2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of toluene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

2.2.1 Inhalation Exposure

Adverse effects on the nervous system are the critical effects of concern from inhalation exposure to toluene as evidenced by results from studies of workers acutely or chronically exposed to toluene in workplace air, studies of volunteers under controlled acute exposure conditions, and studies of chronic solvent abusers predominantly exposed to toluene. Observed effects include reversible neurological symptoms from acute exposure progressing from fatigue, headache, and decreased manual dexterity to narcosis with increasing exposure level, degenerative changes in white matter in chronic solvent abusers, and subtle changes in neurological functions including cognitive and neuromuscular performance, hearing, and color discrimination in chronically exposed workers. Studies of toluene-exposed animals provide supporting data showing changes in behavior, hearing loss, and subtle changes in brain structure, brain electrophysiology, and brain chemistry. Case reports of birth defects and developmental delays in children of mothers who abused solvents, including toluene, during pregnancy suggest that exposure to high levels of toluene may be toxic to the developing fetus. A number of developmental toxicity studies with rats, mice, and rabbits exposed to airborne toluene indicate that toluene is not a potent teratogenic agent at exposure levels below those inducing maternal toxicity, but can retard fetal growth and skeletal development and alter development of behavior in offspring.

2.2.1.1 Death

Limited data are available on toluene-associated deaths due to solvent abuse or occupational exposure and these studies do not indicate exposure concentrations. Paterson and Sarvesvaran (1983) reported on a teenager who died following an episode of glue sniffing. In Japan, a man died of cardiac arrest after painting a bathroom using a sealer containing 65% toluene (Shibata et al. 1994) and a woman died of adrenal hemorrhage after sniffing thinner containing 67% toluene (Kamijo et al. 1998). In Great Britain, approximately 80 deaths per year have been associated with solvent abuse (Anderson et al. 1985). Approximately half these cases were attributed to cardiac arrhythmias, central nervous system depression, asphyxia, and hepatic and renal failure (Anderson et al. 1982). Among the 52 cases with a toxicological report, 42 mentioned toluene(Anderson et al. 1982).

There are only a few animal inhalation studies that have examined the lethality of toluene, and there is evidence from an intermediate-duration study suggesting that mice may be more sensitive than rats. An inhalation LC_{50} value (concentrations causing death in of 50% of the animals) of 5,320 ppm has been reported for mice (Svirbely et al. 1943). In 14 to 15 week studies, exposure to 3,000 ppm toluene for 6.5 hours/day, 5 days/week, caused 80% mortality in male rats, 60% mortality in male mice, and 100% mortality in female mice, but no deaths among female rats (NTP 1990). Death also occurred among female mice exposed to 625 (10%), 1,250 (10%), and 2,500 (40%) ppm toluene (NTP 1990).

LOAEL values for deaths in the NTP (1990) study and the LC_{50} from the Svirbely et al. (1943) report are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.2 Systemic Effects

Data are available pertaining to respiratory, cardiovascular, hematological, musculoskeletal, hepatic, renal, endocrine and ocular effects in humans and animals after inhalation exposure to toluene. In addition, there are data on gastrointestinal, dermal, body weight, and other systemic effects in animals after inhalation exposure to toluene. All systemic 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 2-1 and plotted in Figure 2-1.

Respiratory Effects. In humans, respiratory tract irritation is experienced from exposure to toluene. Irritation of the upper airways and degeneration of the nasal epithelium have been observed in animal studies.

Exposure of volunteers to 40 ppm of toluene for 6 hours did not produce statistically significant differences in the results of tests measuring nasal mucus flow and lung function or in subjective evaluations of air quality, but irritation of the nose was noted at 100 ppm (Andersen et al. 1983). No changes in lung function were reported for volunteers exposed to 100 ppm toluene for 6 hours, 30 minutes of which were spent exercising (Rahill et al. 1996). Individuals exposed to 800 ppm toluene for 3 hours (von Oettingen et al. 1942) or 1,862 ppm for 2 hours (Meulenbelt et al. 1990) had no self-reported respiratory effects. However, irritation of the nose and throat was reported in printers exposed to 100 ppm toluene for 6.5 hours (Baelum et al. 1985), and in volunteers exposed to 200 ppm toluene for 7–8 hours (Carpenter et al. 1944). Eight workers from a print factory exposed to <200 ppm toluene for

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL: Less serious (ppm)	LOAEL: Serious (ppm)	Reference
ACUTE EXPOSURE								
Death								
1	Mouse (Swiss-Webster)	7 hr					5320 (LC_{50})	Svirbely et al. 1943
Systemic								
2	Human	6 hr	Resp	40	M	100 M (irritation of the nose)		Andersen et al. 1983
			Ocular	40	M	100 M (irritation of the eyes)		
3	Human	6.5 hr	Resp			100 M (irritation of the nose and throat)		Baelum et al. 1985
			Ocular			100 M (irritation of the eyes)		
4	Human	7-8 hr	Resp			200 M (mild throat irritation)		Carpenter et al. 1944
			Ocular			200 M (eye irritation)		
5	Human	3 hr	Resp	1862	M			Meulenbelt et al. 1990
			Cardio			1862 M (sinus bradycardia)		
			Hemato			1862 M (elevated anion gap)		
			Hepatic	1862	M			
			Ocular			1862 M (mucosal irritation)		
6	Human	6.5 hr	Renal	102	M			Nielsen et al. 1985
7	Human	6 hr/d	Resp	100				Rahill et al. 1996
8	Human	3 hr	Resp	800				von Oettingen et al. 1942
			Cardio	800				
			Hemato	800				

