Benson et al. 2001). The CE concentrations at that

time were highest in the liver of mice and hamsters but were highest in fat for rats; 48 hours later, CE concentrations in all three species were highest in the liver.

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to carbon tetrachloride.

Studies of the time-course of tissue distribution in male rats given oral doses of carbon tetrachloride reported that concentrations in the blood, striated muscle, brain, and liver were maximal 2 hours after dosing (Marchand et al. 1970). The peak concentrations in the liver and brain were significantly higher than in the muscle and blood. Peak levels in the fat were not reached until 5.5 hours post dosing, at which time they were more than 50-fold greater than peak blood levels. A similar time-course of tissue deposition of carbon tetrachloride has been observed in female rats (Teschke et al. 1983) and rabbits (Fowler 1969) dosed orally with carbon tetrachloride. Higher carbon tetrachloride levels were found consistently in the liver than in the brain of rats dosed orally (Marchand et al. 1970; Watanabe et al. 1986). This may be because carbon tetrachloride absorbed from the gastrointestinal tract enters the portal circulation, which initially passes through the liver. A significant proportion of the carbon tetrachloride is likely taken up from the portal blood during the first pass, resulting in the high liver levels following ingestion. One week after exposure to ^{14}C-carbon tetrachloride, the concentrations of radiolabel (expressed as mmol carbon tetrachloride/g tissue) were about 1.5 in plasma, 5–6.5 in soleus and white vastus lateralis muscle, 8 in liver, 10 in kidney and diaphragm, and 13 in adipose tissue (Weber et al. 1992). It is interesting to note that phenobarbital pretreatment, often used to hasten or intensify the toxic effects of carbon tetrachloride exposure, was found not only to nearly double the amount of radiolabel retained in the examined tissues, but also to significantly alter its distribution. Liver, kidney, and plasma concentrations were elevated to 600, 350, and 150% of their respective control (carbon tetrachloride alone) levels, while the muscle, diaphragm, and adipose levels were reduced to 40–70%. This observation is consistent with higher levels of the administered dose being metabolized (largely in the liver) and subsequently entering the carbon pool.

Sanzgiri et al. (1997) exposed rats by bolus dosing or gastric infusion over 2 hours to a dose of carbon tetrachloride that was equivalent to the amount absorbed during a 2-hour exposure at 1,000 ppm. In rats receiving a dose of 179 mg/kg by infusion over 2 hours, the maximal concentration of carbon tetrachloride was reached by 120 minutes in the liver, kidney, and heart, 150 minutes in the brain, muscle, and spleen, 180 minutes in lung, and by 360 minutes in fat (Sanzgiri et al. 1997). The AUC for the first

30 minutes of exposure was 3, 4, 28, and 157 µg per minute/mL, respectively, for the liver, kidney, brain, and fat in infused rats. Absorption of carbon tetrachloride was more rapid and organ concentrations of carbon were higher in rats that received the same dose as a single bolus by gavage. The maximal concentration was reached by 1 minute in the liver, 5 minutes in the kidney, heart, and spleen, 15 minutes in lung and brain, 60 minutes in muscle, and 120 minutes in fat. The AUC for the first 30 minutes was 680, 380, 423, and 306 µg per minute/mL, respectively, for the liver, kidney, brain, and fat in the bolus-treated rats. The authors indicated that the bolus-delivery resulted in high 30-minute AUC values because the capacity of first-pass hepatic and pulmonary elimination was exceeded. The half-life of clearance from different organs (based on the AUC over 24 hours) ranged from 190 minutes for the kidney and 269 minutes for the liver to 358 minutes for fat in the infused rats, and from 278 minutes for the kidney and 323 minutes for the liver to 780 minutes for fat in the bolus-treated rats. The maximum tissue concentrations (µg/g tissue) achieved in the kidney were 24% of the value for the liver following bolus dosing, but 8 times higher than the liver following gastric infusion.

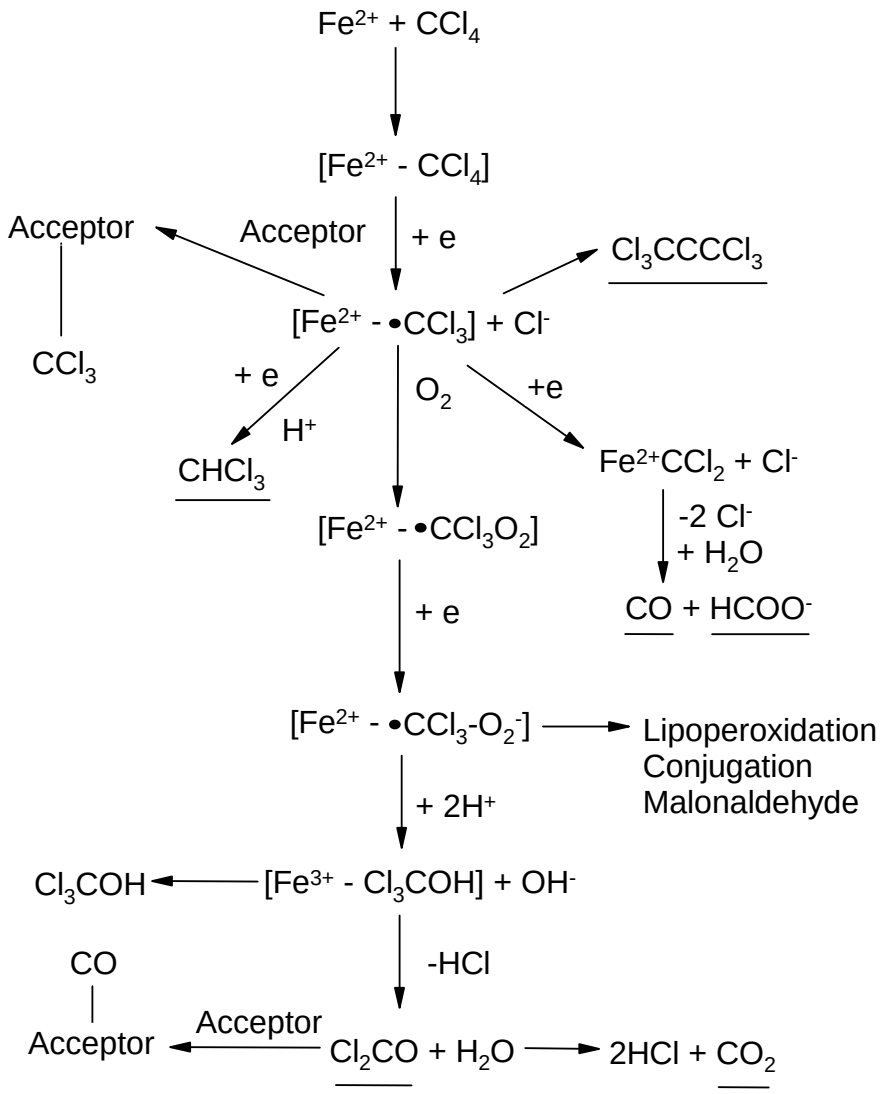
3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to carbon tetrachloride.

3.4.3 Metabolism

The metabolism of carbon tetrachloride in humans has not been investigated, but a great deal of information is available from studies in animals. Pathways of carbon tetrachloride metabolism are illustrated in Figure 3-3, and metabolites that have been identified are underlined. Bioactivation of carbon tetrachloride proceeds by cytochrome P-450-dependent reductive dehalogenation (Sipes et al. 1977). Ethanol inducible CYP2E1 is the primary enzyme responsible for metabolizing carbon tetrachloride in humans at environmentally relevant concentrations, but others, particularly CYP3A, are also involved at higher concentrations (Castillo et al. 1992; Zangar et al. 2000). Studies with CYP2E1 genetic knockout mice *(cyp2e1$^{-/-}$)* demonstrated that hepatic toxicity of carbon tetrachloride in mice is entirely dependent on CYP2E1 (Wong et al. 1998). A large body of experimental data indicates that the first step involves homolytic cleavage of one carbon chlorine bond in carbon tetrachloride to yield chloride ion and the trichloromethyl radical (Lai et al. 1979; Poyer et al. 1978). Anerobically, the

Figure 3-3. Pathways of Carbon Tetrachloride Metabolism*



*Adapted from Shah et al. 1979. Fe2+ and Fe3+ denote the reduced and oxidized forms of cytochrome P-450, and brackets indicate an enzyme substrate complex. Electrons are donated from NADPH or NADH.

trichloromethyl radical may undergo several reactions, including (1) direct binding to microsomal lipids and proteins (Ansari et al. 1982; Gordis 1969; Rao and Recknagel 1969; Villarruel et al. 1977); (2) addition of a proton and an electron to form chloroform (Glende et al. 1976; Uehleke et al. 1973); (3) dimerization to form hexachloroethane (Fowler 1969; Uehleke et al. 1973); and (4) further reductive dechlorination to form carbon monoxide (Wolf et al. 1977). Aerobically, trichloromethyl radical (CCl_3•) may be trapped by oxygen to form trichloromethylperoxy radical (CCl_3OO•), which decomposes to phosgene ($COCl_2$) (Pohl et al. 1984). Hydrolytic cleavage of phosgene is likely the major pathway by which carbon dioxide is formed from carbon tetrachloride (Shah et al. 1979). The trichloromethylperoxy radical is more reactive than the trichloromethyl radical towards amino acids (Packer et al. 1978).

Metabolism of carbon tetrachloride by CYP2E1 may result in the destruction of the enzyme during the metabolic process (Noguchi et al. 1982a, 1982b) CYP2E1 may be lost by either a direct attack (i.e., covalent binding) of radicals on the cytochrome(s) (Manno et al. 1992; Vittozzi and Nastainczyk 1987), or highly localized lipid peroxidation resulting in detachment of P-450 proteins from the microsomal membranes. Cytochrome P-450 mediated homolytic cleavage of the carbon-chlorine bond in carbon tetrachloride is thought to be followed by hydrogen abstraction by the trichloromethyl radical at a methylene group of polyenic fatty acids in the microsomal lipids, thus forming organic free radicals. These organic free radicals then rapidly react with molecular oxygen, leading to the formation of organic peroxy free radicals and eventually organic peroxides (Rao and Recknagel 1969; Recknagel 1967; Recknagel and Glende 1973; Recknagel et al. 1977). The unstable organic peroxides cleave homolytically to form new free radicals, which attack methylene groups of neighboring polyenic lipids in the membrane. This autocatalytic process occurs very rapidly; hepatic microsomal lipid peroxidation is more than half of its maximum value at 5 minutes, and is complete within 15 minutes after oral administration of carbon tetrachloride to fasted rats (Rao and Recknagel 1968). Lipid peroxidation can contribute to breakdown of membrane structure and loss of organelle and cell functions. Connor et al. (1986) conducted a study in which they detected the trichloromethyl radical and a second free radical, the carbon dioxide anion radical, by electron spin resonance spectroscopy in liver perfusate and in urine of female rats. Adducts of both radicals have also been detected in blood of male rats (Reinke and Janzen 1991).

Cytochrome P-450 from rat or human liver microsome preparations is inactivated when incubated anaerobically with carbon tetrachloride in the presence of NADPH and an oxygen-scavenging system (Manno et al. 1988, 1992). Inactivation involved destruction of the heme tetrapyrrolic structure, and followed pseudo first-order kinetics with fast and slow half-lives of 4.0 and 29.8 minutes. When

compared with rat liver microsomes, the human preparations were 6–7 times faster at metabolizing carbon tetrachloride and only about one-eighth as susceptible to self-destructing ("suicidal") inactivation (about 1 enzyme molecule lost for every 196 carbon tetrachloride molecules metabolized).

The rate of carbon tetrachloride metabolism *in vivo* has been estimated primarily by indirect methods. Male rats were exposed to carbon tetrachloride vapor in a desiccator jar with a recirculating atmosphere. The decline in the chamber concentration was monitored over time as the index of carbon tetrachloride uptake into the animals (Gargas et al. 1986). The shapes of the uptake curves were a function of tissue partition coefficients and the metabolism of carbon tetrachloride. The uptake kinetics of carbon tetrachloride were accurately described by a physiological pharmacokinetic model with a single, saturable metabolic pathway. The maximum rate of reaction (Vmax) was calculated to be 0.14 mg/hour (0.62 mg/kg/hour), while the half-maximum rate concentration of carbon tetrachloride (K_m, the Michaelis-Menten constant) was calculated to be 1.62 µM (0.25 mg/L). Carbon tetrachloride was metabolized more slowly than other halocarbons studied (methyl chloroform, 1,1-dichloroethylene, bromochloromethane). Another indirect method was evaluated for estimating the rate of carbon tetrachloride metabolism in male rats, based on arterial blood:inhaled air concentration ratios (Uemitsu 1986). Results of this study suggest that carbon tetrachloride metabolism was limited by the rate of blood perfusion of the liver at concentrations below 100 ppm, and was saturated at concentrations above 100 ppm. The estimated Vmax was 2.8 mg/kg/hour. The rate of metabolism gradually decreased during the exposure period, apparently the result of carbon tetrachloride-induced loss of cytochrome P-450.

Based on comparative PBPK modeling, which incorporated *in vivo* gas uptake data and *in vitro* data, Thrall et al. (2000) calculated that the rates of metabolism (V_{max}/K_m) by milligrams of liver protein differed across species, with hamster > mouse > rat > human. The human *in vivo* metabolic rates for carbon tetrachloride were estimated as 1.49 mg/hour/kg body weight (V_{max}) and 0.25 mg/L for K_m.

The extent of metabolism of ^{14}C-carbon tetrachloride in rats was assessed by measuring the amounts of unchanged carbon tetrachloride, carbon dioxide, and chloroform exhaled in the breath, ^{14}C-metabolite excreted in urine and feces, and ^{14}C-metabolite bound to liver macromolecules within a 24-hour period post oral dosing (Reynolds et al. 1984). The major metabolite in this study was carbon dioxide at all dose levels, ranging from 85% of total metabolites recovered at 15 mg/kg to 63% at 4,000 mg/kg. The modest 22% (from 85 down to 63%) reduction in carbon dioxide production when the dose was increased 28-fold (15 versus 4,000 mg/kg) suggests that excess amounts of P-450 are available in the liver for metabolism of carbon tetrachloride. Intermediate amounts of nonvolatile ^{14}C-labeled material were recovered from

the urine and feces, although none of the metabolites were identified by these investigators. About 2–4% of the label was found covalently bound to liver macromolecules. The relative amount of chloroform formed depended on dose, with chloroform being the least abundant metabolite formed at the lowest dose, but the second most abundant metabolite at the highest dose. As the dose of carbon tetrachloride increased, the fraction of the dose recovered decreased for each metabolite except chloroform. A major change in the overall extent of carbon tetrachloride metabolism occurred as the dose was increased from 15 to 46 mg/kg, the nature of which suggests that the oxidative metabolism of carbon tetrachloride was saturated and/or impaired by destruction of cytochrome P-450 in this dosage range. The fraction recovered in the expired air as unchanged carbon tetrachloride increased from 20 to 80% of the administered dose, and the peak carbon tetrachloride exhalation rate increased 40-fold. Thus, this study indicated that when oxidative metabolism of carbon tetrachloride was saturated or inhibited, more of the parent chemical was exhaled and increased amounts of chloroform were formed by a reductive pathway. Low levels of carbon tetrachloride metabolism to CO_2 were also indicated by other studies showing that 6 hours after intraperitoneal injection of 128–159 mg/kg carbon tetrachloride to rats or gerbils, <1% (approximately 0.2% for rats, and 0.7% for gerbils) of the dose had been expired as CO_2, while approximately 80–90% had been expired as unchanged carbon tetrachloride (Cai and Mehendale 1990; Mehendale and Klingensmith 1988; Young and Mehendale 1989).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Little quantitative information was located regarding the amount or fraction of absorbed carbon tetrachloride that is subsequently excreted in air, urine, or feces in humans exposed by inhalation. Studies of the rate of excretion of carbon tetrachloride in the expired air indicate that samples taken immediately after exposure may be contaminated with environmental carbon tetrachloride, but realistic values may be obtained by 15 minutes (Stewart et al. 1961). Among volunteers who breathed 10–49 ppm carbon tetrachloride for 1–3 hours, expired air concentrations in the range of 1–3 ppm were detectable within 15-25 minutes, steadily declining to about 0.3 ppm 5 hours after exposure (Stewart et al. 1961). No carbon tetrachloride was detected in blood or urine of the subjects where the limit of detection was 5 ppm. In subjects who inhaled 2.5 mg radiochlorinated carbon tetrachloride vapor in a single breath (subsequently held for 20 seconds to maximize absorption), 33% of the administered dose was exhaled within 1 hour (Morgan et al. 1970). The urinary excretion rate during the first hour was less than 0.01% of the administered dose per minute.

Studies in animals indicate about 30–40% of an inhaled dose of carbon tetrachloride is excreted in expired air and about 32–62% is excreted in feces (McCollister et al. 1951; Paustenbach et al. 1986a). Relatively low amounts are excreted in urine. Nearly all of the material in expired air is parent carbon tetrachloride, with only small amounts of carbon dioxide. The identity of the nonvolatile metabolites in feces and urine was not determined.

During the 48 hours following nose-only inhalation exposure to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, rats, mice and hamsters eliminated 65–83% of the initial body burden of ^{14}C activity as CO_2 or volatile organic compounds in exhaled breath (Benson et al. 2001). Elimination in expired air was described as a single-order negative exponential function. Elimination half-times for carbon tetrachloride equivalents (CEs) in exhaled breath were 4.3, 0.8, and 3.6 hours for volatile organic compounds and 7.4, 8.8, and 5.3 hours for CO_2 for rats, mice, and hamsters, respectively. The fraction of the initial body burden of CEs eliminated in urine and feces combined was <10% in rats and >20% in mice and hamsters. Clearance of CEs from various tissues was characterized as being best described by single- or two-component negative exponential functions (Benson et al. 2001). Clearance of CEs from the blood was complete within 48 hours and was described by a single-component function for all three species. The half-life for clearance ($T_{1/2}$) from blood was shortest for rats (1.8 hours) and longest for hamsters (23 hours). Clearance of CEs from the lung was also described by a single-component function for all three species, but was only about 80% complete after 48 hours; the $T_{1/2}$ ranged from 7 hours for rats to 17 hours for mice. Clearance of CEs from the liver in hamsters was complete and best described by a single-component function; the $T_{1/2}$ was 33 hours. In rats and mice, clearance from the liver was best described by a two-component function; a large fraction was cleared with a $T_{1/2}$ of 3 hours and the remainder cleared with a $T_{1/2}$ of 35 hours. Clearance of CEs from the kidney in rats was complete and best described by a two-component function. In mice and hamsters, the $T_{1/2}$ for clearance from the kidney for the largest fraction (70–80%) of carbon tetrachloride was <10 hours, but no additional clearance occurred up to 48 hours.

As in humans, the rate of carbon tetrachloride excretion in rats appears to be biphasic, with an initial half-life value of 7–10 hours (Paustenbach et al. 1986a). The rapid phase was judged to reflect clearance from blood, while the slower phase was related to clearance from fatty tissue and metabolic turnover of covalent adducts (Paustenbach et al. 1988). In support of this, exposure for longer periods of time led to higher concentrations of carbon tetrachloride in fat and a decreased rate of clearance (Paustenbach et al. 1986a, 1986b, 1988).