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
9	Rat (Sprague-Dawley)	48 hr	Hemato		2000 M (increased hematocrit and blood glucose)		Tahti et al. 1983
			Hepatic		2000 M (increased serum ALT and AST)		
			Bd Wt		2000 M (body weight decrease 10%)		
10	Rat	7 d 8 hr/d	Hepatic		795 F (increased liver weight 12%, increased smooth and rough endoplasmic reticulum)		Ungvary et al. 1982
11	Rat (Wistar)	6 hr	Hepatic		4000 (increased CYP2E1 and decreased CYP 2 C11 in liver)		Wang et al. 1996
12	Mouse	7 d 8 hr/d	Hepatic		795 F (increased liver weight 11% and cytochrome P-450 30%)		Ungvary et al. 1982
13	Dog	1 hr	Hemato	200	500 (decreased leukocytes)		Hobara et al. 1984a
14	Rabbit	7 d 8 hr/d	Hepatic		795 F (increased liver weight 14%, cytochrome P-450-35%, and cytochrome b5-25%)		Ungvary et al. 1982
Immunological/Lymphoreticular							
15	Rat (Sprague-Dawley)	Gd 7-17 6 hr/d			600 F (significant decrease thymus weights in dams)		Ono et al. 1995
16	Mouse (CD-1)	3 hr		1 F	2.5 F (increased susceptibility to infections)		Aranyi et al. 1985

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL: Less serious (ppm)	LOAEL: Serious (ppm)	Reference
Neurological							
17	Human	6 hr		40[b] M	100 M (headaches, dizziness, intoxication)		Andersen et al. 1983
18	Human	6.5 hr			100 M (intoxication, dizziness, decreased manual performance and color perception)		Baelum et al. 1985
19	Human	7 hr			75 (dose-related impairment of performance on recognition, pattern memory, and one-hole test results)		Echeverria et al. 1991
20	Human	4 hr		80 M			Iregren 1986
21	Human	28-41 min		1250 M	(color vision impairment)		Muttray et al. 1999
22	Human	6 hr			100 (decreased perfomance on neuropsychological tests)		Rahill et al. 1996
23	Human	3 or 8 hr			200 (drowsiness and headache)		von Oettingen et al. 1942
24	Monkey (Cynomolgus)	50 min		1000 F	2000 F (cognitive and motor skills impaired)		Taylor and Evans 1985
25	Rat	8 hr			900 M (altered patterns of sleep and wakefulness)		Arito et al. 1988
26	Rat (Fischer- 344)	2 hr			110 M (decreased REM sleep)		Ghosh et al. 1989

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
27	Rat (Fischer- 344)	2 hr			110 M (changes in sleep pattern)		Ghosh et al. 1990
28	Rat (Wistar)	4 hr			2000 M (increased lever presses during exposure)	4000 M (decreased shock avoidance behavior)	Harabuchi et al. 1993
29	Rat (Long- Evans)	1 hr		2500 M	5000 M (increased locomotor activity)		Hinman 1987
30	Rat (Sprague-Dawley)	10 d 16 hr/d			1000 M (loss of auditory sensitivity)		Johnson 1992
31	Rat (Sprague-Dawley)	8 d 14 hr/d			1400 M (hair loss in cochleae)		Johnson and Canlon 1994
32	Rat (Sprague-Dawley)	2 wk 5 d/wk 16 hr/d			1000 M (diminished auditory response)		Johnson et al. 1988
33	Rat	20 min			1000 (increased dopamine in the cerebellum and striatum and norepinephrine and 5-hydroxytryptamine in the cerebellum and cortex)		Kim et al. 1998
34	Rat (Wistar)	4 hr			125 M (a temporary decline in conditioned avoidance response)		Kishi et al. 1988
35	Rat	14 d 1 hr/d			1500 (nystagmus)		Larsby et al. 1986

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
36	Rat (Fischer- 344)	6 h/d, for 3 or 7 d			1000 M (decreased GFAP in thalamus and increased corticosterone)		Little et al. 1998
37	Rat (Fischer- 344)	0, 1600, 3200 ppm for 4 hr			1600 M (reduced lever press response accuracy)		Miyagawa et al. 1998
38	Rat (CD)	4 hr			810 M (decr lift reflex, vertical bar placing, and horizontal rod grasping)		Mullin and Krivanek 1982
39	Rat (Fischer- 344)	7 d 8 hr/d			1929 M (diminished auditory response)		Pryor et al. 1991
40	Rat (Long- Evans)	30 min			8000 M (changes in sensory-evoked potentials, brainstem auditory-evoked responses and flash-evoked potentials and oscillations in the visual cortex)		Rebert et al. 1989a
41	Rat (Fischer- 344)	30 min			500 M (changes in flash-evoked and somatosensory-evoked potentials)		Rebert et al. 1989b
42	Rat	4 hr			1000 M (sleep pattern disturbances- reduced slow wave sleep and increased paradoxical phase)		Takeuchi and Hisanega 1977

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL: Less serious (ppm)	LOAEL: Serious (ppm)	Reference
43	Rat	7+3 d 6 hr/d			80 M (decreased dopamine concentration and noradrenaline utilization)		Von Euler et al. 1989b
44	Rat	2-4 hr			480 (decreased performance in rewarded task)		Wood et al. 1983
45	Mouse CFW (ChasRiver Swiss) albino	30 min		250	500 (increased locomotor activity)		Bowen and Balster, 1998
46	Mouse (NS)	0.5-3 hr			2600 M (central nervous system depression)		Bruckner and Peterson 1981a
47	Mouse (ICR)	8x/d 5 min			12000 M (narcosis)		Bruckner and Peterson 1981b
48	Mouse (C57BL/6N)	5 d 72 min/d		100 M	1000 M (increased locomotor activity)		Bushnell et al. 1985
49	Mouse (CD-1)	Gd 7-16 7 hr/d		200 F	400 F (increased total dehydrogenase activity in brain)		Courtney et al. 1986
50	Mouse (CBA/CA; C57BL/6J)	7 d 12 hr/d			1000 F (accelerated hearing loss in genetically predisposed mice)		Li et al. 1992
51	Mouse ddy	500 ppm for 8 hr		500 M			Matsuoka et al 1997
52	Mouse (CD-1)	6x 1 hr/x		300 M	560 M (increased activity)		Wood and Colotla 1990

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
Reproductive										
53	Rat (Wistar)	7 d 8 hr/d				3000 F	(structural variations in antral follicles in ovary)			Tap et al. 1996
54	Rat (Wistar)	Gd 9-21 6 hr/d		1200						Thiel and Chahoud 1997
55	Rabbit	Gd 7-20 24 hr/d		133	F	266	(4/8 dams aborted)			Ungvary and Tatrai 1985
Developmental										
56	Rat CFY	gd 1-8, 24 hr/d						399	(5/14 dams died, increased incidence of fetuses with skeletal retardation)	Hudak and Ungvary 1978
57	Rat CFY	gd 9-14, 24 hr/d						399	(2/21 dams died, increased incidence of fetuses with skeletal anomalies)	Hudak and Ungvary 1978
58	Rat (Crl:CD (SD) BR VAF/Plus)	Gd 6-15 6 hr/d		750		1500	(decreased fetal bodyweight)	3000	(decreased fetal body weight and increased incidence of fetuses with unossified sternebrae)	Huntingdon Research Centre 1992b
59	Rat (Crl:CD BR VAF/Plus)	Gd 6-15 6 hr/d						3500	(20% decrease in fetal body weight, total absorption at higher exposure levels)	Huntington Research Centre 1992a
60	Rat (Sprague-Dawley)	Gd 7-17 6 hr/d		600		2000	(decreased fetal body weight)			Ono et al. 1995
61	Rat (Wistar)	Gd 9-21 6 hr/d		600		1000	(decreased body weight of fetus and delayed vaginal opening)	1200	(significantly increased postnatal/preweening mortality in offspring)	Thiel and Chahoud 1997

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL: Less serious (ppm)	LOAEL: Serious (ppm)	Reference
62	Mouse (CD-1)	Gd 7-16 7 hr/d			200 (increased number of litters with fetuses with dilated renal pelvis)		Courtney et al. 1986
63	Mouse (CD-1)	Gd 7-16 7 hr/d			400 (increased activity of brain lactate dehydrogenase in 21-day-old weanling pups)		Courtney et al. 1986
64	Mouse CFLP	gd 6-13, 24 hr/d			133 (decreased fetal body weight)	399 (maternal mortality)	Hudak and Ungvary 1978
65	Mouse (CD-1)	Gd 12-17 3x/d 60 min		400		2000 (performance deficits in tests of reflexes, muscle strength and motor coordination in offspring)	Jones and Balster, 1997
66	Mouse	gd 6-15 3-4 hr/d		133	266 (decreased fetal body weight and retardation of fetal skeletal development)		Ungvary and Tatrai 1985
67	Rabbit	gd 6-18 6 hr/d		500			Klimisch et al. 1992
68	Rabbit	Gd 7-20 14 d 24 hr/d		133 F		266 F (4/8 does aborted)	Ungvary and Tatrai 1985
INTERMEDIATE EXPOSURE							
Death							
69	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d				3000 M (8/10 or 80% died)	NTP 1990

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL: Less serious (ppm)	LOAEL: Serious (ppm)	Reference
70	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d					625 F (1/10 died)	NTP 1990
							3000 M (6/10 died)	
	Systemic							
71	Human	2-14 mo 8 hr/d	Hepatic	167	F			Seiji et al. 1987
72	Rat (CD)	95 d 7 d/wk 6 hr/d	Endocr	2000				API 1985
			Dermal	2000				
			Ocular	2000				
			Bd Wt	2000				
73	Rat (Fischer- 344)	42 d 5 d/wk 6 hr/d	Bd Wt	1000	M			API 1997
74	Rat (albino)	8 wk 5 d/wk 70 min/d	Resp	12000	M			Bruckner and Peterson 1981b
			Cardio	12000	M			
			Hepatic			12000 M (decreased liver weight-11%, elevated liver enzymes in serum)		
			Renal			12000 M (decreased kidney weight-27%)		
			Bd Wt				12000 M (20% reduction in body weight gain)	
75	Rat (Sprague-Dawley)	30 d 24 hr/d	Hepatic	320	M			Kyrklund et al. 1987
			Bd Wt			320 M (10% decreased body weight)		