3.4.4.2 Oral Exposure

The concentration of carbon tetrachloride was measured in the expired air of a person who swallowed a large amount of carbon tetrachloride (Stewart et al. 1963). Excretion in expired air was found to decrease exponentially in a biphasic or multiphasic fashion, but no quantitative estimate of the elimination half-life of carbon tetrachloride or of the fraction of the dose excreted by this pathway was provided. Visual inspection of their graphed data suggests very approximate half-lives of less than several hours initially, 40 hours (75–150 hours post exposure), and 85 hours (300–400 hours post exposure).

A detailed investigation of carbon tetrachloride excretion was performed in rats exposed by gavage to a range of doses (Reynolds et al. 1984). At doses of 50 mg/kg or higher, most of the dose (70–90%) was recovered in expired air as unchanged carbon tetrachloride. Lower amounts were recovered as expired carbon dioxide or chloroform, or as nonvolatile metabolites in feces or urine. As would be expected for a saturable or self-destructing metabolic system, the proportion of each dose recovered as metabolites tended to decrease as the dose increased. For example, 12% of the lowest dose (15 mg/kg) was recovered as carbon dioxide, while only 0.7% of the highest dose (4,000 mg/kg) was recovered as carbon dioxide. The time-course of excretion also depended on dose, tending to become slower as doses increased. For example, the half-life for exhalation of carbon tetrachloride was 1.3 hours at a dose of 50 mg/kg, but was 6.3 hours at a dose of 4,000 mg/kg. This is consistent with the concept that an increased proportion of a dose enters fat as the dose level increases, with clearance from fat being slower than from blood and other tissues. Increased hepatotoxicity in the form of greater cytochrome P-450 destruction (and thus reduced carbon tetrachloride metabolism) may also be a significant factor. Studies evaluating the rate of excretion over the first 12 hours described a one-compartment model, but did not deduce that a two-compartment model was inappropriate (Reynolds et al. 1984). Approximately 24 hours after receiving an oral dose of 3,985 mg/kg, rats were observed to excrete elevated levels of various lipid peroxidation products (formaldehyde, acetaldehyde, malondialdehyde, and acetone) in their urine, presumably as a result of carbon tetrachloride-induced oxidative stress (Shara et al. 1992).

3.4.4.3 Dermal Exposure

Carbon tetrachloride was rapidly excreted in expired air of volunteers who immersed their thumbs in liquid carbon tetrachloride (Stewart and Dodd 1964). The half-life of expiration was about 30 minutes,

but no quantitative estimate of the fraction of the absorbed dose excreted in air was performed. No studies were located regarding excretion in animals after dermal exposure to carbon tetrachloride.

3.4.4.4 Other Routes of Exposure

After what was described as either intragastric or intraduodenal administration of carbon tetrachloride to rats under various conditions, evidence from electron paramagnetic resonance experiments using phenyl-N-t-butyl nitrone as a spin trap suggested that trichloromethyl free-radical adducts are secreted into the bile without being concentrated, and in concentrations which reflect those concurrently found in the liver (Knecht and Mason 1991). Expressed in arbitrary concentration units, spin-trap-bound adduct quantities found in the liver, in the bile, and liver/bile concentration ratios under the various experimental conditions were as follows: carbon tetrachloride alone (93, 28, 3.4 ratio), carbon tetrachloride plus hypoxia (161, 50, 3.2 ratio), carbon tetrachloride with phenobarbital pretreatment (118, 69, 1.7 ratio), and carbon tetrachloride with intravascular infusion of the bile salt dehydrocholate to double the bile flow rate (85, 13, 6.8 ratio). Taken together, these results from conditions that vary bile flow or reductive metabolic generation of free radical seem to indicate that carbon tetrachloride free-radical adducts are secreted rather than merely diffused into bile, and in amounts proportional to their generation in the liver. The drop in liver/bile ratio observed with phenobarbital pretreatment (from 3.4 to 1.7) was attributed to the liver's phenobarbital-enhanced ability to destroy many of the induced free-radical adducts. These results are supported by findings in bile duct-cannulated rats and in perfused rat liver systems, where spin-trapped free-radical adducts were observed in bile, but not in blood or urine (Hughes et al. 1991).

As noted above, within 6 hours of intraperitoneally injecting rats or gerbils with 128–159 mg/kg of carbon tetrachloride, 80–90% of the administered dose was expired as unchanged carbon tetrachloride, while less than 1% was expired as CO_2 (Cai and Mehendale 1990; Mehendale and Klingensmith 1988; Young and Mehendale 1989). After rats were injected intraperitoneally with 3 mL carbon tetrachloride per kg body weight, volatile carbonyl compounds released into expired air over 24 hours were evaluated by gas chromatography (Dennis et al. 1993). Injected rats exhaled significantly higher levels of acetone and a compound tentatively identified as formyl chloride than control rats; the amounts of acetaldehyde and formaldehyde were not significantly different in the two groups.

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

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

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for

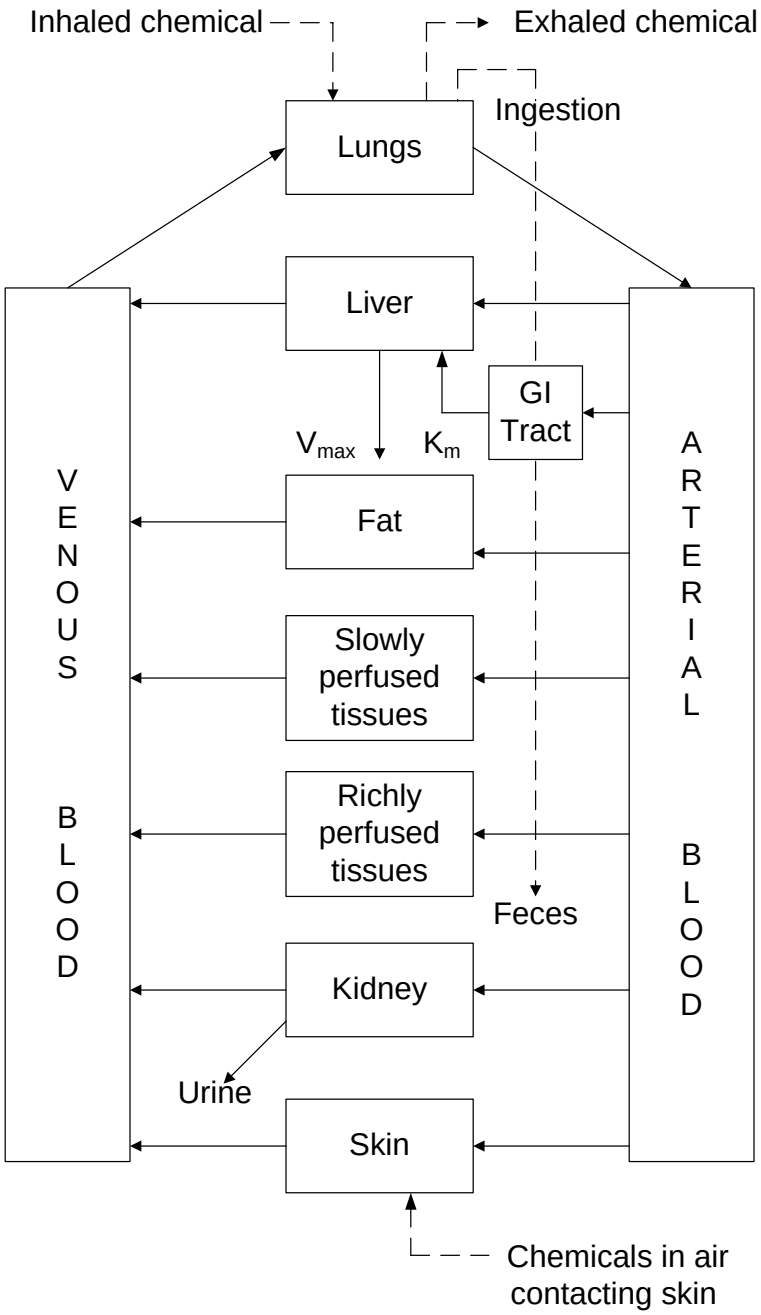
many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for carbon tetrachloride exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

A detailed physiologically based pharmacokinetic model (Figure 3-5) has been developed that describes the metabolism of carbon tetrachloride following inhalation exposure (Paustenbach et al. 1988). The model was based on and validated against a previous study in rats in which 1–2 weeks of inhalation exposure to 100 ppm ^{14}C-labeled carbon tetrachloride for 8–11.5 hours/day, 4–5 days/week apparently resulted in 40–60% of the absorbed dose being metabolized (Paustenbach et al. 1986a). The model incorporated partition characteristics of carbon tetrachloride (blood:air and tissue:blood partition coefficients), anatomical and physiological parameters of the test species (body weight, organ weights, ventilation rates, blood flows), and biochemical constants (V_{max} and K_m) for carbon tetrachloride metabolism. Rat and human parameters used in the model are listed in Table 3-7. The model accurately predicted the behavior of carbon tetrachloride and its metabolites, both the exhaled unmetabolized parent compound and $^{14}CO_2$ and the elimination of radioactivity in urine and feces. In agreement with other studies (Gargas et al. 1986; Uemitsu 1986), Paustenbach et al. (1988) found that metabolism was best described as a single saturable pathway, with a V_{max} of 0.65 mg/kg/hour and a K_m of 0.25 mg/L. Metabolites were partitioned in the model to three compartments: the amounts to be excreted in the breath (as $^{14}CO_2$), urine, and feces. Of total carbon tetrachloride metabolites, 6.5% was excreted as CO_2, 9.5% was excreted in urine, and 84.0% was excreted in feces. Based on this model, the authors estimated that about 4% of initially metabolized carbon tetrachloride is converted directly to carbon dioxide and is promptly excreted, while the remainder forms adducts with proteins and other cellular molecules. These adducts are then degraded with a half-life of about 24 hours, and the products are excreted mainly in the

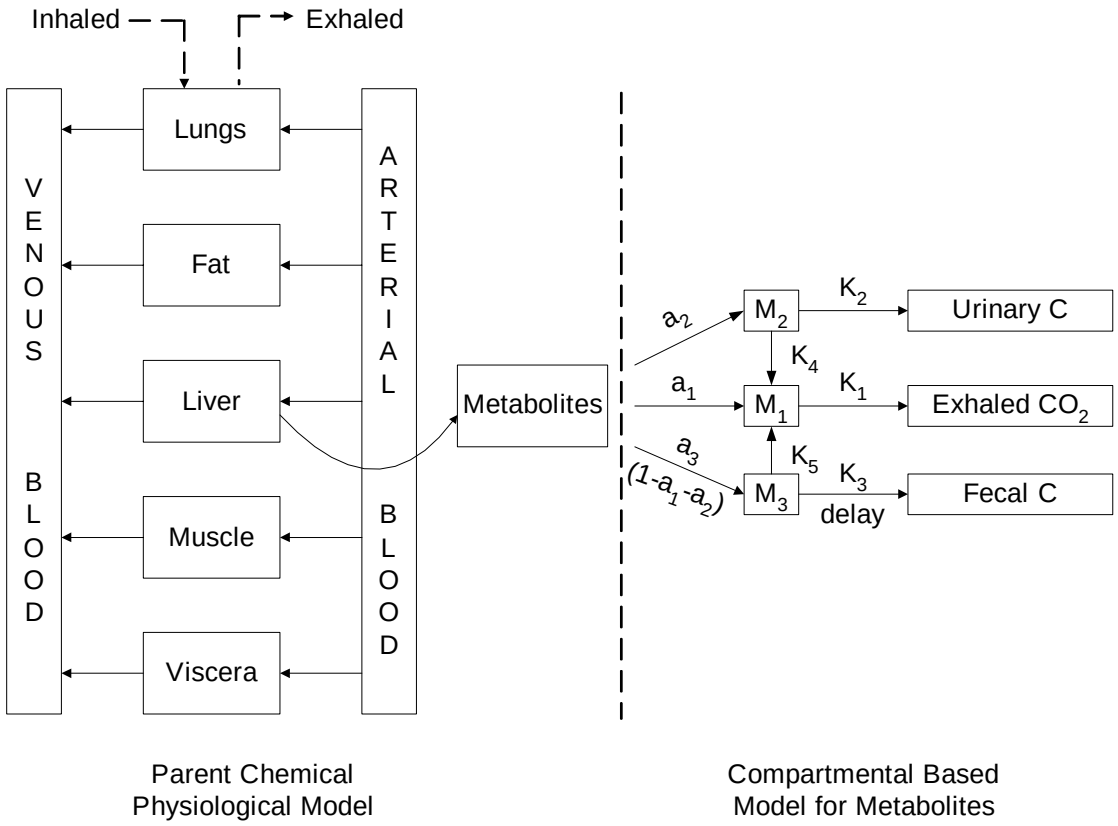
Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Figure 3-5. Physiologically Based Pharmacokinetic Model for Inhaled Carbon Tetrachloride*



*Adapted from Paustenbach et al. 1988

Table 3-7. Parameters in PBPK Models for Carbon tetrachloride

Parameter	Rat	Human
Physiological parameters (as per Paustenbach et al. 1988)		
Body weight (kg)	0.42	70
Percentages of body weight		
Liver	4	4[a]
Fat	8	10
Rapidly perfused	5	5
Slowly perfused	74	62
Flows (L/hour)		
Cardiac output (QC)	8.15	348
Alveolar ventilation (QP)	7.91	254
Blood flow (percentages of cardiac output)		
Liver	25	25[a]
Fat	4	6
Rapidly perfused	51	51[a]
Slowly perfused	20	18
Partition coefficients		
Blood:air	4.52	2.64
Liver:blood	3.14[b]	3.14[a]
Fat:blood	79.4	79.4[a]
Rapidly perfused:blood	3.14[c]	3.14[a]
Slowly perfused:blood	1	1[a]
Metabolism		
Vmax (mg/hour)	0.35	12.72[d]
Km (mg/L)	0.25	0.25[a]

[a]Human value set equal to rat value
[b]Gargas et al. 1986
[c]Rapidly perfused blood: set equivalent to liver blood
[d]Human value scaled up using allometric equation $V_{max} = V_{maxc} \times (\text{body weight})^{0.7}$ using V_{maxc} for rat = (maximum rate of metabolism of 0.65 mg/hour-kg body weight) and human body weight of 70 kg

urine and feces, with small amounts eliminated as carbon dioxide. The amount of carbon tetrachloride metabolized is limited by the saturable enzyme system, with high exposures (e.g., 100 ppm) leading to saturation within a short time. Following cessation of exposure, considerable metabolism may occur as carbon tetrachloride emerges from fatty tissue. The model successfully described elimination using a V_{max} of 0.65 mg/kg/hour and a K_m of 0.25 mg/L. The model was scaled up to predict the expected behavior of carbon tetrachloride in monkeys and humans. The results were consistent with data collected by McCollister et al. (1951) and Stewart et al. (1961). The earlier study by Paustenbach et al. (1986) showed that rats did not have significant day-to-day accumulations in the blood or fat following repeated exposure to 100 ppm for 8 or 11.5 hours/day; this was accurately described in the model. In contrast, humans exposed to 5 ppm for 8 hours/day would be expected to show day-to-day increases in fat because of physiological differences.

Thrall et al. (2000) adapted the model of Paustenbach et al. (1988) to compare the metabolism of carbon tetrachloride in male rats, mice, and hamsters exposed to 40–1,800 ppm in a recirculating closed-chamber gas-uptake system. For each species, an optimal fit of the uptake curves was obtained by adjusting the metabolic constants V_{max} (capacity) and K_m (affinity) using the model. The mouse had a slightly higher capacity and lower affinity for metabolizing carbon tetrachloride than the rat, whereas the hamster had a higher capacity and lower affinity than either the rat or mouse. A comparison of V_{max}/K_m normalized for milligrams of liver protein (L/hour/mg) indicated that hamsters metabolize more carbon tetrachloride than rats or mice. The species comparisons were evaluated against toxicokinetic studies conducted in animals exposed by nose-only inhalation to 20 ppm ^{14}C-labeled carbon tetrachloride for four hours. Rats eliminated a lower fraction of the dose as metabolites and more as parent compound compared to mice or hamsters. The use of the model was expanded to include *in vitro* constants using liver microsomes from rat, mouse, hamster, and human in order to estimate *in vivo* metabolic rates for humans: a V_{max} of 1.49 mg/hour/kg body weight and a K_m of 0.25 mg/L. Normalizing the rate of metabolism (V_{max}/K_m), the rate of metabolism differed across species, with hamster > mouse > rat > human.

Yoshida et al. (1999) estimated rates of absorption of carbon tetrachloride and three trihalomethanes in low-level inhalation exposures by rats using a pharmacokinetic analysis. A three-compartment model, consisting of a tank with barium chloride to trap the chemical, the exposure chamber, and the rat, was employed for carbon tetrachloride, which was injected into the chamber. The model estimated that the amounts of carbon tetrachloride metabolized by rats in µmol/hour/kg were 0.000053, 0.0053, and 0.53 for exposures at 1 ppb, 10 ppb, and 10 ppm, respectively.

Semino et al. (1997) adapted the model of Paustenbach et al. (1988) to develop a PBPK model to describe the oral uptake of carbon tetrachloride administered to male Fischer 344 rats in corn oil or 0.25% Emulphor, an aqueous vehicle. The gastrointestinal model used a series of subcompartments with an absorption constant (K_a, L/hour), a bioavailability term (A, unitless), and a compartment emptying time (T, hours). The model was optimized by varying the values of the constants for the experimental data. Higher values of K_a and A were needed to fit data from aqueous gavage compared to that for corn oil. The model provided precise fits of multipeak blood and exhaled breath carbon tetrachloride concentration-time profiles. A pulsatile pattern noted following corn oil gavage was attributed to discontinuous emptying of the stomach into the small intestine. Initial absorption of the bolus occurs rapidly in the stomach, especially for aqueous vehicles; subsequently, stomach absorption slows and uptake from the small intestine determines the absorption profile.

Gallo et al. (1993) developed a PBPK model for blood concentration of carbon tetrachloride in rats following intravenous delivery in aqueous polyethylene glycol 400. Subsequently, absorption input functions were added to the model to describe blood concentration profiles resulting from administration of 25 mg carbon tetrachloride per kg body weight alone, in aqueous vehicles (water or 0.25% Emulphor emulsion), or in corn oil. Absorption was 91.9% for administration in water, 85.4% in Emulphor, 62.8% for the pure compound, and 93.1% for administration in corn oil. A pulsatile pattern was obtained for absorption in corn oil.

Andersen et al. (1996) developed a pharmacokinetic model to calculate the concentration of carbon tetrachloride in microsomal suspensions from male Fischer 344 rats under anerobic conditions. Dose-response curves revealed a nonlinear, biphasis appearance of trichloromethane. One experiment compared microsomes from fasted or unfasted rats; fasting did not alter the shape of the dose-response curve, but increased the production of trichloromethane in microsomes.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. As a small volatile haloalkane, carbon tetrachloride diffuses passively across cell membranes, leading to rapid absorption from the lungs and gastrointestinal tract into the circulatory system (Sanzgiri et al. 1995, 1997). Pulmonary absorption is ventilation limited.