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
76	Rat (Fischer- 344)	13 wk 5 d/wk 15-35 min, 4-9 x/d	Bd Wt					8000 M	(23% decreased body weight gain)	Mattsson et al. 1990
77	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d	Resp	1250		2500	(9-15% increased relative lung weight)			NTP 1990
			Cardio	1250		2500	(6-11% increased relative heart weight)			
			Gastro	3000						
			Hemato	625	F	1250 F	(decreased leukocytes)			
				3000	M					
			Hepatic	625	M	1250 M	(9% increase in relative liver weight)			
				1250	F	2500 F	(16% increase in relative liver weight)			
			Renal	625		1250	(increased relative kidney weights)			
			Endocr	3000						
			Bd Wt	1250		2500	(final body weights 15% lower than controls in males and females)	3000 M	(final body weights 25% lower than controls)	
78	Rat (Sprague-Dawley)	21 d 6 hr/d	Ocular	600		2000 F	(lacrimation)			Ono et al. 1996
79	Rat (Sprague-Dawley)	90 d 6 hr/d	Hemato	2000	M					Ono et al. 1996
			Renal	600	M	2000 M	(increase in kidney weights, necrosis of kidney tubules)			
			Bd Wt	2000	M					

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
80	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d	Resp		30 F (submucosal edema of tracheal epithelium)		Poon et al. 1994
			Hemato	300			
			Hepatic	30	300 (significantly increased serum alkaline phosphatase in males & variation hepatocellular size in females)		
			Renal	300			
			Endocr		30 F (mild reduction in follicle size in thyroid)		
81	Rat (Fischer- 344)	23 wk 7 d/wk 8 hr/d 60, 30, 15 min/hr	Bd Wt		2200 M (decreased body weight gain)		Pryor 1991
82	Rat (Fischer- 344)	11 wk 7 d/wk 8 hr/d	Bd Wt		2000 M (decreased body weight gain)		Pryor 1991
83	Rat (NS)	5 wk 5 d/wk 7 hr/d	Resp		600 (irritation of the lung)	2500 (pulmonary lesions)	von Oettingen et al. 1942
			Hemato	600	2500 (transient decrease in leukocytes)		
			Hepatic	5000			
			Renal		600 (renal casts)		
			Endocr	5000			

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
84	Mouse (ICR)	8 wk 3 hr/d 5 d/wk	Resp	4000	M			Bruckner and Peterson 1981b
			Cardio	4000	M			
			Hepatic			4000 M (increased relative liver weight, elevated serum glutamic oxaloacetic transaminase)		
			Renal	4000	M			
			Bd Wt			4000 M (significant decrease, 5-10%, in body weight gain)		
85	Mouse (ICR)	8 wk 5 d/wk 70 min/d	Resp	12000	M			Bruckner and Peterson 1981b
			Cardio	12000	M			
			Hepatic			12000 M (decreased liver weight)		
			Renal			12000 M (decreased kidney weight)		
			Bd Wt			12000 M (20% reduction in body weight)		
86	Mouse	20 d 6 hr/d	Hemato			10 M (decreased white blood cells and thrombocytes)		Horiguchi and Inoue 1977
			Bd Wt	1000	M			
87	Mouse	30 d 24 hr/d	Hepatic			150 F (increased liver weight-9.6%)		Kjellstrand et al. 1985

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
88	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d	Resp			100 F (12% increase in relative lung weight)		NTP 1990
				1250	M	2500 M (5% increase in relative lung weight)		
			Cardio	1250	F	2500 F (14% increase in relative heart weight)		
				2500	M			
			Gastro	2500				
			Hemato	2500				
			Hepatic	100	F	625 F (6% increase in relative liver weight)		
				625	M	1250 M (9% increase in relative liver weight)		
			Renal	625	F	1250 F (7% increase in relative kidney weight)		
				2500	M			
			Endocr	2500				
			Bd Wt			100 F (13% lower body weight than controls)		
				1250	M	2500 M (12% lower body weight than controls)		
Immunological/Lymphoreticular								
89	Rat (Fischer- 344)	42 d 5 d/wk 6 hr/d		1000	M			API 1997
90	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d		3000				NTP 1990
91	Rat (Sprague-Dawley)	90 d 6 hr/d		600		2000 M (decrease in thymus weights)		Ono et al. 1996

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
92	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		300			Poon et al. 1994
93	Rat (NS)	5 wk 5 d/wk 7 hr/d		5000			von Oettingen et al. 1942
94	Mouse (CD-1)	4 wk 5 d/wk 3 hr/d		1 F	2.5 F (increased susceptibility to infections)		Aranyi et al. 1985
95	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d		2500			NTP 1990
	Neurological						
96	Rat (Fischer- 344)	42 d 5 d/wk 6 hr/d			100 M (changes in GFAP levels in brain)	3000 M (overt signs of neurotoxicity)	API 1997
97	Rat	3 wk 5 d/wk 8 hr/d			900 M (prolonged slow-wave sleep and paraoxical sleep latencies)		Arito et al. 1988
98	Rat	4 wk 8 hr/d			50 M (changes in neurotransmitter-related parameters)		Bjornaes and Naalsund 1988
99	Rat (Long- Evans)	4 wk 5 d/wk 6 hr/d			1000 M (loss of hair cells in organ of Corti)		Campo et al. 1997
100	Rat (Long- Evans)	6h/d, 5 d/wk, 4 wk			1750 (hearing damage)		Campo et al. 1998

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
101	Rat (Wistar)	30 d 15 min/d			35000 M (increased latency of initial response of escape and latency to escape)		Castilla-Serna et al. 1991
102	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		40 M	80 M (decrease in wet weight & increase in dopamine binding in caudate-putamen)		Hillefors-Berglund et al. 1995
103	Rat (Wistar)	30 d 24 hr/d		200 M	400 M (reduced noradrenaline or dopamine in selected areas of the brain)		Ikeda et al. 1986
104	Rat (Wistar)	6 mo 5 d/wk 6 hr/d				1500 M (signficantly fewer neurons in hippocampus)	Korbo et al. 1996
105	Rat (Sprague-Dawley)	30 d 24 hr/d			320 M (decreased phospholipids in cerebral cortex; decreased weight of brain and cerebral cortex)		Kyrklund et al. 1987
106	Rat (Long- Evans)	4 wk 5 d/wk 6 hr/d			2000 M (loss of hearing)		Lataye and Campo 1997
107	Rat (Wistar)	ppd 3-56 5 d/wk 15 min/d			10000 M (increased righting reflex latency and decreased hypnotic latency over weeks 5-8)		Lorenzana-Jimenez and Salas 1990

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
108	Rat (Fischer- 344)	13 wk 5 d/wk 15-35 min, 4-9 x/d				8000 M (decreased auditory brainstem response & flash-evoked potential & other neurobehavioral changes)		Mattsson et al. 1990
109	Rat (Fischer- 344)	50 d 24 hr/d		600	M			Miyagawa et al. 1995
110	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d		1250		2500 (ataxia, increased relative brain weight)		NTP 1990
111	Rat (Fischer- 344)	11 wk 7 d/wk 8 hr/d				2273 M (gait and stride abnormalities, impaired hearing)		Pryor 1991
112	Rat (Fischer- 344)	23 wk 7 d/wk 8 hr/d 60, 30, or 15 min/hr				2200 M (gait and stride abnormalities; diminished auditory response)		Pryor 1991
113	Rat (Fischer- 344)	9 wk 7 d/wk 14 hr/d				1200 M (hearing loss, motor disturbances)		Pryor and Rebert 1992
114	Rat (Fischer- 344)	5 wk 7 d/wk 14 hr/d				1200 M (high-frequency hearing loss, more severe in rats exposed as weanlings)		Pryor et al. 1984a
115	Rat (Fischer- 344)	16 wk + 5 wk 7 d/wk 14 hr/d		700	M	1000 M (diminished auditory response)		Pryor et al. 1984b

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
116	Rat (Sprague-Dawley)	13 wk 8 hr/d		1000	M					Tahti et al. 1983
117	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d				80	M (increased dopamine D2 receptors)			Von Euler et al. 1993
118	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d				80	(increase in serum prolactin levels)			Von Euler et al. 1994
119	Rat (NS)	5 wk 5 d/wk 7 hr/d		600				2500	(incoordination)	von Oettingen et al. 1942
120	Rat (Long- Evans)	3 wk 2x/wk 2 hr/d				178	M (increased nose poking)			Wood and Cox 1995
121	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d		2500						NTP 1990
	Reproductive									
122	Rat (CD)	95 d 7 d/wk 6 hr/d		2000						API 1985
123	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d		1250 3000	M F	2500	M (15% increased testis weight)			NTP 1990
124	Rat (Sprague-Dawley)	90 d 6 hr/d				600	M (slightly decreased (13%) sperm count)	2000	M (significantly decreased (26%) sperm count and decrease (15%) in wts of epididymes, but no effect on indices of fertility)	Ono et al. 1996

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
125	Mouse (CD-1)	8 wk 5 d/wk 6 hr/d		400	M			API 1981
126	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d		2500				NTP 1990
Developmental								
127	Rat CFY	gd 1-21, 8hr/d				266 (increased incidence of fetuses with skeletal retardation)		Hudak and Ungvary 1978
128	Rat (Wistar)	ppd 1-28 12 hr/d				100 (decreased growth of hippocampus)		Slomianka et al. 1990
129	Rat (Wistar)	ppd 1-28 12 hr/d				500 M (reversible decrease in growth of hippocampus)		Slomianka et al. 1992
CHRONIC EXPOSURE								
Systemic								
130	Human	NS	Renal	80	M	106 M (urine albumin increased)		Askergren et al. 1981a
131	Human	>3 yr	Hemato	600				Banfer 1961
132	Human	>18 months 2-8 hr/d	Resp	200	M			Guzelian et al. 1988
			Hepatic			200 M (elevated alanine aminotransferase to aspartate aminotransferase ratios, fatty infiltration)		

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
133	Human	>3 yr	Hemato	100	F			Matsushita et al. 1975
			Hepatic	100	F			
134	Human	16 +/- 13 years	Renal	44				Stengel et al. 1998
135	Human	25 yr (median) 0.5-37 yr	Endocr			36 M (thyroid stimulating hormone levels were inversely proportional to cumulative toluene exposure)		Svensson et al. 1992a
136	Human	3-39 yr	Hepatic			29 M (increased levels of alkaline phosphatase)		Svensson et al. 1992b
137	Human	>10 yr	Hemato			110 (increased leukocytes)		Tahti et al. 1981
138	Human	NS	Hemato	24.7				Ukai et al. 1993
			Hepatic	24.7				
139	Human	73-96 mo	Hemato			41 (significant decrease in lymphocytes)		Yin et al. 1987
140	Rat (Fischer- 344)	106 wk 5 d/wk 6 hr/d	Resp	300				CIIT 1980
			Cardio	300				
			Hemato			100 (decreased hematocrit)		
			Hepatic	300				
			Renal	300				
			Bd Wt	300				