Distribution. Being somewhat lipophilic, absorbed carbon tetrachloride diffuses from the blood to the liver, kidney, brain, and other organs and accumulates in adipose tissue. Following absorption by the gastrointestinal tract, a first-pass effect is apparent through the liver, where carbon tetrachloride is biotransformed and adducts are formed from reactive metabolites binding to cell macromolecules. Clearance of unmetabolized carbon tetrachloride is limited by passive diffusion; the rate of clearance is slowest for adipose tissue compared to internal organs (Benson et al. 2001; Sanzgiri et al. 1997). Quantitative differences between maximal tissue concentrations of carbon tetrachloride in the kidney or liver occur following administration of equivalent absorbed doses (179 mg/kg) by inhalation, oral bolus dosing, or gradual gastric infusion (Sanzgiri et al. 1997). Inhalation exposure resulted in similar values for kidney (25 µg/g) and liver (20 µg/g). Delivery of carbon tetrachloride as a single bolus can exceed first-pass hepatic and pulmonary elimination, resulting in higher blood levels and more severe hepatic injury compared to gradual delivery of the same dose over a longer period of time (Sanzgiri et al. 1997); bolus delivery resulted in a maximal tissue concentration for the kidney (14 µg/g) that is only 24% of the hepatic value (58 µg/g). Gastric infusion of the equivalent dose over 2 hours resulted in much lower tissue concentrations, with the kidney value (4 µg/g) 8 times higher than the value for the liver (0.5 µg/g). These results suggest that gradual oral intakes, such as might occur from contaminated drinking water, might result in less hepatotoxicity than equivalent exposure by bolus dosing or inhalation.

Metabolism. Carbon tetrachloride is primarily metabolized in tissues that express CYP2E1. The metabolic pathways are described in detail in Section 3.4.3 and depicted in Figure 3-3.

Excretion. In humans and animals, carbon tetrachloride is eliminated by passive diffusion primarily through exhaled breath, with a smaller fraction eliminated in urine and feces (Benson et al. 2001; Thrall et al. 2000).

3.5.2 Mechanisms of Toxicity

Unmetabolized carbon tetrachloride, as a volatile halogenated alkane, depresses the central nervous system. All other toxic effects of carbon tetrachloride are related to its biotransformation catalyzed by cytochrome P-450 dependent monooxygenase, specifically CYP2E1 (Azri et al. 1991; Hughes et al. 1991; Lindros et al. 1990; Raucy et al. 1993; Wong et al. 1998; Zangar et al. 2000). The liver and kidney (especially in humans) are especially vulnerable because of the abundance of CYP2E1, which is also present in the respiratory and nervous systems, and various isoforms of CYP3A (Haehner et al. 1996; Koch et al. 2002; Martin et al. 2003; Warrington et al. 2004; Wauthier et al. 2004). Considerable data

are available for hepatic toxicity, but similar cellular damage would be expected in other tissues with a high abundance of CYP2E1. There is considerable evidence that hepatic injury produced by carbon tetrachloride is mediated by two major processes resulting from bioactivation in the endoplasmic reticulum and mitochondria of centrilobular hepatocytes, which have the highest concentration of CYP2E1 (Buhler et al. 1992; Raucy et al. 1993): haloalkylation of cellular macromolecules by reactive metabolites such as trichloromethyl free radical or trichloromethyl peroxyl free radical (Link et al 1984; Mico and Pohl 1983; Poyer et al. 1980; Recknagel et al. 1977; Slater et al. 1986) and lipid peroxidation, which impairs cellular functions dependent on membrane integrity (Benedetti et al. 1982; Comporti 1985; Lee et al. 1982; Slater 1981; Slater and Sawyer 1970; Tribble et al. 1987; Weber et al. 2003). Both haloalkylation and lipid peroxidation contribute to loss of cellular functions and subsequent cell death as discussed in greater detail in the following paragraphs. In response to parenchymal cell damage, perisinusoidal cells may be stimulated to release extracellular matrix proteins (type-I collagen) that contribute to hepatic fibrogenesis, which is largely mediated by hepatic macrophages (Kupffer cells) (Belyaev et al. 1992; Ishiki et al. 1992; Johnson et al. 1992; Luckey and Petersen 2001; Muriel and Escobar 2003). Kupffer cells activated by carbon tetrachloride release tumor necrosis factor-alpha (TNF-alpha), nitric oxide, transforming growth factor-beta (TGF-beta) (Date et al. 1998), and interleukins (IL)-1, -6, and -10 (Weber et al. 2003). TNF-alpha elicits an inflammatory response and may generate aptoptosis or contribute to the development of steatosis in heptocytes (Morio et al. 2000); however, impaired secretion of lipoproteins, possibly because of lipid peroxidation, has also been proposed as a mechanism for steatosis (Boll et al. 2001c). TNF-alpha may also stimulate genes involved in hepatic mitogenesis (Bruccoleri et al. 1997). TNF-alpha is also responsible for the activity of hepatocyte nuclear factor 1 (HNF-1) in down-regulating genes for organic anion transporters (*Ntcp*, *Oatp1*, and *Oatp2*) that operate in the basolateral domain of parencymal cells following carbon tetrachloride treatment (Geier et al. 2003). Nitric oxide modulates intra-hepatic vascular tone in normal rats (Loureiro-Silva et al. 2003) and generally protects against apoptopic tissue damage (Muriel 1998), but it can also react with the O_2^- radical (formed during carbon tetrachloride-induced oxidative stress) to form an aggressive peroxynitrite radical, resulting in more severe hepatic injury (Morio et al. 2001; Weber et al. 2003). Inhibition of Kupffer cell activation with gadolinium prevented hepatic lipid peroxidation and histopathology produced by carbon tetrachloride (Muriel et al. 2001). Interleukin-6 pathways that use the signal transducer gp130 have been proposed as protective against the progression of carbon tetrachloride-induced fibrosis (Streetz et al. 2003). As shown by the results of experiments with IL-10 knockout mice, IL-10 reduces neutrophilic infiltration (inflammation) following injury with carbon tetrachloride and limits the proliferative response of hepatocytes and the development of fibrosis during the recovery phase (Louis et al. 1998). TGF-beta1 promotes hepatic fibrogenesis following carbon tetrachloride treatment, as shown

by the inhibition of fibrosis by peptides that block the cytokine's Type III receptor (Ezquerro et al. 2003). Lipid peroxidation may be at least partially independent of cytochrome P-450, as iron-dependent peroxidation occurred in cultured mammalian cells even in the presence of P-450 inhibitors (Dickens 1991). While carbon tetrachloride-induced liver damage was mitigated by treatment with allopurinol, an inhibitor of xanthine oxidase (a free radical-generating enzyme), prolonged administration of the free radical scavenger superoxide dismutase actually aggravated hepatocellular damage (Dashti et al. 1992). Some inflammatory processes in the liver following carbon tetrachloride treatment appear to be mediated by the activation of prothrombin by the prothrombinase complex (Mizuguchi et al. 2002). In acute injury from carbon tetrachloride, hydrolytic enzymes such as calpain are released from dying hepatocytes and are activated by the higher calcium levels in the extracellular space (Limaye et al. 2003). As a result, the activated proteases begin to hydrolyze proteins in neighboring cells, leading to a progression of the lesion. Necrotic responses in the liver to carbon tetrachloride are apparently mediated by the Cdk inhibitor p21, as shown by an absence of necrosis in p21-knockout mice treated with the chemical (Kwon et al. 2003).

Overall responses of different organs to carbon tetrachloride will be mediated, in part, by variations in gene expression patterns. DNA array studies in rodents demonstrated slightly different and dose-related patterns of gene expression/repression in the liver and kidney; activated genes involved heat shock proteins, oxidative stress, and DNA damage responses (Bartosiewicz et al. 2001; Fountoulakis et al. 2002; Jessen et al. 2003; Kier et al. 2004). In human hepatoma G2 cells, treatment with carbon tetrachloride increased expression and activity of interleukin-8 (IL-8), which *in vivo* directs the migration of neutrophils, resulting in release of reactive oxygen species (Holden et al. 2000). Injection of carbon tetrachloride into rats activated the expression of c-fos and c-jun within 30 minutes and also increased in hepatic nuclei the levels of the transcription factor NF-kB, which regulates transcription related to inflammation, apoptosis, and regenerative processes (Gruebele et al. 1996). Suppression of CYP3A1 and activation of multiple drug resistance gene1 (MDR1) were observed in livers of Sprague-Dawley rats following acute exposure (Kier et al. 2004). Gene expression changes associated with fibrosis (see below) may persist for weeks after cessation of treatment (Jiang et al. 2004).

Hepatic microsomal lipid peroxidation damages cellular functions by disturbing the integrity and hence the function of membranes and by covalent binding of reactive intermediates. The trichloromethyl radical is sufficiently reactive to bind covalently to CYP2E1, a process sometimes referred to as the "suicidal inactivation" of CYP2E1 (De Groot and Haas, 1981; Fernandez et al. 1982; Fujii 1997; Manno et al. 1988, 1992). It is also possible that reactive intermediates formed during the process of lipid peroxidation contribute to the loss of CYP2E1, but some *in vitro* studies have indicated that carbon tetrachloride-

induced lipid peroxidation is not required for the inactivation of CYP2E1 (Dai and Cederbaum 1995; De Groot and Haas 1980). Nevertheless, it is still not clear how these initial events are related to subsequent triglyceride accumulation, polyribosomal disaggregation, depression of protein synthesis, cell membrane breakdown and eventual death of the hepatocytes. Carbon tetrachloride can inhibit triglyceride secretion from hepatocytes in the absence of lipid peroxidation, and polyribosomal dissociation and decreased protein synthesis can occur when no ^{14}C-labelled carbon tetrachloride has been incorporated into ribosomal fractions (Waller et al. 1983). When rats were pretreated with a chemical that reduced lipid peroxidation by 85%, only small recoveries from carbon tetrachloride-induced decreases in hepatocellular viability, cytochrome P-450 content, aniline hydroxylase activity, and carbon tetrachloride metabolism capacities were observed (Kostyuk and Potapovich 1991). This suggests that free radical binding to critical cellular macromolecules (e.g., microsomal oxidation system enzymes) may be more critical for these effects than lipid peroxidation. On the other hand, inhalation exposure to carbon tetrachloride produced a direct correlation between lipid peroxidation and proline hydroxylase (a collagen biosynthetic enzyme) in rats, and dietary zinc supplementation was associated with decreases in lipid peroxidation, collagen deposition, and proline hydroxylase activity, together with an increase in collagenase activity (Camps et al. 1992). Carbon tetrachloride-induced lipid peroxidation apparently requires the presence of Fe^{2+} ions, as demonstrated by the inhibitory effect of the iron-chelating agent deferrioxamine on lipid peroxidation and hepatotoxicity in rats (Younes and Siegers 1985). One product of lipid peroxidation, 4-hydroxynonenal, has been shown to act as a pro-fibrogenic stimulus following acute hepatic injury from carbon tetrachloride (Zamara et al. 2004).

Intrinsic tissue levels of antioxidants such as glutathione influence the degree to which oxidative damage progresses following exposure to carbon tetrachloride. In 11 selected human cells types, steady-state levels of oxidative DNA base modifications (e.g., 8-hydroxyguanine) were inversely proportional to intrinsic glutathione levels (Will et al. 1999). An age-related decline in the activity of nuclear factor erythroid2-related factor (Nrf2), the factor regulating the transcription of gamma-glutamylcysteine ligase (GGCL), is the ultimate cause of the age-related decline in hepatic glutathione levels in rats (Suh et al. 2004). The hepatic activity of GGCL, which synthesizes gamma-glutamylcysteine, a precursor to glutathione, is 54.8% lower in old (24-month) rats compared to young (3-month) rats, resulting in a 35% decline in glutathione content in older rats. Agents such as buthionine sulphoximine, which inhibit GGCL, also deplete glutathione levels (Edgren and Revesz 1987). Conversely, S-adenosylmethionine (SAM), which is required for the synthesis of precursors to glutathione (homocysteine and cysteine), is also depleted by liver injury and its loss is exacerbated by the concomitant inactivation of SAM synthetase (Gasso et al. 1996). Exogenous administration of SAM or cysteine reduced carbon

tetrachloride-induced liver injury by the increase in glutathione levels (De Ferreyra et al. 1974; Gasso et al. 1996). Reduced glutathione levels concomitant with renal histopathology have also been demonstrated in the kidney of rats injected with carbon tetrachloride (Dogukan et al. 2003; Ozturk et al. 2003).

Another factor that may be of importance in carbon tetrachloride-induced hepatotoxicity is the perturbation of normal cellular calcium homeostasis following exposure. A number of studies have reported data that suggest carbon tetrachloride exposure inhibits the capacity of the hepatocyte endoplasmic reticulum or microsomal fraction to sequester (or keep sequestered) calcium, under either *in vivo* (Kodavanti et al. 1993; Long and Moore 1986a; Long et al. 1989; Lowrey et al. 1981b; Moore 1980; Moore et al. 1976) or *in vitro* (Long and Moore 1987; Long et al. 1989; Lowrey et al. 1981a; Srivastava et al. 1990; Waller et al. 1983) exposure conditions. This inhibition of sequestration capacity is considered to be a key contributor to the rise in cytosolic calcium concentration that is generally observed following carbon tetrachloride exposure (e.g., Kodavanti et al. 1990b, 1993; Long and Moore 1987), and that is postulated to play a central role in the induced cytotoxicity. The suppression of calcium uptake by microsomes occurred in the liver, (but not the kidney) of rats receiving a single oral dose of 2,500 mg/kg carbon tetrachloride (Moore et al. 1976). While some *in vivo* (Long and Moore 1986a) and *in vitro* (Srivastava et al. 1990) data suggest that carbon tetrachloride intoxication actually promotes the release of calcium to the cytosol from the endoplasmic reticulum or microsomes, other *in vivo* studies with carbon tetrachloride alone (Yamamoto 1990b) or in conjunction with chlordecone (Agarwal and Mehendale 1984a, 1984b, 1986) indicate that microsomal calcium content in fact rises, though generally to a lesser extent than cytosolic or total calcium content. Such microsomal increases presumably occur despite diminished calcium sequestration capacity. In isolated hepatocytes, immediate (<1 minute) alterations in calcium sequestation following treatment have been attributed to the solvent effect of unmetabolized carbon tetrachloride (Hemmings et al. 2002). It should be noted that another *in vitro* study found that membrane effects (membrane fusion) only occurred at concentrations that are unlikely to be achieved during inhalation exposure, but might occur following bolus gavage dosing at high levels (Johnston and Kroening 1998).

Studies have indicated that increased intracellular calcium may mediate cytotoxicity by activating phospholipase A2 (Chiarpotto et al. 1990; Glende and Recknagel 1991, 1992; Simon et al. 1986; van den Bosch et al. 1990), which might contribute to irreversible plasma membrane damage. Lipid damage from phospholipase A2 may result from increased lipid hydrolysis and from the initiation of the arachidonic acid cascade that generates toxic prostanoids (Basu 2003; Glende and Pushpendran 1986). Elevated

phospholipase A2 activity has been detected in the renal cortex and medulla of rats with carbon tetrachloride/phenobarbital-induced hepatic cirrhosis (Niederberger et al. 1998). Elevated intracellular calcium may also be associated with elevated levels of phosphorylase and altered intracellular levels and distribution of calmodulin (Kodavanti et al. 1990), but was reported not to result in any DNA degradation—a potential result of calcium-activation of endonuclease activity (Long et al. 1989).

The finding that carbon tetrachloride is converted to reactive metabolites that bind to nuclear protein, lipids, and DNA may be relevant to the understanding of carbon tetrachloride carcinogenicity. Binding of radiolabel to liver cytoplasmic and nuclear proteins was found in Wistar rats and Swiss mice dosed with ^{14}C-carbon tetrachloride (Rocchi et al. 1973). Pretreatment of the animals with 3-methylcholanthrene (an inducer of cytochrome P-450 IA [P-448]) resulted in ^{14}C binding to hepatic DNA of mice, but not rats. Similarly, Diaz Gomez and Castro (1980a) found significantly greater ^{14}C binding to the liver DNA of A/J mice than to that of Sprague-Dawley rats given a tracer dose of ^{14}C-carbon tetrachloride. A/J mice are among the most susceptible of strains tested with respect to liver tumor induction by carbon tetrachloride. Administration of a high dose (3,200 mg/kg) of ^{14}C-carbon tetrachloride, having the same total radioactivity as the tracer dose, resulted in much more intensive binding to hepatic DNA. Presumably, the fewer reactive metabolites formed from the tracer dose react primarily with microsomal lipids and proteins in close proximity to their formation. With the higher dose, more ^{14}C-carbon tetrachloride can apparently reach the nucleus and be metabolically activated there, subsequently reacting with nuclear lipids, proteins, and DNA. This scenario receives support from the finding that highly purified rat liver nuclear preparations were able to anaerobically activate ^{14}C-carbon tetrachloride in the presence of an NADPH generating system (Diaz Gomez and Castro 1980b). Under microsome-mediated aerobic conditions, it was observed that ^{14}C-carbon tetrachloride bound more to histone than to nonhistone chromosomal proteins from livers of B6C3F$_1$ mice (Oruambo and Van Duuren 1987). These findings may be relevant to the understanding of carbon tetrachloride hepatocarcinogenicity, since reactive metabolites of carbon tetrachloride appear capable of binding to targets of putative relevance to cancer induction (chromosomal DNA and nucleosome proteins), and may even be generated within the nucleus itself. Since lipid peroxidation products such as malonaldehyde also have the ability to form adducts with DNA (Chaudhary et al. 1994; Chung et al. 2001; Wacker et al. 2001), it is possible that the genotoxic effect of carbon tetrachloride is partly indirect. Malonaldehyde-initiated tumors have been reported in Swiss mice (Shamberger et al. 1974). It is also worth noting that data from a variety of congenic mouse strains suggest that both the toxicity of, and recovery from, carbon tetrachloride exposure are under genetic control (an Ah gene, and H-2 genes) (Bhathal et al. 1983; Biesel et al. 1984).

Results of chronic bioassays in rats and mice exposed orally or by inhalation indicate that hepatocellular carcinomas are induced at hepatotoxic doses, suggesting that there may be a threshold for carcinogenicity of carbon tetrachloride (Japan Bioassay Research Centre 1998; NCI 1976a, 1976b, 1977). The results of these chronic rodent studies are consistent with the idea that hepatic carcinogenicity directly related to the increase in cellular replication that occurs in response to hepatocyte lethality. Enhanced cellular replication increases the possibility that unrepaired DNA errors will become fixed mutations, possibly resulting in an initiated preneoplastic cell. Exposures at levels lower than those eliciting hepatic regeneration would not be expected to result in hepatic carcinogenicity.

Interesting data from other studies illustrate that the hepatotoxic effects of carbon tetrachloride (or carbon tetrachloride plus chlordecone) depend not merely on its metabolic activation, but also to a substantial degree on the liver's hepatocellular regenerative capacity (e.g., Mehendale 1990, 1991, 1992). For example, the auto protection conferred by a low nontoxic dose of carbon tetrachloride against the toxic effects of a subsequent high dose seem not to be completely accounted for by mere destruction of cytochrome P-450 activation capacity, but appear also to involve the early (2–6 hours after pretreatment) stimulation of hepatocellular regeneration (Rao and Mehendale 1991; Thakore and Mehendale 1991). This early, low-dose stimulation, which leads to much greater hepatocellular regenerative activity (DNA-synthesis and mitosis) following the high-dose exposure, and the autoprotection phenomenon are both inhibited by a colchicine-induced mitotic block (Rao and Mehendale 1991, 1993). It has been hypothesized that the low dose of carbon tetrachloride and/or the resulting minimal injury induces hepatocytes into the cell cycle from an arrested G_2 state (Calabrese et al. 1993). Further, partial hepatectomy in rats has been shown to confer resistance to carbon tetrachloride-induced hepatotoxicity, presumably via enhanced regenerative capacity, as hepatic uptake and metabolism of carbon tetrachloride was not significantly altered (Young and Mehendale 1989). The particular sensitivity of gerbils to carbon tetrachloride-induced hepatotoxicity appeared related not only to extensive bioactivation, but also to a sluggish hepatocellular regenerative and tissue repair response, and was mitigated by partial hepatectomy that stimulated this response in the absence of any significant effect on carbon tetrachloride bioactivation or induced lipid peroxidation (Cai and Mehendale 1990, 1991a, 1991b). Finally, in rats, pretreatment with nontoxic levels of chlordecone has been shown to substantially potentiate the hepatotoxicity of low doses of carbon tetrachloride without affecting its hepatic metabolism to a similarly significant degree, whereas phenobarbital pretreatment induced greater bioactivation, but less hepatotoxicity (Mehendale and Klingensmith 1988; Young and Mehendale 1989). This chlordecone potentiation phenomenon has been attributed to its inhibitory effect on the level of hepatocellular regeneration and tissue repair normally induced by low-dose carbon tetrachloride, with death resulting from hepatic failure and hepatic

encephalopathy (renal toxicity was not affected) (Kodavanti et al. 1992; Soni and Mehendale 1993). Where chlordecone cannot inhibit this regenerative response, as in cultured rat hepatocytes (Mehendale et al. 1991) or gerbils (Cai and Mehendale 1990), it does not potentiate cellular or hepatic toxicity.

The signaling factor leptin apparently plays a role in the initiation of hepatic regenerative processes following acute injury by a sublethal dose of carbon tetrachloride (Leclercq et al. 2003). Hepatic changes occurring in mice treated with carbon tetrachloride (in temporal order) include induction of nuclear factor kappaB (NF-kB) and interleukin-6 (IL-6) at the time of G1/S transition, increased DNA binding by STAT3 (signal transducer and activator of transcription), induction of cyclin D1 expression consistent with an increase in mitosis, and a time-dependent increase in tumor necrosis factor (TNF) consistent with the appearance of necrosis. NF-kB also regulates genes involved in inflammation, apoptosis, proliferation, and regeneration in the liver (Gruebele et al. 1996). Mice (*ob/ob)* transgenic for the loss of expression of leptin and treated with carbon tetrachloride had an exaggerated expression of STAT3 and NF-kB, impaired activation of TNF and IL-6 release, failure of induction of cyclin D1, and reduced hepatocyte proliferation. Exogenous leptin restored the regenerative capacity of *ob/ob* mice. Hepatic regeneration following acute injury by carbon tetrachloride is mediated by the type 1 TNF receptor, but not the type 2 receptor, in mice (Yamada and Fausto 1998).

Fibrotic processes in the liver following carbon tetrachloride treatment are modulated by the adipocytokine adiponectin (Kamada et al. 2003). Repeated (twice weekly) intraperitoneal dosing with 1,594 mg/kg but not 478 mg/kg carbon tetrachloride significantly reduced plasma concentrations of adiponectin. Although showing the same degree of initial hepatic injury (serum ALT, inflammation) as wild type mice following injection with carbon tetrachloride, adiponectin-knockout mice showed more extensive liver fibrosis (hydroxyoproline content) and enhanced expression of TGF-beta1 and connective tissue growth factor (CTGF) compared to wild type mice. Replacement dosing with adiponectin reduced hepatic fibrosis. The effect of adiponectin on cultured hepatic stellate cells stimulated with platelet-derived growth factor-BB (PDGF-BB) was to inhibit proliferation and migration and counteract the TGF-beta1-induced activation of TGF-beta1 and CTGF genes by interfering with the nuclear translocation of Smad2. Overall, a reduction of adiponectin levels following carbon tetrachloride treatment would be expected to foster fibrotic processes in the liver.

Telomere shortening resulting in chromosomal instability has been associated with hepatocellular carcinoma in humans, with loss of regenerative capacity in chronic liver injury. Intraperitoneal injection of carbon tetrachloride into wild type mice increased the initiation of hepatic foci and the development of

hepatocellular carcinoma (Farazi et al. 2003). The number and size of hepatic nodules were significantly lower in treated mice that were transgenic for aberrant telomerase. The authors suggest that telomere dysfunction impedes the progression to malignancy. Estradiol protected against telomere shortening, fibrosis, and senescence in hepatocyes of rats injected intramuscularly with carbon tetrachloride (Sato et al. 2004). The authors attributed the protective effect of estradiol on its transactivation of the telomerase gene.

3.5.3 Animal-to-Human Extrapolations

Patterns of toxicity and metabolism of carbon tetrachloride in laboratory animals are very similar in humans and animals. In both, similar effects are observed in the major target organs, the liver and kidney, as well as in the nervous system during acute inhalation exposures. There are some minor species differences in metabolic parameters following exposure to carbon tetrachloride. Benson et al. (2001) reported that the fraction of carbon tetrachloride (equivalents following inhalation of radiolabeled carbon tetrachloride) partitioning to the liver after a 4-hour inhalation exposure was higher in hamsters and mice than in rats, which show an immediate accumulation in fat. . Rats eliminated less radioactivity associated with metabolism and more associated with the parent compound in exhaled air than mice or hamsters. Thrall et al. (2000) estimated that humans at low inhalation concentrations metabolized less of the dose than rats, and would be less sensitive than rats at equivalent exposures; the rate of metabolism was highest in mice, followed by rat, and then humans. In humans, rats, and mice, CYP2E1 is the major enzyme responsible for bioactivation of carbon tetrachloride; thus, similar effects of reactive metabolites could be expected in rodents and humans.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in

1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is no reported direct effect of carbon tetrachloride on hormones in humans or animals. Fertility was reduced in an inhalation bioassay in rats, but it is not known whether the cause was hormonal disruption or a necrotic effect on the gonads (Smyth et al. 1936). Testicular degeneration, possibly resulting from necrosis, was observed in rats exposed by inhalation (Adams et al. 1952). Adrenal pheochromocytomas were induced in mice exposed to carbon tetrachloride vapor for 2 years (Japan Bioassay Research Center 1998). It is possible that catecholamine balances were affected in these animals (Landsberg and Young 1998).

It is possible that the loss of hepatic function caused by carbon tetrachloride could indirectly impair hormone metabolic processes that are regulated by the liver. Functions that could be affected by reduced liver function include inactivation of some hormones (e.g., insulin and glucagon) by proteolysis or deamination, deiodination of thyroxine and triiodothyronine, inactivation of steroid hormones (e.g., glucocorticoids and aldosterone) followed by glucuronidation, metabolism of testosterone to 17-ketosteroids and sulfonation, conversion of estrogens to estriol and estrone followed by conjugation to glucuronic acid or sulfate, and removal of circulating vasoactive substances such as epinephrine and

bradykinin (Podolsky and Isselbacher 1998). In humans, chronic liver disease not caused by carbon tetrachloride is known to result in signs of hormonal imbalance such as testicular atrophy (Podolsky and Isselbacher 1998). The development of ascites in chronic liver disease may be facilitated by the elevated levels of epinephrine (Podolsky and Isselbacher 1998).

No *in vitro* studies were located regarding endocrine disruption of carbon tetrachloride.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many

xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

One epidemiological study reported associations between maternal exposure to carbon tetrachloride at levels higher than 1 ppb in drinking water and adverse developmental outcomes (low full-term birth weight and small for gestational age) in humans (Bove et al. 1992a, 1992b, 1995). However, the same effects were associated with exposure to trihalomethanes, which occurred at higher concentrations in drinking water. Associations between exposure and incidences of central nervous system defects, cleft-lip or cleft-palate, or heart conotruncal defects were not statistically significant (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997). In general, exposure to other chemicals by the study population raises uncertainty as to the causative role of carbon tetrachloride in the observed adverse developmental effects. Animal studies did not report adverse developmental effects in the absence of maternal toxicity. No teratogenic effects (morphological anomalies) were observed in rats exposed to carbon tetrachloride by inhalation (Gilman 1971; Schwetz et al. 1974) or in rats or mice exposed by ingestion (Hamlin et al. 1993; Wilson 1954). Complete litter loss occurred in some rats given oral doses that produced clear maternal toxicity (Narotsky et al. 1997a, 1997b; Wilson 1954). It is not known whether litter loss is the result of toxicity to the fetus or to the placenta, but the critical site of injury is likely related to the abundance of cytochrome proteins that metabolize carbon tetrachloride.

An *in vitro* fertilization assay in mice reported significant adverse effects on fertilization at concentrations ≥1 mM (154 µg/mL), but not ≤0.5 mM (77 µg/mL) (Hamlin et al. 1993). These levels are significantly higher than those encountered by the general population (see Section 6.5). An assay in mice intraperitoneally injected with carbon tetrachloride found no increase in sperm head abnormalities at doses as high as 3,180 mg/kg (Topham 1990).

Fetal tissues and the placenta appear to have the capacity for bioactivating carbon tetrachloride, although the levels of cytochrome enzymes are lower than in neonates or adults (EPA 2001). Total fetal liver CYP content is a relatively constant 30% of the adult level from the end of the first trimester of gestation up to 1 year of age (EPA 2001). mRNA for CYP2E1 has been detected in human first-trimester placentas (Hakkola et al. 1996). Low levels of CYP2E1 protein have been detected in human fetal brain as early as gestational day 46, substantially increasing around day 50 (Boutelet-Bochan et al. 1997; Brzezinski et al. 1999). In the fetal liver, CYP2E1 protein was not detectable at 10 weeks of gestation, but was present at 16 weeks (Carpenter et al. 1996). Therefore, it would appear that there is a period early in gestation during which the fetal brain might be more vulnerable than the liver to the effects of carbon tetrachloride. However, no developmental studies are available that specifically examined neurological or neurobehavioral effects of exposure to carbon tetrachloride during gestation. Additionally, there is some evidence that maternal alcohol consumption induces placental CYP2E1 in humans (Rasheed et al. 1997b). If maternal alcohol exposure also increases levels of CYP2E1 in fetal tissues, the likelihood of fetal injury from exposure to carbon tetrachloride would be increased. Induction of fetal hepatic CYP2E1 by maternal ethanol consumption has been confirmed in rats (Carpenter et al. 1997). Transcription of the CYP2E1 gene in human placenta and fetal lung and kidney is regulated in part by hypermethylation of dinucleotide CG residues within the promoter (Viera et al. 1998).

Hepatic levels of CYP2E1 mRNA increase significantly during the first 24 hours after birth, largely resulting from demethylation that allows transcription to proceed (Viera et al. 1996). Major accumulations of CYP2E1 occur between 1 and 3 months of age and values comparable to those of adults are achieved sometime between 1 and 10 years of age (EPA 2001; Viera et al. 1996). Thus, children exposed to carbon tetrachloride would be expected to experience similar effects as in adults.

Fisher et al. (1997) have calculated that maternal exposure to carbon tetrachloride is likely to result in its transfer to breast milk, which would be a possible means of exposure for nursing infants.

Effects of metabolism of carbon tetrachloride on late (day 20) rat fetal hepatic microsomes have been measured *in vitro* (Cambron-Gros 1986). Fetal microsomes had the ability to metabolize the compound as evidenced by inhibition of cytochrome P-450 (to a greater degree than maternal microsomes), inhibition of calcium uptake (similar to maternal microsomes), and the increased amount of carbon tetrachloride bound to protein (less than maternal microsomes). The production of trichloromethyl radicals by fetal microsomes did not induce the membrane phospholipid peroxidation observed with maternal microsomes. The absence of lipid peroxidation in fetal liver would be expected to result in a qualitatively different pattern of hepatic toxicity following exposure to carbon tetrachloride compared to adults. The authors suggest that necrotic effects would be less in fetuses than in adults. The basis for the lack of lipid peroxidation by fetal microsomes was not determined in that study.

3.8 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 tetrachloride are discussed in Section 3.8.1.

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 tetrachloride are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations that are Unusually Susceptible.”

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Carbon Tetrachloride

Measurement of parent carbon tetrachloride and its metabolites in expired air has been the most convenient way to determine exposure. Levels of 9.5 ppm carbon tetrachloride were detected in expired air of one worker who had been exposed to carbon tetrachloride vapors for several minutes (Stewart et al. 1965). In another case, expired air levels were over 2,000 ppm in a person exposed by ingestion to a pint of carbon tetrachloride mixed with methanol (Stewart et al. 1963). Levels fell below 2 ppm after 16 days. Depending on dose and length and route of exposure, the half-life of carbon tetrachloride in expired air initially appears to range from 1 to several hours, later lengthening to 40–>85 hours. Measurement of carbon tetrachloride in blood has also been used as an indicator of exposure.

Covalent adducts between reactive carbon tetrachloride metabolites (trichloromethyl radical) and cellular protein, lipids, and nucleic acids are known to occur. Although measurements of such adducts may provide data on past exposure, the method's overall usefulness in assessing exposure in the general population is severely limited since it requires the use of radiolabeled carbon tetrachloride. Further, metabolite compounds and their adducts may originate in ways other than from carbon tetrachloride, or they may undergo reduction and thus require some reoxidation procedure prior to being detectable by *in vivo* spin trapping techniques (Sentjure and Mason 1992).

3.8.2 Biomarkers Used to Characterize Effects Caused by Carbon Tetrachloride

As discussed in Section 3.2, the effects that are most often observed in humans exposed to carbon tetrachloride are liver and kidney injury and central nervous system depression. Exposure levels leading to these effects in humans are not well-defined. The threshold for central nervous system effects following exposures of 8 hours or more is probably in the range of 20–50 ppm (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960). On the other hand, kidney and liver effects can occur following exposure (15 minutes to 3 hours) to vapor concentrations of 200 and 250 ppm, respectively (Barnes and Jones 1967; Norwood et al. 1950). These exposures correspond to an absorbed dose of approximately 100–200 mg/kg.

Detection of liver injury has commonly been associated with alterations in serum levels of certain hepatic enzymes and proteins. Elevation in bilirubin levels following exposure (Barnes and Jones 1967) has been detected in humans, as have decreased serum levels of secreted liver proteins (e.g., albumin and fibrinogen) (Ashe and Sailer 1942; McGuire 1932; New et al. 1962; Norwood et al. 1950; Straus 1954). Elevations in serum levels of enzymes (alkaline phosphatase and gamma-glutamyltransferase) released from damaged hepatocytes have been reported in occupational exposures above 1 ppm lasting months to years (Tomenson et al. 1995). Similar enzyme elevations were observed following acute-, intermediate-, and chronic-duration exposures to carbon tetrachloride in animals (Bruckner et al. 1986; Hayes et al. 1986; Japan Bioassay Research Center 1998; Sakata et al. 1987). Typically, ALT, AST, alkaline phosphatase, and LDH have been monitored, but these are also produced in nonhepatic tissues. Ikemoto et al. (2001) investigated serum levels of several urea-cycle enzymes that are more exclusively found in the liver: liver-type arginase (ARG), ornithine carbamoyltransferase (OCT), and arginosuccinate synthase (AS). After rats were injected with carbon tetrachloride, serum ARG levels were immediately elevated at the first 15-minute timepoint and within 30 minutes, were about 45-fold higher than normal; after 300 minutes, the increase in serum ARG levels had not reached a plateau. All other enzymes (AST, ALT, OCT, and AS) measured had maximally 10-fold increases. The authors propose that ARG is a sensitive biomarker for acute exposure to carbon tetrachloride and attribute its pattern of appearance in serum to the fact that it is a cytosolic enzyme (having only the plasma membrane as a barrier to the extracellular compartment) and to its smaller molecular mass compared to the other enzyme biomarkers.

Yamaguchi et al. (2002) proposed that serum concentrations of regulcalcin, a Ca^{2+}-binding protein that is especially abundant in the liver but not abundant in the kidney, heart, or brain of rats, would be a sensitive measure of hepatitis following exposure to carbon tetrachloride. In rats that received 5 doses of

15,940 mg/kg at 3-day intervals, standard serum markers for AST and ALT were significantly elevated compared to controls in samples taken during the first week of exposure, but not later, whereas significant elevations in serum regucalcin were detectable both during the first week and, at lower levels, 18 and 30 days after the first exposure.

In rats acutely treated with 1,275 mg/kg carbon tetrachloride, histological evaluations of the liver showed peak scores for necrosis at 24–36 hours and for inflammation at 48 hours, with resolution largely evident by 60 hours and 4 days, respectively (Giffen et al. 2003). Detection of serum markers for necrosis and inflammation demonstrated a pattern consistent with the histological results. Serum markers of hepatic damage (AST, ALT, glutamate dehydrogenase) showed peak elevations at 36 hours and a subsequent decline. Serum haptoglobin, a marker for inflammation, peaked at 48 hours (400% over control), and after declining, was significantly elevated at 4 days but not later. The authors suggest that haptoglobin would be a sensitive marker for hepatic inflammation, since that protein is primarily synthesized by centrilobular hepatocytes, which are vulnerable to injury by carbon tetrachloride.

In the rat, carbon tetrachloride-induced liver cytolysis has been associated with elevated serum activities of glutamate dehydrogenase, sorbitol dehydrogenase, and glucose-6-phosphatase (microsomal glucose-6-phosphatase activity was decreased) (Brondeau et al. 1991, 1993), while serum procollagen III peptide was demonstrated to be a valuable indicator of liver fibrogenesis, and serum prolidase was shown to be a limited signal of accelerated liver collagen metabolism (Jiang et al. 1992). Serum immunoassay for the 7S fragment of type IV collagen may be an even more sensitive indicator of hepatic fibrosis in man (Ala-Kokko et al. 1992). Another sensitive (but nonspecific) indicator of liver injury is the serum levels of individual bile acids (Bai et al. 1992). Lipid peroxidation, increased erythrocyte membrane cholesterol/phospholipid ratio, and decreased erythrocyte ATPase activity were all associated with the onset of carbon tetrachloride-induced liver cirrhosis (Mourelle and Franco 1991). Also, lipid peroxidation accompanying carbon tetrachloride-induced hepatotoxicity has been monitored by quantitating hepatic levels of hydroperoxy- and hydroxy-eicosatetraenoic acids (Guido et al. 1993).

Renal injury has been associated with acute exposure of humans to carbon tetrachloride. Impaired renal function as evidenced by oliguria and anuria have been reported (Barnes and Jones 1967; Norwood et al. 1950). Proteinuria, hemoglobinuria, and glycosuria have also been reported in other cases involving acute exposure of humans to the compound (Forbes 1944; Guild et al. 1958; New et al. 1962; Smetana 1939; Umiker and Pearce 1953). Although acute renal failure induced in rats by carbon tetrachloride apparently did not involve activation of the circulating active renin-angiotensin system, increased

prorenin levels were associated with decreased renal function (Cruz et al. 1993). Long-term inhalation exposure to carbon tetrachloide increased the incidence and severity of chronic nephropathy in the rat, and significantly increased proteinuria levels and serum biomarkers such as blood nitrogen (Japan Bioassay Research Center 1998). These renal effects can occur following exposure to chemicals other than carbon tetrachloride.