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
141	Rat (Fischer- 344)	2 yr 5 d/wk 6.5 hr/d	Resp		600 (nasal inflammation, degeneration of olfactory and nasal respiratory epithelium)		NTP 1990
			Cardio	1200			
			Gastro	1200			
			Hemato	1200			
			Musc/skel	1200			
			Hepatic	1200			
			Renal		600 (increased severity of nephropathy)		
			Endocr	1200			
			Bd Wt	1200			
142	Rat (Fischer- 344)	15 mo 5 d/wk 6.5 hr/d	Resp		600 (mild-to-moderate degeneration of the olfactory and respiratory epithelium)		NTP 1990
			Hemato	1200			
			Hepatic	1200			
143	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d	Resp	1200			NTP 1990
			Cardio	1200			
			Gastro	1200			
			Hemato	1200			
			Musc/skel	1200			
			Hepatic	1200			
			Renal	1200			
			Endocr	1200			
			Bd Wt	1200			

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)		LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
144	Mouse (B6C3F1)	15 mo 5 d/wk 6.5 hr/d	Resp	600	F	1200 F (minimal hyperplasia of the bronchial epithelium)		NTP 1990
			Hepatic	1200	M			
Immunological/Lymphoreticular								
145	Human	13 yr (average)		1170	M			Pelclova et al. 1990
146	Human	16 +/- 13 yr				44 (increased IgE levels in blood)		Stengel et al. 1998
147	Human	73-96 mo				41 (significantly decreased lymphocytes and increased eosinophils)		Yin et al. 1987
148	Rat (Fischer- 344)	2 yr 5 d/wk 6.5 hr/d		1200				NTP 1990
149	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d				120 M (increased incidence of pigmentation of the spleen)		NTP 1990
				1200	F			
Neurological								
150	Human	12-14 yr				97 M (increased wave latencies for BAEPs)		Abbate et al. 1993
151	Human	4.9 yr (average)				90.9 (statistically significant performance deficits on neurobehavioral tests)		Boey et al. 1997
152	Human	5.7 yr				88 F (statistically significant performance deficits on neurobehavioral tests)		Foo et al. 1990

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
153	Human	>18 mo 2-8 hr/d			200 M (mild intoxication)		Guzelian et al. 1988
154	Human	1-25 yr			122 M (hearing loss)		Morata et al. 1997
155	Human	1-36 yr			83 M (lowered coefficient of variation in electrocardiographic R-R intervals and maximal motor and sensory nerve conduction velocities in median nerve)		Murata et al 1993
156	Human	4-43 yr (median= 29 yr)			140 M (increased incidenec of self-reported neurological symptoms)		Orbaek and Nise 1989
157	Human	21.4 yr (average) range 4-30 yr			50 (increased wave latency of visual evoked potentials)		Vrca et al. 1995
158	Human	21.6 yr (range 4-30 yr)			50 (increased latency of BAEPs and decreased latency of visual evoked potentials)		Vrca et al. 1997a
159	Human	73-96 mo			41 (headaches, dizziness)		Yin et al. 1987
160	Human	17 yr (average)			35[c] (increased color confusion index)		Zavalic et al. 1998a, c
161	Human	16.8 +/- 5.94 yr			120 (increased color confusion index)		Zavalic et al. 1998b

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)		Reference
162	Rat (Fischer- 344)	2 yr 5 d/wk 6.5 hr/d		1200				NTP 1990
163	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d		1200				NTP 1990
	Reproductive							
164	Human	6 yr		150 F				Ng et al. 1992a
165	Human	10 yr				88	(increased incidence of spontaneous abortions (12.4/100) compared to 2 control groups (2.9/100 and 4.5/100))	Ng et al. 1992b
166	Rat (Fischer- 344)	106 wk 5 d/wk 6 hr/d		300				CIIT 1980
167	Rat (Fischer- 344)	2 yr 5 d/wk 6.5 hr/d		1200				NTP 1990

Table 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
168	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d		1200			NTP 1990

[a]The number corresponds to entries on Figure 2-1.
[b]Used to derive an acute inhalation minimal risk level (MRL); concentration was adjusted to a continuous exposure basis (40 ppm x 5d/7d x 8hr/24hr = 9.5 ppm) and divided by an uncertainty factor of 10 (for human variability), resulting in an MRL of 1 ppm (3.8 mg/m3).
[c]Used to derive chronic inhalation MRL; concentration was adjusted to a continuous exposure basis (35 ppm x 5d/7d x 8hyr/24hr = 8.3 ppm) and divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability), resulting in an MRL of 0.08 ppm (0.3 mg/m3).

ALT = alanine amino transferase; AST = aspartame aminotransferase; BAEP = brainstem auditory evoked potential; Bd Wt = body weight; Cardio - cardiovascular; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Gd = gestational day; GFAP = glial fibrillary acidic protein; Hemato = hematological; hr = hour(s); LC50 = lethal concentration, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; ppd = post-partum day; Resp = respiratory; wk = week(s); yr = year(s); x = times

Figure 2-1. Levels of Significant Exposure to Toluene - Inhalation

Acute (≤14 days)