Neurotoxicity, as evidenced by central nervous system depression, has been associated with acute exposure to carbon tetrachloride in humans. Clinical signs and symptoms that may be monitored include headache, dizziness, fatigue, and coma (Cohen 1957; Stevens and Forster 1953; Stewart et al. 1961). Impaired visual functions have also been observed (Johnstone 1948; Smyth et al. 1936; Wirtschafter 1933). It should be noted that central nervous system effects disappear rapidly as carbon tetrachloride is eliminated from the body. Therefore, they will be detectable for only relatively short periods after exposure. The neural effects are not specific to carbon tetrachloride exposure and may occur following exposure to other chemicals.

Lipid peroxidation products appearing in urine following exposure to carbon tetrachloride offer the possibility of noninvasive monitoring for hepatic damage (de Zwart et al. 1998). As measured by gas chromatography, the urinary levels in rats of the following lipid peroxidation products showed statistically significant increases over normal values within 12 hours of an intraperitoneal injection with 0.5 or 1.0 mL/kg carbon tetrachloride: formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, and malondialdehyde (MDA). The 0.25 mL/kg dose elicited significant increases only in acetaldehyde. The level of MDA returned to normal after 48 hours, at which time the levels of the other chemicals remained elevated. The same study found that neither coproporphyrin III nor 8-hydroxy-2'-deoxy-guanosine were suitable urinary biomarkers for exposure to carbon tetrachloride.

Metabonomics is a new technology combining high resolution nuclear magnetic resonance (NMR) and pattern recognition technology that is starting to be applied to the evaluation of *in vivo* toxicology. Robertson et al. (2000) treated rats with single intraperitoneal or oral doses of carbon tetrachloride and evaluated the changes in NMR spectra of urine as displayed by principal component analysis (PCA), a statistical method that reduces multidimensional data to a two- or three-dimensional pattern. The PCA pattern was most altered compared to the pretreatment state on the first and second days after treatment, but had returned to normal within 10 days. PCA patterns were detectable in rats treated with 0.5 mg/kg, but not in rats treated with 0.1 mg/kg.

Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

There is substantial evidence that the toxicity of carbon tetrachloride is dramatically increased by alcohols, ketones and a variety of other chemicals. Many of these might be found at hazardous waste sites also containing carbon tetrachloride. Although the precise mechanisms for this marked potentiation are not always known, it is likely that most potentiators act, at least in part, by increasing the metabolic activation of carbon tetrachloride to its toxic intermediates and metabolites, thus increasing the induced injury. Other agents may affect the toxic outcome by altering cellular regenerative and tissue repair capacities. The extent to which either or both of these mechanisms are involved in the interaction will substantially affect the relationships among induced injury, duration of toxic damage, and animal survival. Interactions with agents enhancing lipid peroxidation would be expected to increase the severity of cell injury due to increased permeability of cell membranes.

Ethanol. Alcohol (ethanol) ingestion has often been associated with potentiation of carbon tetrachloride-induced hepatic and renal injury in humans (Manno et al. 1996). In two cases in which men cleaned furniture and draperies with carbon tetrachloride, one man, a heavy drinker, became ill and died, whereas his coworker, a nondrinker, suffered a headache and nausea, but recovered quickly after breathing fresh air (Smetana 1939). Both men were subjected to the same carbon tetrachloride exposure, as they had been working in the same room for the same amount of time. In 19 cases of acute renal failure due to carbon tetrachloride inhalation or ingestion, 17 of 19 patients had been drinking alcoholic beverages at about the time of their carbon tetrachloride exposure (New et al. 1962). Many other cases of carbon tetrachloride-induced hepatic and/or renal injury associated with ethanol ingestion have been described in the medical literature (Durden and Chipman 1967; Guild et al. 1958; Jennings 1955; Lamson et al. 1928; Markham 1967; Tracey and Sherlock 1968). These clinical reports establish that occasional or frequent ingestion of alcoholic beverages can increase the danger from exposure to carbon tetrachloride at levels that otherwise do not result in significant toxicity. As ethanol is known to induce microsomal mixed-function oxidase activity in man (Rubin and Lieber 1968), the mechanism of potentiation may involve ethanol-induced enhancement of the metabolic activation of carbon tetrachloride.

Numerous studies in animals confirm that ethanol is a strong potentiator of carbon tetrachloride-induced hepatotoxicity (Ikatsu et al. 1991; Kniepert et al. 1991; Reinke et al. 1988; Sato and Nakajima 1985; Strubelt 1984; Teschke et al. 1984; Wang et al. 1997a). Ethanol administration 16–18 hours before carbon tetrachloride exposure potentiated hepatotoxicity (Cornish and Adefuin 1966; Towner et al. 1991); however, enhancement was less when ethanol was given 2 hours before carbon tetrachloride (Cornish and Adefuin 1966). This is consistent with the idea that ethanol increases carbon tetrachloride toxicity by inducing the synthesis of one or more enzymes, such as cytochrome P-450 2E1 (Castillo et al. 1992), that are involved in the metabolic activation of carbon tetrachloride; or by acting as a competitive inhibitor of carbon tetrachloride metabolism during concurrent exposure. Thus, the precise timing of exposure to each agent is likely to critically influence the observed effects. For example, a single dose of ethanol 18 hours prior to intraperitoneal administration of 1,275 mg/kg carbon tetrachloride in rats did not increase either trichloromethyl free-radical adducts or p-nitrophenol hydroxylase activity, whereas 2 weeks of dietary exposure to ethanol significantly increased the generation of trichloromethyl radicals (Reinke et al. 1988, 1992). Threshold levels also appear involved, as 14 days of 0.05–0.5 mL/kg/day ethanol did not result in a statistically significant increase in any effects of a subtoxic 20 mg/kg/day dose of carbon tetrachloride (Berman et al. 1992). Ethanol exposure intensified carbon tetrachloride toxicity in pregnant rats and caused decreased postnatal survival of offspring (Gilman 1971). For the most part, these studies involved short-term exposures to ethanol. Inhalation studies involving longer-term pretreatment exposures to ethanol (5–10 weeks) prior to carbon tetrachloride exposure raised the possibility of increased susceptibility to chronic liver injury at low doses of carbon tetrachloride that have not been shown to cause significant liver damage (Hall et al. 1990). On the other hand, when ethanol pretreatments increased in duration (30 or 52 weeks), there was a decrease in ethanol potentiation of carbon tetrachloride toxicity (Kniepert et al. 1990). Factors contributing to this diminished potentiation were not determined. It has also been reported that despite substantial potentiation of carbon tetrachloride-induced hepatotoxicity in ethanol pretreated rats, no increase in lethality was observed (Ray and Mehendale 1990). The authors speculated that this result occurred due to the treatment's concomitant stimulation of hepatic regenerative capacity—to a degree sufficient to overcome the induced injury. In addition to enhanced hepatotoxicity pretreatments with ethanol have been reported to enhance certain immunosuppressive effects of carbon tetrachloride (Kaminski et al. 1990).

Other Alcohols and Ketones. Secondary alcohols can also potentiate carbon tetrachloride hepatorenal toxicity in humans. Eighteen workers in an isopropyl alcohol packaging plant became ill after inhalation of carbon tetrachloride (Folland et al. 1976). Four of these people were hospitalized; one with liver injury, one with kidney damage, and the other two with both kidney and liver injury. Air samples taken

at the plant during a subsequent investigation revealed relatively high concentrations of isopropanol and acetone, and these were thought to play a major role in potentiation of toxicity. Potentiation of carbon tetrachloride hepatoxicity in mice by isopropanol far exceeded that caused by an equal dose of ethanol, though both exerted their maximum effect when given 18 hours before carbon tetrachloride (Traiger and Plaa 1971). In rats, isopropanol potentiated hepatic injury caused by carbon tetrachloride, but lethality was not increased because of the augmentation of hepatic tissue repair mechanisms (Rao et al. 1996). Methanol co-treatment in rats potentiated the hepatotoxicity of carbon tetrachloride by inducing CYP2E1 in rat liver (Allis et al. 1996). Methanol was found to be markedly less effective on an equimolar basis than either isopropanol or tertiary-butanol in enhancing carbon tetrachloride-induced hepatotoxicity in rats (Harris and Anders 1980). These differences likely reflect the substantially longer half-lives of the secondary and tertiary compounds (relative to their primary congeners), which makes them more potent and persistent inducers of cytochrome P-450 activities. Methanol, ethanol, isopropanol, or decanol in combination with carbon tetrachloride caused massive liver damage, but failed to increase carbon tetrachloride induced lethality. On the other hand, tert-butanol, pentanol, hexanol, and octanol not only potentiated liver damage when administered prior to carbon tetrachloride, but also significantly increased the lethal effects of carbon tetrachloride (Ray and Mehendale 1990). Thus, potentiated hepatotoxicity, as measured by various endpoints, may not be a very reliable predictor of the eventual survival outcome. Other experiments in rats demonstrated that both isopropanol and acetone (the major metabolite of isopropanol) are apparently responsible for the marked enhancement of carbon tetrachloride hepatotoxicity (Plaa and Traiger 1972). Similarly, the metabolism of 2-butanol to 2-butanone contributed to the marked ability of this alcohol to potentiate carbon tetrachloride hepatotoxicity in rats (Traiger and Bruckner 1976).

Investigations in rats indicate that ketosis, caused either by diabetes or administration of ketones, can potentiate carbon tetrachloride hepatotoxicity. Pre-treatment with methyl isobutyl ketone, acetone, or metyl ethyl ketone increased hepatotoxicity in rats treated with a single dose of carbon tetrachloride, essentially reducing the ED_{50} for carbon tetrachloride by 80, 73, or 89%, respectively (Raymond and Plaa 1995). Hepatotoxicity (fibrosis and cirrhosis) and nephrotoxicity were increased in rats exposed to both acetone and carbon tetrachloride (Charbonneau et al. 1986). Carbon tetrachloride hepatotoxicity increased in diabetic rats (Hanasono et al. 1975), while 1,3-butanediol induced ketosis and potentiated carbon tetrachloride hepatoxicity (Pilon et al. 1986). In both studies, ketosis was a better index for prediction of liver injury than glycemic status. Interestingly, the same specific form of cytochrome P-450 was reported to be induced in rats by chronic ethanol administration (Joly et al. 1977) and by diabetes (Past and Cook 1982). The bulk of available evidence suggests that elevated levels of ketone bodies

induce the enzyme system responsible for biotransformation of carbon tetrachloride to its reactive metabolites (Pilon et al. 1986). Methyl isobutyl ketone significantly increased total levels of cytochrome P-450 in rat liver microsomes (Raymond and Plaa 1995).

Phenobarbital, Metamphetamine, DDT, PBB, Chlordecone. Phenobarbital (PB) has been shown to produce a marked increase in carbon tetrachloride hepatotoxicity in rats and it is widely used to provide experimental animal models of carbon tetrachloride-induced cirrhosis (Abraham et al. 1999; Cornish et al. 1973; Garner and McLean 1969; Hocher et al. 1996; Sundari et al. 1997). This is not surprising, in that cytochrome P-450 PB-B (CYP2B1), the isozyme that can be induced at least 50-fold in rats by PB, participates in the metabolic activation of carbon tetrachloride (Vittozzi and Nastainczyk 1987). Lethal effects of carbon tetrachloride are not potentiated by even large doses of phenobarbital in spite of increased liver injury. Thus, as with the alcohols, manifestations of bioactivation capacity or hepatic injury do not appear to reliably predict the eventual survival outcome. The mechanism underlying this phenomenon appears to be the stimulation of hepatic regeneration and tissue repair. Although the early phase of hepatic regeneration was postponed from 6 to 24 hours, it was greatly increased at 24 and 48 hours. Therefore, in spite of remarkably increased liver injury, the animals are able to overcome injury and survive the potentiated liver toxicity (Kodavanti et al. 1992; Mehendale 1990, 1991, 1992). Some data suggest that the PB-induced P-450 isozyme(s) are more rapidly inactivated by carbon tetrachloride, and that PB pretreatment may alter the target lipids and/or the initiating metabolites involved in lipid peroxidation and diene conjugate formation (Moody 1992). DDT increased the sensitivity of rats to carbon tetrachloride poisoning (McLean and McLean 1966), and mice fed 100 ppm polybrominated biphenyls (PBBs) or 200 ppm polychlorinated biphenyls (PCBs) in their diet for 28 days experienced increased carbon tetrachloride hepatotoxicity (Kluwe et al. 1979). Potentiation of renal dysfunction was also found in the PBB-pretreated mice. All of these compounds are broad-spectrum mixed-function oxidase (MFO) inducers.

Concurrent treatment with methamphetamine at doses between 5 and 15 mg/kg increased hepatotoxicity in rats treated with carbon tetrachloride (Roberts et al. 1994). No potentiation occurred when metamphetamine was administered several hours before or after administration of carbon tetrachloride.

Low dietary doses (10 ppm) of the insecticides chlordecone or mirex (a structural analog of chlordecone) have been demonstrated to potentiate carbon tetrachloride hepatotoxicity. Chlordecone greatly enhanced the hepatotoxicity of carbon tetrachloride in rats, producing cholestasis as well as hepatocellular damage (Curtis et al. 1979). The investigators conclude that there is the likelihood of severe liver damage

resulting from interaction of carbon tetrachloride and chlordecone at exposure levels which may independently be nontoxic. Chlordecone has been reported not to potentiate the renal toxicity in rats (Kodavanti et al. 1992) or neurotoxicity in gerbils (Desaiah et al. 1991) of carbon tetrachloride, so its enhancing effects may be liver-specific. Chlordecone potentiation of carbon tetrachloride hepatotoxicity and lethality appears due to incapacitation of hepatocytes to regenerate and initiate the early phase of tissue repair. The authors also suggest that this is due to a precipitous depletion of cellular ATP that results from increased intracellular accumulation of Ca^{2+}, which in turn leads to a depletion of glycogen (Bell and Mehendale 1987; Mehendale 1990, 1991, 1992; Soni and Mehendale 1993). Mirex pretreatment of carbon tetrachloride-dosed rats was found not to produce cholestasis, but to produce a relatively modest increase in carbon tetrachloride hepatotoxicity (Bell and Mehendale 1985). Pretreatment of carbon tetrachloride-dosed rats with both mirex and chlordecone did not increase hepatotoxicity above that seen with chlordecone alone, indicating that chlordecone influenced susceptibility to carbon tetrachloride in a way independent of that of mirex. As proposed for phenobarbital, the mechanism underlying only limited and low-grade potentiation of carbon tetrachloride by mirex may involve a stimulation of hepatic regeneration and tissue repair that offsets cytochrome P-450 induction (Mehendale 1990, 1991, 1992). A single oral dose of chlordecone enhanced the oxidative metabolism of carbon tetrachloride in rats, but to a lesser degree than PB, which was in inverse relationship to these agents' effects on potentiation of the lethal and hepatotoxic effects of carbon tetrachloride (Mehendale and Klingensmith 1988). The investigators suggested the involvement as of yet unidentified factors, in addition to the modest enhancement of carbon tetrachloride metabolism, in chlordecone's unusually strong potentiating capacity. As discussed above, subsequent studies have suggested that chlordecone potentiates carbon tetrachloride-induced hepatotoxicity by depleting cellular energy stores, and consequently by inhibiting hepatocellular regeneration and liver tissue repair (e.g., Kodavanti et al. 1992; Mehendale 1991, 1992; Soni and Mehendale 1993).

Haloalkanes. Certain haloalkanes and haloalkane-containing mixtures have been demonstrated to potentiate carbon tetrachloride hepatotoxicity. Pretreatment of rats with trichloroethylene (TCE) enhanced carbon tetrachloride-induced hepatotoxicity, and a mixture of nontoxic doses of TCE and carbon tetrachloride elicited moderate to severe liver injury (Pessayre et al. 1982). The researchers believed that the interaction was mediated by TCE itself rather than its metabolites. TCE can also potentiate hepatic damage produced by low (10 ppm) concentrations of carbon tetrachloride in ethanol pretreated rats (Ikatsu and Nakajima 1992). Acetone was a more potent potentiator of carbon tetrachloride hepatotoxicity than was TCE, and acetone pretreatment also enhanced the hepatotoxic response of rats to a TCE-carbon tetrachloride mixture (Charbonneau et al. 1986). The potentiating

action of acetone may involve not only increased metabolic activation of TCE and/or carbon tetrachloride, but also possible alteration of the integrity of organelle membranes. Carbon tetrachloride-induced liver necrosis and lipid peroxidation in the rat have been reported to be potentiated by 1,2-dichloroethane in an interaction that does not involve depletion of reduced liver glutathione, and that is prevented by vitamin E (Aragno et al. 1992; Danni et al. 1992). Dichloromethane potentiated the hepatotoxicity of carbon tetrachloride in rats by increasing the covalent binding of carbon tetrachloride metabolites to hepatic microsomal lipids (Kim 1997). Several anesthetics (isoflurane, enflurane, halothane, and sevoflurane) enhanced the dechlorination of carbon tetrachloride by guinea pig microsomes by stimulating the reduction of cytochrome P-450 (Fujii 1996; Fujii et al. 1996).

Nicotine. Treatment of rats for 10 days with nicotine in drinking water increased liver histopathology (fatty change, necrosis, and dark-cell change) caused by an injection of carbon tetrachloride (Yuen et al. 1995). It was proposed that the increased hepatotoxicity might have resulted from a synergistic effect of the lipid peroxidation induced by both agents. Pregnant rats showed less severe effects than nonpregnant rats, possibly because of the differential hormonal status or differential expression of CYP-450 enzymes.

Carbon Disulfide and Other Alkyl Sulfides. Just as chemicals that serve to stimulate the metabolism of carbon tetrachloride lead to increased toxicity, chemicals that impair carbon tetrachloride metabolism lead to decreased toxicity. Rats dosed with carbon disulfide together with carbon tetrachloride displayed effects on the liver that resembled those due to carbon disulfide alone, rather than those caused by carbon tetrachloride alone (Seawright et al. 1980). This was judged to be due to destruction of the hepatic P-450 metabolizing system by carbon disulfide, such that activation of carbon tetrachloride was much reduced. Similar results have been reported in workers exposed to "80/20" (a mixture of carbon tetrachloride and carbon disulfide used to fumigate grain) (Peters et al. 1987). The neurological effects observed in these individuals resembled those caused by carbon disulfide alone, and there was no evidence of hepatotoxic effects characteristic of carbon tetrachloride exposure.

Other sulfides administered as pretreatments had different effects on carbon tetrachloride hepatotoxicity as measured by plasma ALT levels (Kim et al. 1996). The increase in plasma ALT levels induced by carbon tetrachloride was blocked by pretreatment with allyl sulfide or allyl disulfide and increased by pretreatment with propyl disulfide and butyl sulfide.