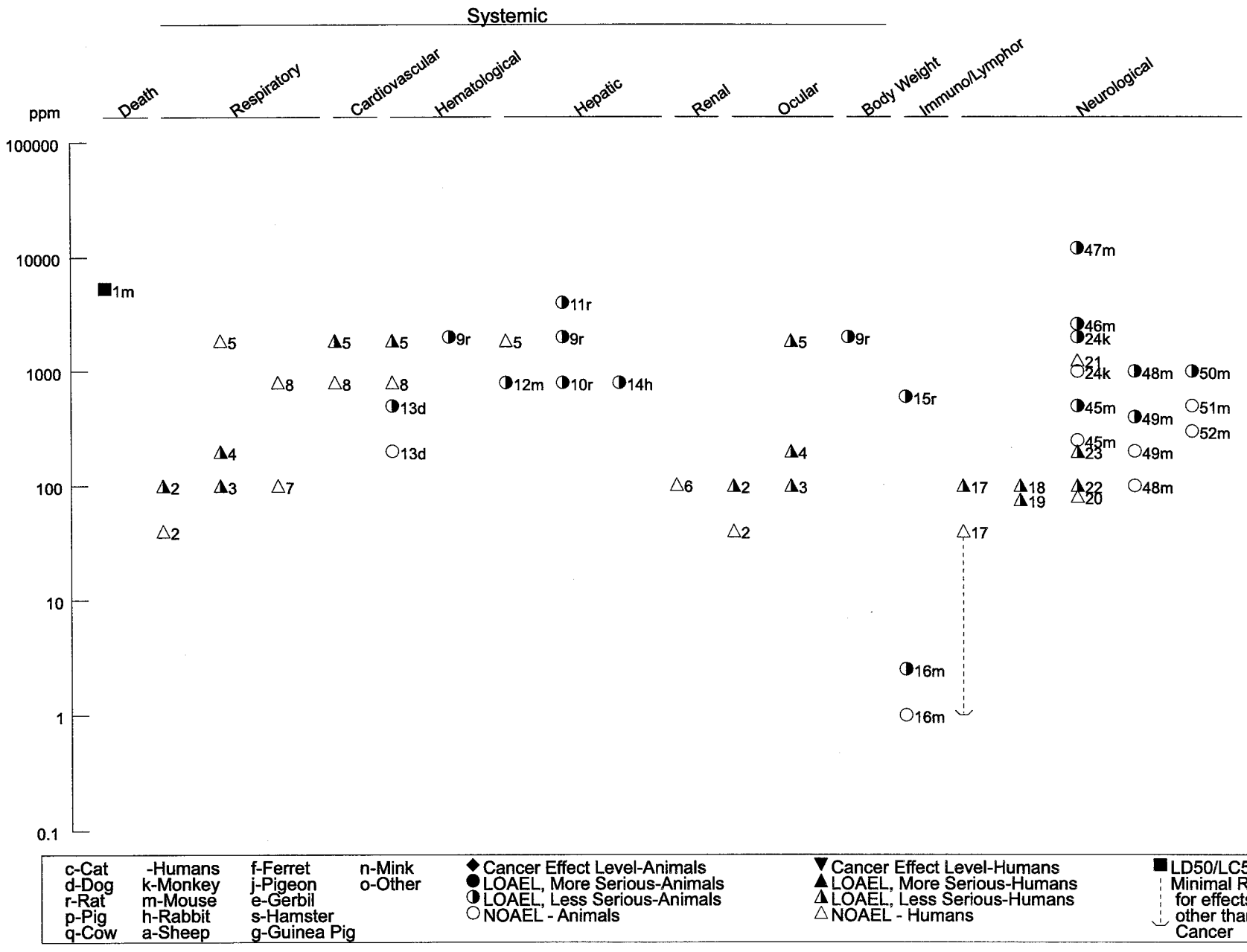


Figure 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Acute (≤14 days)