Dietary Status. Because carbon tetrachloride causes injury through oxidative pathways, depletion of cellular antioxidants such as glutathione, vitamin E, and methionine tend to increase the toxicity of carbon

tetrachloride. For example, feeding rats a diet low in vitamin E, selenium (a required cofactor for glutathione reductase), and methionine led to increased lipid peroxidation, while feeding a diet supplemented with one or more of these antioxidants tended to decrease lipid peroxidation (Hafeman and Hoekstra 1977) and oxidative liver damage (Parola et al. 1992). Similar results have been obtained by Taylor and Tappel (1976) and Sagai and Tappel (1978). In mice, retinoic acid or retinol inhibited the carbon tetrachloride-induced increase in serum alanine transaminase activity and liver histopathology, suggesting a protective effect of vitamin A in mice (Kohno et al. 1992; Rosengren et al. 1995). However, pretreatment with retinol increased hepatocyte injury in rats exposed to carbon tetrachloride (Badger et al. 1996; ElSisi et al. 1993a, 1993b).

Food deprivation has also been shown to have a substantial effect on carbon tetrachloride hepatotoxicity. A 24-hour fast significantly depressed hepatic glutathione (GSH) levels and enhanced carbon tetrachloride hepatotoxicity in rats (Harris and Anders 1980; Sato and Nakajima 1985), and promoted lipid peroxidation as measured by malondialdehyde formation (Ikatsu et al. 1991). A 1-day fast also increased hepatic injury as measured by increases in serum enzymes 2–2.5-fold compared to fed rats following 4-hour exposures at 500–2,500 ppm (Jaeger et al. 1982); the dietary status had no effect at 5,000 ppm. Diurnal decreases in hepatic GSH levels were found to coincide with periods of maximal susceptibility to carbon tetrachloride hepatotoxicity (Bruckner et al. 1984; Harris and Anders 1980). Even though the role of GSH in carbon tetrachloride cytotoxicity is poorly understood, it appears that more than GSH depletion is involved in fasting-induced enhancement of carbon tetrachloride hepatotoxicity. A 1-day fast stimulates the capacity of liver microsomes from male and female rats to metabolize carbon tetrachloride, although fasting did not produce a significant increase in hepatic microsomal protein or cytochrome P-450 levels (Nakajima and Sato 1979). Thus, short-term food deprivation may enhance the biotransformation of carbon tetrachloride to cytotoxic metabolites. Another factor in fasted animals was demonstrated in mice fasted for 24 hours that showed an 8-fold increase in hepatic triglycerides (steatosis) compared to untreated mice (Pentz and Strubelt 1983); it is likely that the increase in lipid content in the livers of fasted mice was responsible for their greater hepatic accumulation of injected carbon tetrachloride compared to fed mice. It should be recognized that food deprivation or consumption of a protein-free diet for several days diminishes MFO activity and makes rats more resistant to carbon tetrachloride (McLean and McLean 1966; Seawright and McLean 1967). Food restriction (25 or 50% lower caloric than control intake) for 30 days prior to administration of carbon tetrachloride and increased the carbon-tetrachloride-induced elevations in some serum enzymes in carbon-tetrachloride-treated rats. (Ramkumar et al. 2003; Seki et al. 2000). For example, the chemical-induced increase in serum AST was elevated 11-fold in female rats fed *ad libitum* but 27-fold in those on

a restricted diet (-25%) compared to controls. Food restriction (reduced by 25%) also increased the severity of lesions of the liver (hepatic cellular degeneration and fibrosis) and kidney (proximal tubular vacuolation and glomerular sclerosis) in treated rats compared to those fed *ad libitum* (Seki et al. 2000).

Metals. Pre-exposure to single doses of various metals (hexavalent chromium, mercuric chloride or silver) had no synergistic effect on lipid peroxidation in rats treated with carbon tetrachloride (Rungby and Ernst 1992). In mice fed a diet augmented with 3% carbonyl iron and intraperitoneally injected with carbon tetrachloride for 12 weeks, there were significant increases in parameters of hepatic injury (serum ALT, absolute and relative liver weight, severity of necrosis) compared to controls that were numerically larger than for groups treated with iron or carbon tetrachloride alone (Arezzini et al. 2003). Rats fed a low-copper diet were reported to be more sensitive to hepatic plasma membrane injury 24 hours following an intraperitoneal injection of carbon tetrachloride, possibly due to reduced Cu-Zn superoxide dismutase activities (DiSilvestro and Medeiros 1992). Rats fed a diet mildly deficient in zinc showed elevated levels of hepatocyte injury, as assessed by serum sorbitol dehydrogenase activity (DiSilvestro and Carlson 1994). In rats injected with lead nitrate and then carbon tetrachloride, hepatoxicity, as measured by serum ALT and AST, was lower than in rats injected with carbon tetrachloride alone (Calabrese et al. 1995); the authors attributed this effect to the ability of lead to inhibit cytochrome P-450.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to carbon tetrachloride than will most persons exposed to the same level of carbon tetrachloride in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of carbon tetrachloride, or compromised function of organs affected by carbon tetrachloride. Populations who are at greater risk due to their unusually high exposure to carbon tetrachloride are discussed in Section 6.7, Populations with Potentially High Exposures.

Section 3.9 discusses several types of compounds that can exacerbate the toxicity of carbon tetrachloride. Individuals exposed to these compounds may, therefore, be more sensitive to carbon tetrachloride exposure. As noted above, persons who are moderate to heavy drinkers are at significantly increased risk of liver and/or kidney injury following ingestion or inhalation of carbon tetrachloride (Manno et al. 1996). Occupational exposure to isopropanol has also been reported to markedly potentiate the hepatic or renal toxicity of carbon tetrachloride in men and women (Folland et al. 1976). This report and numerous

animal studies indicate that primary, secondary, and tertiary alcohols, as well as their ketone analogues, can substantially enhance the toxic potency of carbon tetrachloride. Substantial exposures to alcohols and ketones may occur in occupational settings or in certain instances in the use of household products containing these chemicals.

Drugs and other chemicals that significantly induce microsomal MFO activity can significantly increase the toxicity of carbon tetrachloride by enhancing its biotransformation to reactive, cytotoxic metabolites. A number of drugs such as phenobarbital, pentobarbital, and phenylbutazone are MFO inducers in animals and humans. Thus, individuals taking such medications may be at substantially greater risk of carbon tetrachloride toxicity. Other unusually susceptible individuals are those who have had significant exposures to insecticides such as DDT, chlordecone, or mirex, or to industrial chemicals such as PCBs or PBBs. All of these chemicals are potent MFO inducers and have been shown to markedly potentiate the hepatotoxicity of carbon tetrachloride in animals. Exposures to these chemicals can occur in industrial and agricultural settings, as well as in the general population via environmental media (i.e., contaminated water, food, air, and soil). Other widely used chemicals such as TCE have been found to enhance carbon tetrachloride toxicity in animals. Thus, persons with substantial exposure to TCE and other haloalkanes may be at greater risk of carbon tetrachloride toxicity.

Nutritional status can also influence the toxic potency of carbon tetrachloride. Animal studies have clearly demonstrated that brief fasting or consumption of diets low in antioxidants (vitamin E, selenium, methionine) can lead to increased carbon tetrachloride hepatotoxicity. The same may be true for humans, although this is not known for certain. Another aspect of nutritional status affecting carbon tetrachloride toxicity is hepatic energy status. Hepatic ATP levels might influence the ultimate outcome of toxicity (low levels may inhibit recovery mechanisms).

A variety of conditions may predispose certain segments of the population to carbon tetrachloride toxicity. Persons with alcoholic cirrhosis, or other liver diseases that have significantly diminished the functional reserve of the liver, have a reduced capacity to tolerate carbon tetrachloride-induced hepatotoxicity. The same is true for carbon tetrachloride-induced nephrotoxicity in people with significant renal dysfunction from other causes. Diabetics may be particularly susceptible to carbon tetrachloride poisoning, in light of animal studies that indicate elevated levels of ketone bodies induce the MFO system, which converts carbon tetrachloride to reactive, cytotoxic metabolites. Animal models for diabetes suggest different outcomes from exposure to carbon tetrachloride, depending on whether the disease is type 1 or type 2 (Sawant et al. 2004). Mice with type 1 diabetes, induced by intraperitoneal

injection with 200 mg/kg streptozotocin, showed no mortality after receiving a dose of carbon tetrachloride (1,594 mg/kg) that was lethal to half of non-diabetic mice (Shankar et al. 2003). Conversely, rats with type 2 diabetes, induced by administration of a high-fat diet and 45 mg/kg streptozotocin, showed 100% lethality at a dose of 3,188 mg/kg carbon tetrachloride that was not lethal to untreated controls or rats receiving a high-fat diet or streptozotocin alone (Sawant et al. 2004). The type 2 diabetic group had more severe hepatic necrosis between hours 12 and 36, greater depletion of hepatic glutathione at 6 hours, and a significant delay in the stimulation and progression of the S-phase of the cell division cycle compared to the other groups; CYP2E1 levels and rates of lipid peroxidation were not affected by type 2 diabetes in this animal model. Individuals with genetically-determined high MFO activity may be more susceptible to carbon tetrachloride toxicity, as may be persons with habits (e.g., smoking, consumption of smoked meats) that can produce increased MFO activity.

The organ-content of microsomal enzymes responsible for metabolizing carbon tetrachloride may change during different stages of the life cycle, indicating a potential for differing age-related susceptibilities following exposure. A number of studies on drug metabolism reported declines in hepatic activities of CYP2E1 and CYP3A3/4 in the elderly (>65 years) compared with earlier adult stages (as reviewed in Tanaka 1998). *In vitro* studies of human microsomes indicated that total immunoreactive CYP3A (the sum of CYP3A4 and CYP3A5) was significantly higher in livers from individuals aged 21–40 years compared to those aged 14–20 or 61–72 years (Patki et al. 2004). The observed lower rates of biotransformation of triazolam in the adolescent and elderly microsomal preparations was consistent with the reduced CYP3A content. In 18-month-old rats, no statistically significant reduction was observed in the mRNA or protein content for CYP2E1, but enzyme activity was reduced by 46% compared to 8-month-old rats (Wauthier et al. 2004). The reduced activity was attributed to inactivation of the enzyme over time by reactive metabolites. Reduced hepatic CYP3A content was noted in microsomes from 2-year-old CD-1 mice compared to 1-year-old mice (Warrington et al. 2000). Total CYP3A protein was also reduced in the liver of 25–26-month old F344 rats compared to younger animals, but was elevated by 11% in the kidney, largely because of a 50% upregulation of one isoform (Warrington et al. 2004). These results suggest that in F344 rats, ability of the kidney to generate reactive metabolites increases in the elderly.

Age-related reductions in antioxidant content would also tend to increase vulnerability to reactive metabolites of carbon tetrachloride in the elderly. Reductions in glutathione in old rats compared to younger animals have been noted in the liver and are associated with an age-related reduction in the transcription factor nuclear factor erythroid2-related factor (NrF2) that induces gammaglutamylcysteine

ligase, significantly involved with the synthesis of glutathione (Suh et al. 2004). In Wistar rats, both the liver and kidney of 22-month-old rats had significant decreases in glutathione and glutathione peroxidase, but increases in biomarkers of lipid peroxidation compared to 10-week-old rats (Martin et al. 2003). In F344 rats, glutathione peroxidase activity was significantly reduced in the kidney but not the liver of 24-month-old rats compared to 6-month-old rats (Tian et al. 1998); these results suggest a possible basis for the increasing sensitivity of the kidney in rats exposed to carbon tetrachloride by inhalation for 2 years (Japan Bioassay Research Center 1998). No study, however, has directly measured age-related differences in carbon tetrachloride metabolism in the kidney.

Genetic polymorphisms may confer differing susceptibilities to the effects of carbon tetrachloride exposure. In rat liver, two different forms of glutathione S-transferase 3-3 have been identified (Mayama et al. 2003). Hirosaki hairless rats are homozygous for the NC type gene (encoding Asn^{198}-Cys^{199}) and Sprague-Dawley rats are homozygous for the KS type gene (encoding Lys^{198}-Ser^{199}). When the two strains of rat were given an oral gavage dose of carbon tetrachloride, hepatic glutathione activity 0.5 hours later was reduced more significantly in NC rats compared to KS rats. Electrophoretic and chromatographic studies showed that the polymorphism affected the ability of the two kinds of enzyme to bind to heat shock protein 90-beta. The authors conclude that heat shock protein-beta protects the KS type of enzyme from inactivation by carbon tetrachloride.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to carbon tetrachloride. 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 tetrachloride. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to carbon tetrachloride:

Ellenhorn MJ. 1997. Ellenhorn’s medical toxicology: Diagnosis and treatment of human poisoning. 2nd ed. New York, NY: Elsevier, 1422-1429.

Leikin JB, Paloucek FP. 2002. Poisoning and toxicology handbook. 3rd ed. Hudson, OH: Lexi-Comp, Inc., 334.

Shih RD. 1998. Hydrocarbons. In: Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds Goldfrank's toxicologic emergencies. 6th ed. Stamford, CT: Appleton & Lange, 1383-1398.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to carbon tetrachloride may occur by inhalation, ingestion, or dermal contact. Inhalation or oral exposure to carbon tetrachloride may cause hepatic, renal, and neurological effects. There is evidence, though limited, that dermal contact causes a similar pattern of effects.

If carbon tetrachloride has been inhaled, movement to fresh air is recommended. Humidified supplemental oxygen (100%) may be administered as required.

Ingestion of carbon tetrachloride should be considered a toxic emergency in which treatment should begin immediately. Treatment currently involves gastric emptying, either by gastric lavage (with a small bore nasogastric tube) or by induction of vomiting, preferably within minutes of exposure (Shih 1998). The patient needs to have a gag reflex and should not show signs of seizure, lethargy, or coma because of the risk of pneumonitis from pulmonary aspiration. In infants and young children, the induction of vomiting may induce severe fluid loss. Supportive therapy should be followed in all instances of treatment. A cathartic may be administered to speed fecal excretion (Ellenhorn 1997). Administration of activated charcoal is unlikely to be effective (Ellenhorn 1997). Animal studies revealed peak blood levels of carbon tetrachloride within 3–6 minutes after oral exposure when carbon tetrachloride was ingested undiluted or in aqueous vehicles by fasted rats (Kim et al. 1990a). Chemicals that induce P-450, such as ethanol and phenobarbital, should not be given. The administration of epinephrine is avoided, due to the possibility of inducing ventricular arrhythmias. In order to minimize absorption through the skin, all contaminated clothing should be removed and the skin should be washed with soap and water. In cases where the compound has been splashed into the eyes, irrigation with copious amounts of tepid water for 15 minutes has been recommended. Medical treatment is required if irritation, pain, swelling, lacrimation, or photophobia persist.

3.11.2 Reducing Body Burden

Hemodialysis may be employed in order to lower plasma carbon tetrachloride at the onset of renal failure (Ellenhorn 1997). Although this method is not very effective in removing lipophilic compounds from the blood, it is effective in controlling extracellular fluid composition if renal failure occurs (Ellenhorn 1997; EPA 1989b;). Because a substantial portion of absorbed carbon tetrachloride is exhaled within the first

hour, maintenance of a good tidal volume is recommended; hyperventilation may also be of value (Ellenhorn 1997). Administration of hyperbaric oxygen is an experimental treatment that is also available. Hyperbaric oxygen has been used in treating overdoses of carbon tetrachloride in humans (Larcan and Lorbet 1981; Truss and Killenberg 1982; Zearbaugh et al. 1988). Administration of hyperbaric oxygen following exposure to carbon tetrachloride improved survival from 31 to 96% in rats (Ellenhorn and Barceloux 1988). Hyperbaric oxygen has also been used in treating overdoses of carbon tetrachloride in humans and may correct regional tissue hypoxia and damage, as well as inhibit the P-450-dependent reductive dehalogenation of carbon tetrachloride to the metabolically active trichloromethyl radical in the liver. However, the effectiveness of this method has not been established in humans (Burkhart et al. 1991; Ellenhorn and Barceloux 1988).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Information is limited in humans regarding compounds that interfere with the mechanism of action of carbon tetrachloride. However, there is evidence that liver toxicity associated with exposure to carbon tetrachloride is mediated by reactive metabolites that bind to hepatocytes and initiate lipid peroxidation, thus resulting in loss of cell function. N-acetylcysteine has been suggested to bind the toxic metabolite phosgene and to serve as a precursor for the formation of glutathione (Ellenhorn and Barceloux 1988), and was protective against hepatotoxicity in carbon tetrachloride-exposed rats (Simko et al. 1992; Wong et al. 2003). Glutathione, a cellular antioxidant, tends to decrease lipid peroxidation due to carbon tetrachloride ingestion in rats (Arosio et al. 1997; Hafeman and Hoekstra 1977). Prior oral treatment with glutathione protected against hepatic necrosis, but did not modify lipid peroxidation or prevent covalent binding of carbon tetrachloride metabolites to hepatic microsomes in rats exposed intraperitoneally (Gorla et al. 1983). Agents that foster the maintenance of hepatic reduced glutathione levels have a similar protective effect against carbon tetrachloride: cysteine, a precursor to glutathione (De Ferreyra et al. 1974), taurine (Dincer et al. 2002; Vohra and Hui 2001; Waterfield et al. 1993), constituents of garlic oil such as diallyl trisulfide (Fukao et al. 2004), gamma-glutamylcysteinylethyl ester (Nishida et al. 1998), metformin, a dimethyl biguanide anti-hypoglycemic agent (Poon et al. 2003), and clofibrate (Manautou et al. 1998). Administration of 16,16-dimethyl prostaglandin E2 to block the accumulation of intracellular lipids has also been suggested (Haddad and Winchester 1990; Rush et al. 1986). Administration of fructose 1,6-diphosphate to rats has been shown to decrease carbon tetrachloride liver toxicity by increasing hepatocyte levels of ATP. The ATP thus generated is thought to promote hepatocellular regeneration and tissue repair (Rao and Mehendale 1989). Shertzer and Sainsbury (1991) reported that indole antioxidants 4b,5,9b,10-tetrahydroindeno[1,2-b]indole (THII) and 5,10-dihydroindeno-

[1,2-b]indole (DHII) inhibited carbon tetrachloride initiation of lipid peroxidation in rat liver microsomes, and protected against hepatotoxicity in rats when administered prior to carbon tetrachloride treatment. The authors suggested that these compounds may be suitable candidates for further development as potential chemoprotective and therapeutic agents for use in human disorders that involve free-radicals. Colchicine and trimethylcolchicinic acid, an analog that does not bind tubulin, prevented decreases in Ca^{2+}-ATP-ase activity, and reduced increases in gamma-glutamyl transpeptidase, alanine amino-transferase, and alkaline phosphatase in hepatocyte plasma membranes in rats treated with carbon tetrachloride (Cedillo et al. 1996; Martinez et al. 1995).

Oxygen supplementation improved ratios of ATP/ADP, inorganic phosphate/ATP, and lactate/pyruvate that had been altered in cirrhotic livers of rats previously treated with carbon tetrachloride (Harvey et al. 2000). These results were consistent with the hypothesis that hepatocyte damage in cirrhotic livers is exacerbated by a reduced oxygen supply and may partly explain the efficacy of hyperbaric oxygen therapy as described in Section 3.11.2).

Compounds that suppress the activity or expression of CYP2E1 have been shown to reduce the hepatic necrosis caused by the bioactivation of carbon tetrachloride. Pretreatment with 100–400 µmol/kg (subcutaneous) oleanolic acid, a triterpenoid compound, reduced heptatoxicity in rats and mice injected with carbon tetrachloride (Liu et al. 1998); the protective effect occurred 12–72 hours after pretreatment and was found to be unrelated to metallothionein levels. In mice, the protective effect of oleanolic acid was associated with inhibition of expression and activity of CYP2E1 (Jeong 1999). Another triterpenoid, alpha-hederin similarly reduced expression of CYP2E1 and hepatic injury in mice treated with carbon tetrachloride (Jeong and Park 1998). Methylenedioxybenzenes such as isosafrole, dihydrosafrole, and benzodioxole, administered 1 hour before carbon tetrachloride, prevented increases in plasma AST and ALT in mice (Zhao and O'Brien 1996). Isosafrole co-treatment also prevented the development of liver necrosis. Safrole was partially hepatoprotective, whereas piperonyl butoxide, eugenol, isoeugenol, sesamol, and curcumin were ineffective. Other similar compounds that prevented increases in plasma AST and ALT in rats included tetrahydro-5-methyl bis[1,3]benzdioxide [4,5-*C*: 5',6]-azecin-13 (5*H*)-one (protopine) (Janbaz et al. 1998) and 2-methylaminoethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2-carboxylic acid-2'-carboxylate monohydrochloride (DBB-S) (Oh et al. 2000). A synthetic agent, 2-(allylthio)pyrazine, suppressed constitutive and inducible CYP2E1 expression and also blocked carbon tetrachloride-induced hepatotoxicity in mice (Kim et al. 1997); the compound also elevated hepatic GSH levels.

Tumor necrosis factor alpha (TNF-alpha) has been implicated in the process of hepatocellular injury following exposure to carbon tetrachloride. Co-treatment of rats with the soluble receptor to TNF-alpha reduced hepatocellular necrosis and the elevation in serum enzyme levels caused by carbon tetrachloride (Czaja et al. 1995). Mortality was 16% in the rats co-treated with the soluble receptor and 60% in rats co-treated with IgG.

A number of agents have been shown to reduce the severity of fibrosis induced in animals following intermediate-duration exposure to carbon tetrachloride. A weak but significant reduction in the area of carbon tetrachloride-induced hepatic fibrosis was measured by image analysis in rats co-treated with interferon alpha2a over a period of 9 weeks (Fort et al. 1998). There were concomitant reductions in several biochemical markers of fibrosis (hyaluronate, hydroxyproline, and the mRNAs for procollagen and fibronectin). In mice transgenic for the alpha(2)(I) collagen gene (COL1A2) promoter sequence and receiving a single intraperitoneal injection of carbon tetrachloride, interferon-alpha antagonized the transcription of COL1A2 that is stimulated by transforming growth factor-beta and the coactivator Smad3 (Inagaki et al. 2003); the progression of hepatic fibrosis was also prevented in interferon-treated mice. Administration of interferon-alpha2b also reduced the severity of fibrosis in the kidneys of rats subcutaneously injected with carbon tetrachloride over 7 weeks (Dogukan et al. 2003). Histopathology analysis revealed reductions in necrosis, dilatation and atrophy of renal tubules, hypercellularity of glomeruli, and obliteration of renal capillaries in rats co-treated with interferon compared to placebo-co-treated rats; the level of interstitial fibrosis was also reduced by interferon, although the difference was not statistically significant from the placebo co-treatment group. The kidneys of rats co-treated with interferon had more interstitial inflammation than the rats in the control group or in the placebo-co-treatment group. Pirfenidone (5 methyl-1-phenyl-2-(1H)-pyridone), an anti-fibrotic drug approved by the U.S. FDA for Phase II trials against pulmonary and renal fibrosis, reduced both the number of activated hepatic stellate cells and the severity of hepatic fibrosis when administered to rats with carbon tetrachloride-induced hepatic cirrhosis (Garcia et al. 2002); according to the authors, the anti-fibrotic effect of pirfenidone involves suppression of collagen gene transcription and possibly an inhibition of proline hydroxylase levels that would be expected to reduce the availability of hydroxyproline required for collagen synthesis.

Administration of liver growth factor to rats with hepatic cirrhosis following intraperitoneal injections of carbon tetrachloride for 10 weeks significantly improved the structure and function of the liver (Diaz-Gil et al. 1999). Significant decreases were observed in the levels of serum enzymes, the hepatic collagen content, and microscopic findings of fibrosis, necrosis, and inflammatory infiltration of the liver. In

addition, hepatic hemodynamic measures were improved in rats treated with liver growth factor compared to cirrhotic rats: reduced portal pressure and portosystemic shunting, reduced ascites, and increased mean arterial pressure and systemic vascular resistance. Implantation of rat fibroblasts genetically modified to express hepatic growth factor into the spleens of syngeneic rats significantly reduced hepatic injury (serum enzymes, histopathology) resulting from an intraperitoneal injection of carbon tetrachloride (Kaido et al. 1997). Gene therapy using an adenoviral vector bearing cDNA for a nonsecreted form of human urokinase plasminogen activator (Ad-ΔhuPA) reduced hepatic fibrosis in rats that became cirrhotic following treatment with carbon tetrachloride for 6–8 weeks (Salgado et al. 2000). The beneficial effect of enhanced uPA expression was partly attributed to its induction of hepatocyte growth factor.

Treatment of insulin-like growth factor-I (IGF-I) to rats during the last 3 weeks of exposure to carbon tetrachloride/phenobarbitol partially normalized the expression of 8 of 16 genes that were either up- or down-regulated in the cirrhotic liver (Mirpuri et al. 2002). Three of the genes affected by IGF-I are for protease inhibitors; restoration of the expression of these genes would be expected to protect against necrosis. IGF-I treatment also partially restored the expression of growth hormone receptor and the levels of global genomic DNA methylation, which are reduced during the development of cirrhosis (Mirpuri et al. 2002). Evaluation of hepatic effects following IGF-I administration to cirrhotic rats on the same protocol resulted in reductions in lipid peroxidation, fibrosis, and plasma AST and ALT, and increases in mitochondrial transmembrane potential (a measure of mitochondrial membrane integrity) (Castilla-Cortazar et al. 1997).

Several agents have been shown to ameliorate the effect of carbon tetrachloride on hepatic membranes. When co-administered with carbon tetrachloride, betaine, a mitochondrial metabolite of choline, reduced the extent of centrilobular steatosis and minimized the loss of hepatocyte organelle membranes (rough endoplasmic reticulum) in treated rats (Junnila et al. 2000); the effect was attributed to the enhancement of phospholipid synthesis necessary for maintaining the integrity of cell membranes. Hydroxychalcones, which have a 3,4-dihydroxycinnamoyl structure and inhibit lipoxygenases and cyclooxygenases, were potent inhibitors of lipid peroxidation in cultured rat hepatocytes (Sogawa et al. 1994). Polyenylphosphatidyl choline also reduced hepatic fibrosis induced by carbon tetrachloride in rats and accelerated the regression of existing fibrosis (Ma et al. 1996).

One effect of lipid injury following exposure to carbon tetrachloride is the release of hydrolytic enzymes such as calpain from lysosomes into the extracellular space where activation by calcium occurs (Limaye et al. 2003). As a result, cell necrosis progresses to neighboring cells, extending the hepatic lesion.

Administration of the calpain inhibitor *N*-CBZ-Val-Phe-methyl ester (CBZ) or the cell-impermeable inhibitor E64 1 hour after a toxic, nonlethal intraperitoneal dose of carbon tetrachloride protected against calpain-specific breakdown of alpha-fodrin, a cytoskeletal protein, and reduced the increase in serum ALT. Administration of CBZ 1 hour after a lethal dose (3 g/kg) increased survival from 25 to 75%. The calpain inhibitors have no effect on the metabolism of carbon tetrachloride by CYP2E1 or the generation of metabolites that bind to liver tissue.

As vitamin A (retinol) shows species-specific variations on carbon tetrachloride-related hepatotoxicity, it is not possible to predict whether it would be useful as a therapeutic agent in exposed humans. Pretreatment of male mice with vitamin A for 7 days prior to a single exposure to carbon tetrachloride reduced the elevations in plasma ALT levels as well as the extent of hepatic degeneration (Hooser et al. 1994). Some strain variations were evident in the protective effect of vitamin A, with no hepatocyte damage visible in C3H/He or athymic nude mice and only minimal hepatocyte damage visible near the central vein in Swiss-Webster or Balb/C mice. Conversely, pretreatment with vitamin A increased the hepatotoxicity (plasma ALT levels) of carbon tetrachloride 10-fold in male and female Sprague-Dawley rats, and male nude and Fischer-344 rats. The underlying basis for the species and strain differences is not known, but the possible involvement of Kupffer cells or polymorphonuclear neutrophils is under investigation. Inder et al. (1999) determined that the effect of vitamin A in Swiss-Webster mice does not involve alteration of the constitutive or inducible expression of CYP2E1.

Avid retention of Na^+ is a feature of liver cirrhosis. Icatibant (HOE 140), an antagonist to the bradykinin B_2 receptor, normalized Na^+ retention and reduced the hyperactivity of the renin-angiotensin-aldosterone system in rats that had become cirrhotic following treatment with carbon tetrachloride (Wirth et al. 1997).

Malnutrition is a common result of cirrhosis. Survival was improved in rats with carbon tetrachloride-induced cirrhosis by the dietary administration of branched-chain amino acids in addition to a casein diet (Kajiwara et al. 1998). Supplementation with branched-chain amino acids significantly preserved plasma albumin concentration and inhibited the occurrence of ascites and hyperammonemia without altering liver histopathology. The authors hypothesize that administration of branched-chain amino acids may suppress muscular protein catabolism and aid in detoxifying excess serum ammonia levels, which are characteristic of cirrhotic patients.

The protective effects of gadolinium a rare earth metal (lanthanide) and glycine against carbon tetrachloride injury operate via inactivation of Kupffer cells, which are hepatic macrophages (Rivera et al.

2001). When either compound was administered to rats with carbon tetrachloride-induced cirrhosis, the livers showed reductions in fibrosis, collagen protein, and transforming growth factor-beta-1 caused by carbon tetrachloride (Rivera et al. 2001). The inactivation of Kuppfer cells by glycine is suspected to be related to the inhibition of calcium signaling via glycine-gated chloride channels (Rivera et al. 2001). Gadolinium chloride also prevented liver injury and increased hepatocyte proliferation (as measured by immunostaining for the hepatocyte proliferating cell nuclear antigen) in rats when administered prior to treatment with carbon tetrachloride (Ishiyama et al. 1995). Gadolinium chloride inhibited CYP2E1 activity in cultured hepatocytes, reducing the loss of plasma membrane integrity caused by carbon tetrachloride (Badger et al. 1997).

Other substances that have been demonstrated to be protective against the toxic effects of carbon tetrachloride in animals include disulfiram (Brady et al. 1991), enprostil, an analog of prostaglandin E_2 (Bang et al. 1992), bosentan, and TAK-044, antagonists to the endothelin receptor (Hocher et al. 1995; Thirunavukkarasu et al. 2004), the xanthine oxidase inhibitor allopurinol (Dashti et al. 1992), the prolyl 4-hydroxylase inhibitors S 0885 and HOE 077 (Bickel et al. 1991), pyridoxol L,2-pyrrolidon-5 carboxylate (metadoxine) (Annoni et al. 1992), cyclosporine A (Farghali et al. 1996), the calcium antagonist nifedipine (Cutrin et al. 1992, 1994), alpha-tocopherol and derivatives (Hsiao et al. 2001; Liu et al. 1995), polyamines (Wu et al. 1997), adenosine (Hernandez-Munoz et al. 1992), various phenolic compounds (mostly flavinoids) (Adaramoye and Akinloye 2000; Cholbi et al. 1991; Pavanato et al. 2003), zinc (Camps et al. 1992), and chromium III (but not chromium IV) (Rungby and Ernst 1992; Tezuka et al. 1991a, 1991b). Supplementation with sodium tungstate for 7 weeks significantly reduced lipid peroxidation and necrosis produced by carbon tetrachloride in rats (Pawa and Ali 2004). A combination treatment with hyaluronic acid and chondroitin-4-sulfate (but not either agent alone) partly reduced the effects of carbon tetrachloride treatment (Camp et al. 2004); the therapy reduced hepatic necrosis and the increases in hepatic malondialdehyde, plasma TNF-alpha, and neutrophil-mediated myeloperoxidase and reversed the reduction in glutathione. Exercise has been shown to protect subsequently isolated rat hepatocyte from carbon tetrachloride cytotoxicity, probably by affecting cytochrome P-450-2E1 activity, and perhaps also by stimulating intracellular levels of free radical scavengers and antioxidants (Day and Weiner 1991). Food restriction (25 or 50% lower caloric than control intake) for 30 days prior to administration of carbon tetrachloride reduced the magnitude of blood lipid peroxidation and of increases in serum enzymes in carbon-tetrachloride treated rats (Ramkumar et al. 2003).

3.12 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 tetrachloride 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 tetrachloride.

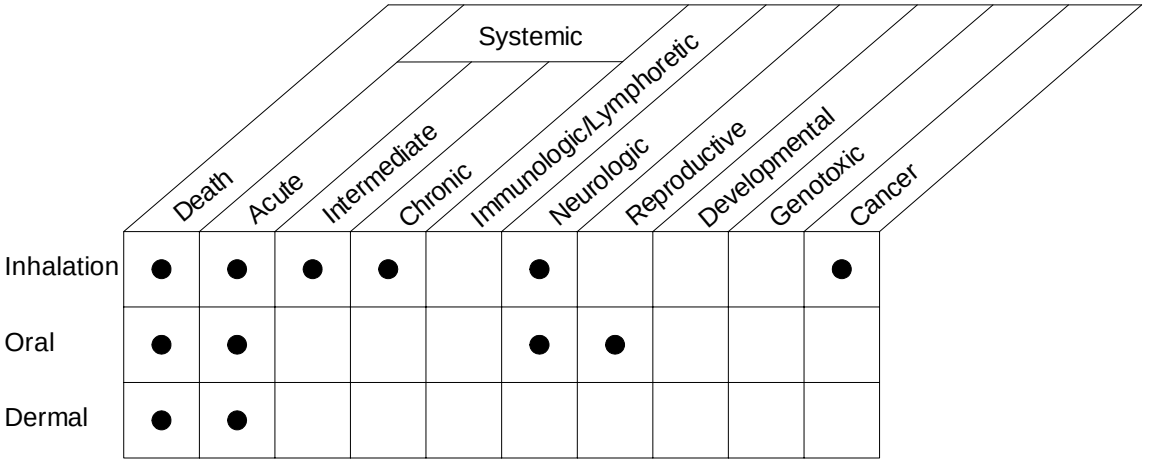
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean 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.

3.12.1 Existing Information on Health Effects of Carbon Tetrachloride

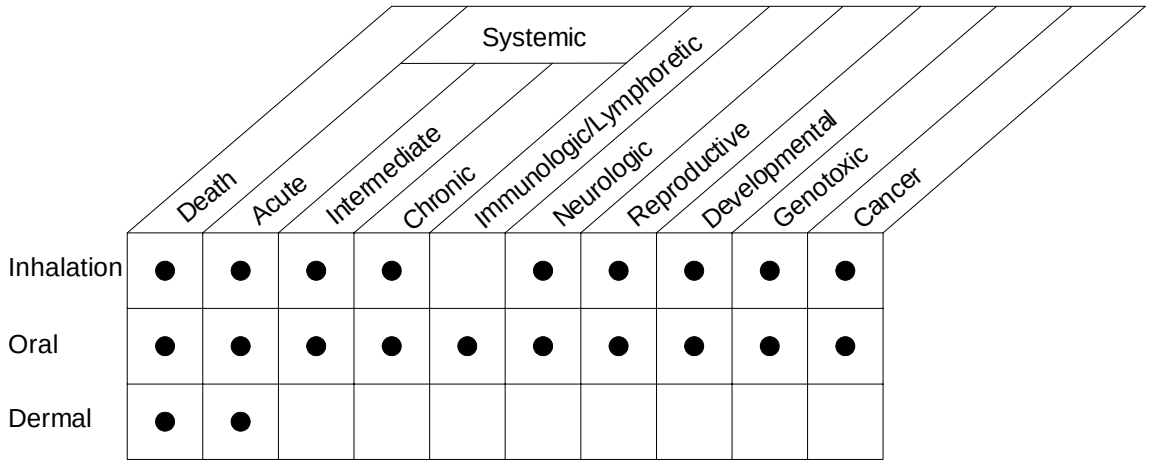
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to carbon tetrachloride are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of carbon tetrachloride. Each dot in the figure indicates 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 (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-6, there is a considerable body of data on the health effects of carbon tetrachloride in humans, especially following acute oral or inhalation exposures. Although many of the available reports lack quantitative information on exposure levels, the data are sufficient to derive approximate values for safe exposure levels. There is limited information on the effects of intermediate or chronic inhalation exposure in the workplace, but there are essentially no data on longer-term oral exposure of humans to carbon tetrachloride. Most toxicity studies have focused on the main systemic effects of

Figure 3-6. Existing Information on Health Effects of Carbon Tetrachloride



Human



Animal

● Existing Studies

obvious clinical significance (hepatotoxicity, renal toxicity, central nervous system depression). There are data on the effects of carbon tetrachloride on the immune system, but there are no reports that establish whether or not developmental, reproductive, genotoxic, or carcinogenic effects occur in humans exposed to carbon tetrachloride.

The toxicity of carbon tetrachloride has been extensively investigated in animals, both by oral and inhalation exposure. While the majority of existing studies in animals have focused on systemic toxicity (hepatic and renal injury), several studies have examined the neurologic, developmental, and reproductive effects of carbon tetrachloride. Effects of carbon tetrachloride on the immune system have been studied following oral, but not after inhalation or dermal exposure. The carcinogenicity of carbon tetrachloride has been studied in animals following inhalation or oral exposure.

3.12.2 Identification of Data Needs

Despite the phase-out of carbon tetrachloride manufacture and use in many areas of the world, its environmental persistence may support the continued practical relevance of many of the data needs identified below.

Acute-Duration Exposure. A large number of studies are available regarding the effects of single exposures to carbon tetrachloride, both in animals and humans. Available data indicate that the central nervous system, liver, and kidneys are primary target organs for carbon tetrachloride. Many of these studies involved exposure to only one dose level (usually high enough to cause clear effects), and the minimum dose needed to produce the characteristic effects of carbon tetrachloride toxicity is not defined with certainty. Although human studies exist, data were not suitable for derivation of an acute inhalation MRL. An acute inhalation MRL was not derived because calculations based on the most suitable data (exposure of rats at 10 ppm, 7 hours/day for 13 exposures over 17 days in the study by Adams et al. 1952), would result in a value (0.02 ppm) lower than the intermediate-duration inhalation MRL. Another assay by Adams et al. (1952) in which rats were exposed for 7 hours on a single day used too small group sizes. The intermediate-duration inhalation MRL of 0.03 ppm is expected to be protective for acute-duration inhalation exposures. An acute-duration oral MRL of 0.02 mg/kg/day was derived based on a LOAEL of 5 mg/kg/day in rats exposed on 10 consecutive days (Smialowicz et al. 1991). Further studies in animals, involving a range of exposure levels and employing sensitive histological and biochemical measurements of injury to liver and kidney, would be helpful in defining the thresholds for acute hepatic

and renal toxicity. Studies on the time-course of changes in the most sensitive parameters would be valuable. Most studies are conducted 18–24 hours after exposure. Because carbon tetrachloride is so rapidly absorbed and distributed to target tissues, significant biochemical and histological changes may occur within minutes. These changes may not be evident 18–24 hours later (e.g., Mehendale 1991, 1992). Data for all exposure routes would be valuable, but further information on inhalation and dermal dose-response relationships would be particularly helpful. In addition, dose-response studies of the effects of acute exposures on other tissues and systems (e.g., nervous, immune, reproductive, developmental) would be useful in determining whether other tissues are injured, especially at doses near the thresholds for injury to the liver and kidney. Furthermore, for purposes of enhancing toxicity and risk assessments related to carbon tetrachloride exposure, dose-response studies in species other than rats and gerbils on induced compensatory mechanisms (e.g., hepatocellular regeneration and tissue repair; see, for example, Calabrese et al. 1993; Kodavanti et al. 1992; Mehendale 1990, 1991, 1992; Rao and Mehendale 1991, 1993) might also prove useful.

Intermediate-Duration Exposure. The effects of repeated exposure to carbon tetrachloride have been investigated in a relatively small number of studies. Similar target organs were reported as those for acute-duration exposure. An intermediate-duration inhalation MRL of 0.03 ppm was derived for liver effects based on a NOAEL of 5 ppm in rats (Adams et al. 1952). An intermediate-duration oral MRL of 0.007 mg/kg/day was derived based on a NOAEL of 1 mg/kg/day in animals (Bruckner et al. 1986). There are a number of areas where further studies would be useful. Most oral studies of carbon tetrachloride toxicity in animals have involved administration of carbon tetrachloride by gavage in corn oil (Condie et al. 1986; Kim et al. 1990b). Since a bolus dose in oil may produce effects somewhat different from those following intermittent exposure in water (e.g., greater hepatotoxicity when administered in oil, Condie et al. 1986), studies involving exposure in drinking water would be valuable, especially since this is a likely exposure pathway for residents using private wells near hazardous waste sites. More information on the adverse effect levels and mechanism of toxicity in tissues other than the liver (e.g., the kidney and nervous system) would be useful; since many oral exposure studies examined only the liver, adverse effect levels for other organ systems are not well characterized.

Chronic-Duration Exposure and Cancer. No definitive studies were located in humans on the noncarcinogenic effects of carbon tetrachloride after chronic-duration exposure. An occupational study by Tomenson et al. (1995) evaluated liver function, as indicated by the levels of hepatic enzymes in serum, in a cross-sectional study of individuals occupationally exposed to carbon tetrachloride. Although the exposed workers were categorized by their length of time on the job (<1, 1–5, and >5 years), this

information was not included in the exposure-response analysis, so the effect of exposure duration is uncertain. A chronic inhalation MRL of 0.03 ppm was derived based on a NOAEL of 5 ppm and a LOAEL of 25 ppm for hepatic effects in rats exposed 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998; Nagano et al. 1998). Increased incidence of chronic progressive nephropathy in rats occurred at the same NOAEL and LOAEL; significantly elevated proteinuria (4+) was observed at a LOAEL of 5 ppm, but was not used for derivation of the MRL because intrinsic levels in controls were so high (>90% scoring 3+ or 4+). At the doses used in chronic oral bioassays, increases in the incidence and severity of non-neoplastic hepatic lesions in rats, the incidence of hepatic cancer in mice, and mortality in both sexes were too high to provide a basis for a chronic oral MRL. A study is needed to determine no-effect levels for hepatotoxicity following chronic-duration oral dosing with carbon tetrachloride.

The carcinogenicity of carbon tetrachloride was evaluated in rats and mice exposed intermittently by inhalation for 2 years (Japan Bioassay Research Center 1998; Nagano et al. 1998). These assays provided sufficient data for hepatic carcinogenicity in both sexes, and some evidence for a threshold effect in both species. The adrenal gland in mice was the only other tissue that had an increased tumor incidence. There is ample evidence that oral (Andervont 1958; Della Porta et al. 1961; Edwards 1941; Edwards et al. 1942; Eschenbrenner and Miller 1944, 1946; NCI 1976a) and parenteral (Della Porta et al. 1961; Reuber and Glover 1967b, 1970) exposure to carbon tetrachloride can lead to increased tumor frequency in animals, but there is currently no dose-response information for carcinogencity at more relevant oral exposure levels. Results of oral gavage studies could be used to plan dose levels for studies in which the chemical is administered in drinking water, as more relevant to actual human exposure scenarios . Current oral data was derived from animals dosed by corn oil bolus gavage, a method of dosing that does not reflect human exposure calculations, and may overestimate the risk as has been suggested by studies of other chlorinated methane and ethane compounds (Jorgenson et al. 1985; Kleming et al. 1986). While the carcinogenic risks of chronic dermal exposure have not been studied, chronic dermal exposure to carbon tetrachloride is not likely for most individuals.

Genotoxicity. Although it is evident that carbon tetrachloride exposure can increase the incidence of tumors in animals, it is not certain whether carbon tetrachloride is acting via a genotoxic mechanism, as a promoter, or some other process. Nearly all studies to date have failed to demonstrate any genotoxicity of unmetabolized carbon tetrachloride, although reactive metabolites and lipid peroxidation products are genotoxic, forming adducts with DNA (Castro et al. 1989; Chaudhary et al. 1994; Chung et al. 2000; Wacker et al. 2001). Since it is believed that carbon tetrachloride toxicity is mediated at least in part

through highly reactive and short-lived metabolites, further studies should focus particular attention on the issue of metabolic activation (especially anaerobic, reductive reactions), with *in vivo* or intact eukaryotic cell systems capable of activation *in situ* being preferred over systems relying on exogenous activation.

Reproductive Toxicity. The effects of carbon tetrachloride on reproduction have not been well investigated. Inhalation of carbon tetrachloride caused testicular degeneration (Adams et al. 1952) and reduced fertility (Smyth et al. 1936) in rats, but at doses higher than the adverse effect level for hepatotoxicity. Oral exposure to carbon tetrachloride did not adversely affect reproduction in one study in rats, but there is uncertainty as to the actual doses administered in this study (Alumot et al. 1976). Additional studies in animals using modern techniques and protocols would be useful to evaluate dose-response relationships for functional reproductive parameters in males and females.

Developmental Toxicity. Epidemiological studies have been published on the developmental effects of carbon tetrachloride in humans (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997). Limited data suggest that carbon tetrachloride has a low potential for developmental toxicity in animals. Fetal size was reduced and viability and lactation indices were decreased following inhalation exposures at or above 250 ppm (Gilman 1971; Schwetz et al. 1974). Fetotoxicity and teratogenicity were not seen in offspring coming to term, but total resorption of fetuses occurred in pregnant rats following oral exposure (Narotsky et al. 1997a, 1997b; Wilson 1954). Metabolic studies suggest that the fetuses of several rodent species, including the rat, lack the enzymes needed for activation of carbon tetrachloride, and that this may explain the low developmental toxicity. However, this phenomenon may not apply to humans, where some drug metabolizing activity takes place *in utero*, especially in the fetal brain (Brezinski et al. 1999). It would be useful to find nonrodent animal models, possibly primates, in which the MFO system also develops *in utero*, and use these to study the developmental toxicity of carbon tetrachloride. Studies are needed to evaluate the possible neurological or neurobehavioral effects of gestational exposure to carbon tetrachloride; parallel groups to evaluate the effect of maternal exposure to ethanol, which induces CYP2E1 would also be relevant to humans.

Immunotoxicity. There are a number of reports that parenteral exposure of animals to carbon tetrachloride can affect the immune system (Kaminski et al. 1989, 1990; Tajima et al. 1985). The effects of carbon tetrachloride on the immune system have been investigated following oral dosing (Smialowicz et al. 1991), but no immunotoxicity was observed at doses much higher than those causing hepatic toxicity. Intermediate- and chronic-duration inhalation bioassays in rodents reported increased spleen

weights, but, as indicated by hemosiderin deposition, this effect appears to be secondary to erythrocyte toxicity and not an immunological effect *per se* (Japan Bioassay Research Center 1998). Although some older reports suggested that dermal exposure to carbon tetrachloride may result in a hypersensitization reaction (McGuire 1932; Taylor 1925), additional dermal exposure studies do not appear to be of high priority.

Neurotoxicity. Available data make it clear that the central nervous system is a target organ for carbon tetrachloride, with the most obvious acute effects being central nervous system depression (Cohen 1957; Stevens and Forster 1953; Stewart et al. 1963). Although our understanding of this important aspect of carbon tetrachloride toxicity might benefit from further study of animals and accidentally exposed humans, of greater concern are the scattered reports that carbon tetrachloride exposure causes focal injury and degeneration of peripheral neurons. Additional studies by inhalation and oral routes would be helpful in defining the dose-response dependency of nerve cell injury, and in determining whether these effects are primary or are secondary to effects on the liver or kidneys.

Epidemiological and Human Dosimetry Studies. Several epidemiological studies have been conducted on the health effects of intermittent workplace exposure to carbon tetrachloride, primarily evaluating the effects on the central nervous system (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960), hepatic (Barnes and Jones 1967; Smyth et al. 1936; Tomenson et al. 1995), and renal (Barnes and Jones 1967) function in relatively small groups of workers. Cancer epidemiological studies have been conducted on significantly larger subject groups (Blair et al. 1998; Bond et al. 1986; Cantor et al 1985; Checkoway et al. 1984; Dumas et al. 2000; Heineman et al. 1994; Kernan et al. 1999; Wilcosky et al. 1984). Epidemiological studies evaluated developmental effects (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997) in populations exposed to carbon tetrachloride in drinking water, which is a route of exposure that may be of concern near hazardous waste sites. A common problem in epidemiological studies is the acquisition of reliable dosimetry data on the exposed populations. For this reason, efforts to improve estimates of past exposures and to define more accurately current exposure levels to carbon tetrachloride would be valuable.

Biomarkers of Exposure and Effect.

Exposure. The presence of carbon tetrachloride in expired air is the most commonly used biomarker of exposure. The rate of excretion in humans appears to be biphasic, with an initial elimination half-life of less than 1 hour, and a second phase of about 30–40 hours. The compound can be detected in expired air

within hours to weeks after exposure. Research on additional biomarkers of exposure would be of value, perhaps in areas such as detection of DNA adducts.

Effect. There are a number of clinical and biochemical tests available that can detect early signs of hepatic and renal injury in humans. However, these tests are not specific for carbon tetrachloride-induced effects. For this reason, studies to identify and measure effects more diagnostic of carbon tetrachloride-specific injury would be helpful. Also, improvements in the sensitivity of these tests, such as accomplished by Ikemoto et al. (2001), would be valuable in evaluating the health status of individuals who have been exposed to low levels of carbon tetrachloride.

Absorption, Distribution, Metabolism, and Excretion. There is relatively little quantitative information on the systemic absorption of inhaled carbon tetrachloride in animals and humans, with estimates ranging from 30 to 60% (Lehmann and Schmidt-Kehl 1936; McCollister et al. 1951). Sanzgiri et al. (1995, 1997) have compared uptake, distribution, and elimination of carbon tetrachloride administered to rats over 2 hours by inhalation or gastric infusion or as a single bolus by gavage and correlated the results with the severity of hepatic injury. This study provides information pertinent to a route-to-route extrapolation.

Although dermal absorption of carbon tetrachloride is relatively modest compared to absorption by the oral or inhalation routes, it would be helpful to quantify the rate and extent of percutaneous absorption of carbon tetrachloride from water. This information would be useful in determining the contribution of dermal exposure to the total dose received by persons using carbon tetrachloride-contaminated drinking water for bathing or showering, or to those who contact carbon tetrachloride-contaminated water near chemical waste sites.

Animal studies reveal that carbon tetrachloride is distributed to tissues according to their rate of blood perfusion and lipid content. Adipose tissue accumulates much higher concentrations of carbon tetrachloride than other tissues, due to the high oil:water partition coefficient of carbon tetrachloride. The animal tissue distribution data are limited, in that carbon tetrachloride levels in tissues in rats have been determined at only a few time-points after a single, high oral dose (Marchand et al. 1970; Teschke et al. 1983). Paustenbach et al. (1986a, 1986b) have measured ^{14}C-carbon tetrachloride levels in tissues of rats at just one time-point following repeated inhalation exposure regimens.

Although numerous studies have investigated the metabolism of carbon tetrachloride in the liver, there is little information about rates of metabolism or relevant CYP-450 proteins in the kidney, to which a substantial proportion of absorbed carbon tetrachloride is distributed under gradual exposures such as inhalation or gastric infusion (Sanzgiri et al. 1997). Investigation of these processes would provide a basis for understanding the increased prominence of renal toxicity in rats under intermediate- and chronic-duration inhalation exposures (Japan Bioassay Research Center 1998).

Comparative Toxicokinetics. Metabolic pathways and mechanisms of hepatotoxicity of carbon tetrachloride have been the subject of many studies in intact animals and *in vitro*, and are therefore better understood than for many other chemicals. However, there are apparently little data on metabolism of carbon tetrachloride in humans. It would be valuable to conduct *in vitro* experiments with human liver samples and hepatocytes to determine whether metabolic pathways and toxic metabolites are similar to those found in animals. It would also be beneficial to identify an animal model in which MFO systems develop in utero as they do in the human fetus.

PBPK models have been developed for a number of drugs and chemicals, in order to better understand and simulate the dynamics of those compounds in the body. Advances made to date indicate that valid PBPK models can accurately predict the concentration of chemicals over time in the blood and specific tissues. Blood and tissue concentration versus time profiles, as well as excretion patterns from animals have been used to validate and adjust PBPK models for carbon tetrachloride (Gallo et al. 1993; Paustenbach et al. 1988). Addition of parameter values for humans has been used to scale-up the PBPK model to predict target tissue uptake, metabolism, and elimination of carbon tetrachloride in humans (Thrall et al. 2000). One limitation of using current models for deriving human equivalent concentrations from animal data for the purposes of MRL derivation is that there is insufficient information on the rates of metabolism for carbon tetrachloride for the general population. Thrall et al. (2000) estimated the *in vivo* metabolic rate (V_{max}) in humans from animal *in vivo* rates and *in vitro* results for animal and human hepatic microsomes. However, the *in vivo* rate derived for humans was based on microsomes pooled from only three individuals (Zangar et al. 2000) and without additional studies, it is not known whether the rate is typical of the general population. Additional studies to characterize the variability of metabolic rates in humans would help to reduce the uncertainty associated with the application of the PBPK model. Quantitative relationships between carbon tetrachloride levels in target organs and organ damage in animals could be used to establish toxicodynamic models. Accurate prediction of ultimate toxicological outcomes will likely also have to account for base-line and inducible levels of compensatory repair

mechanisms. Combined PBPK-toxicodynamic models might then be scaled up and used to predict target organ concentrations and toxicity of carbon tetrachloride in man.

Methods for Reducing Toxic Effects. The usefulness of methods and treatments for reducing peak absorption and reducing the body burden of carbon tetrachloride is rather limited due to the chemical's rapid rates of absorption and tissue disposition. On the other hand, investigations of antidotal therapy based on the mechanism of action have been limited to a few studies involving the administration of compounds to reduce free radical injury. Additional studies would be useful to better establish the effectiveness of both acute and prolonged antidotal therapy, since carbon tetrachloride is persistent in the body.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

The difference between the toxicity of carbon tetrachloride in children and adults is likely to be dependent on the relative expression of microsomal enzymes, such as CYP2E1. Viera et al. (1996) determined that hepatic levels of CYP2E1 in children reach adult levels sometime between the ages of 1 and 10. Additional studies are needed to obtain a precise chronology of the increase. Furthermore, additional studies are needed to clarify fetal expression of CYP2E1 to determine the sensitivity of different fetal tissues and the placenta during gestation.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Numerous current publications on carbon tetrachloride have addressed the efficacy of various agents for reducing or eliminating the toxic effects of exposure; these are mentioned in Section 3.11.3. Additional research programs are focusing on potential therapeutic agents, interacting factors, or mechanisms of toxicity following exposure to carbon tetrachloride. These studies are listed in Table 3-8.

Table 3-8. Ongoing Studies on the Health Effects of Carbon Tetrachloride

Investigator	Affiliation	Research description	Sponsor
Alpini GD	Texas A&M University	Role of protein kinase C in regulating biliary damage from carbon tetrachloride.	NIH-NIDDKD
Anania FA	University of Maryland	Role of leptin in liver fibrogenesis.	NIH-NIDDKD
Bacon BR	St. Louis University	Signaling pathways in hepatic fibrogenesis.	NIH-NIDDKD
Carrasquillo JA	NIH Clinical Center	Development and use of Tc-99m, a radiopharmaceutical as a hepatobiliary agent to visualize effects of carbon tetrachloride *in vivo*.	NIH
Devilliers WJ	University of Kentucky	Role of CD36, a class B scavenger receptor, on activation of hepatic stellate cells and hepatic fibrosis.	NIH-NIAAA
Dranoff JA	DVA, Medical Center, West Haven, Connecticut	Effects of liver injury on nucleotide receptors.	DVA
Gandhi CR	University of Pittsburgh	Endothelin receptor involvement in development of hepatic cirrhosis following exposure to carbon tetrachloride.	NIH- NIDDKD
Kavanagh TJ	University of Washington	Role of glutamate-cysteine ligase in antioxidant defense against hepatic injury.	NIH-NIEHS
Lambris JD	University of Pennsylvania	Role of complement in liver regeneration	NIH-NIDDKD
Lu SC	University of Southern California	Support research on cultured hepatic parenchymal and non-parenchymal cells as models of hepatic injury from carbon tetrachloride and other hepatotoxins.	NIH-NIDDKD
Manautou JE	University of Connecticut	Evaluation of upregulation of canalicular ATP-dependent efflux pump (cMRP or C MOAT) for organic ions and hepatocellular regeneration after carbon tetrachloride exposure.	NIH-NIEHS
Mason R	NIEHS	Role of nitric oxide in metabolism of toxic chemicals.	NIH-NIEHS
Mason R	NIEHS	Biomarkers of oxidative stress in carbon tetrachloride -induced hepatotoxicity.	NIEHS
Mehendale HM	University of Louisiana at Monroe	Investigate factors associated with resiliency in aged rats to hepatotoxicity from chlordecone and carbon tetrachloride.	NIH-NIA

Table 3-8. Ongoing Studies on the Health Effects of Carbon Tetrachloride

Petersen BE	University of Florida	Study subpopulations of oval cells derived from bone marrow for therapeutic purposes following carbon tetrachloride hepatotoxicity.	NIH-NIDDKD
Song BJ	NIAAA	Regulation and role of CYP2E1 in mechanism of hepatic injury.	NIH-NIAAA
Tyner AL	University Illinois at Chicago	Role of cyclin kinase inhibitors p21 and p27 in carbon tetrachloride -induced hepatotoxicity.	NIH-NIDDKD

Sources: CRISP 2004; FEDRIP 2004

CRISP = Computer Retrieval of Information on Scientific Projects; DVA = Department of Veterans Affairs; FEDRIP = Federal Research in Progress database; NIAAA = National Institute on Alcohol Abuse and Alcoholism; NIDDKD = National Institute of Diabetes and Digestive and Kidney Diseases; NIEHS = National Institute of Environmental Health Services; NIH = National Institute of Health