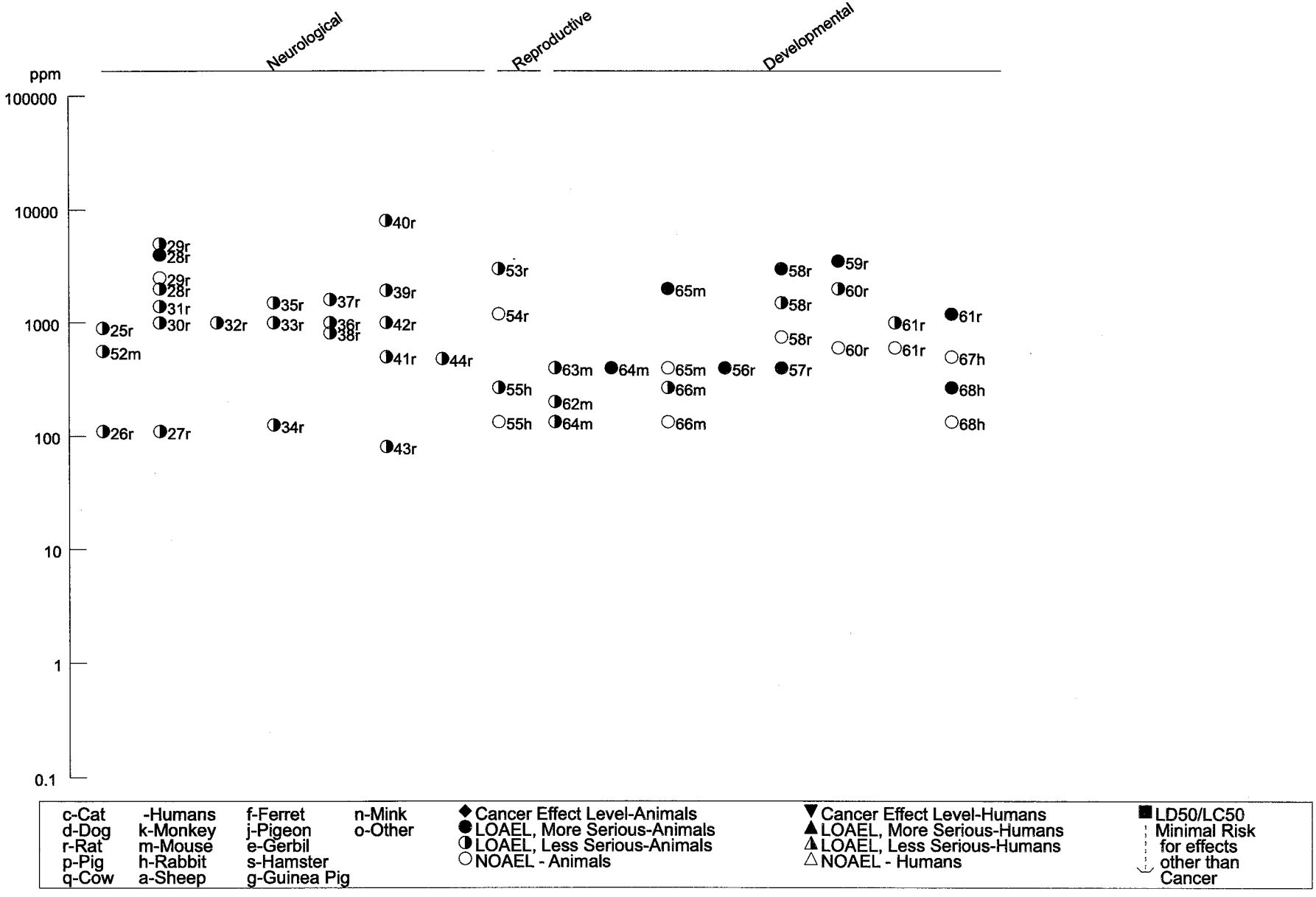


Figure 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Intermediate (15-364 days)

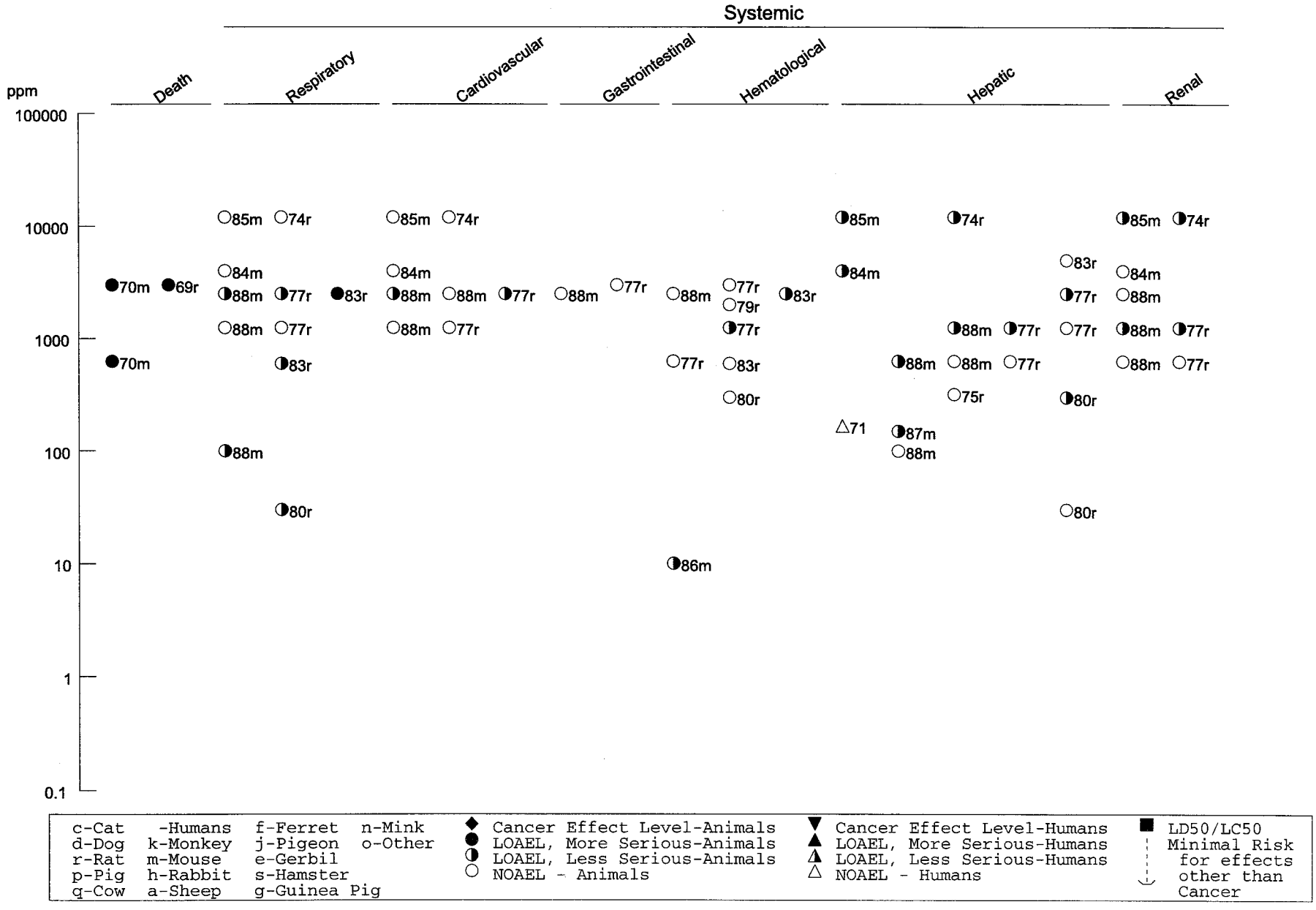


Figure 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Intermediate (15-364 days)

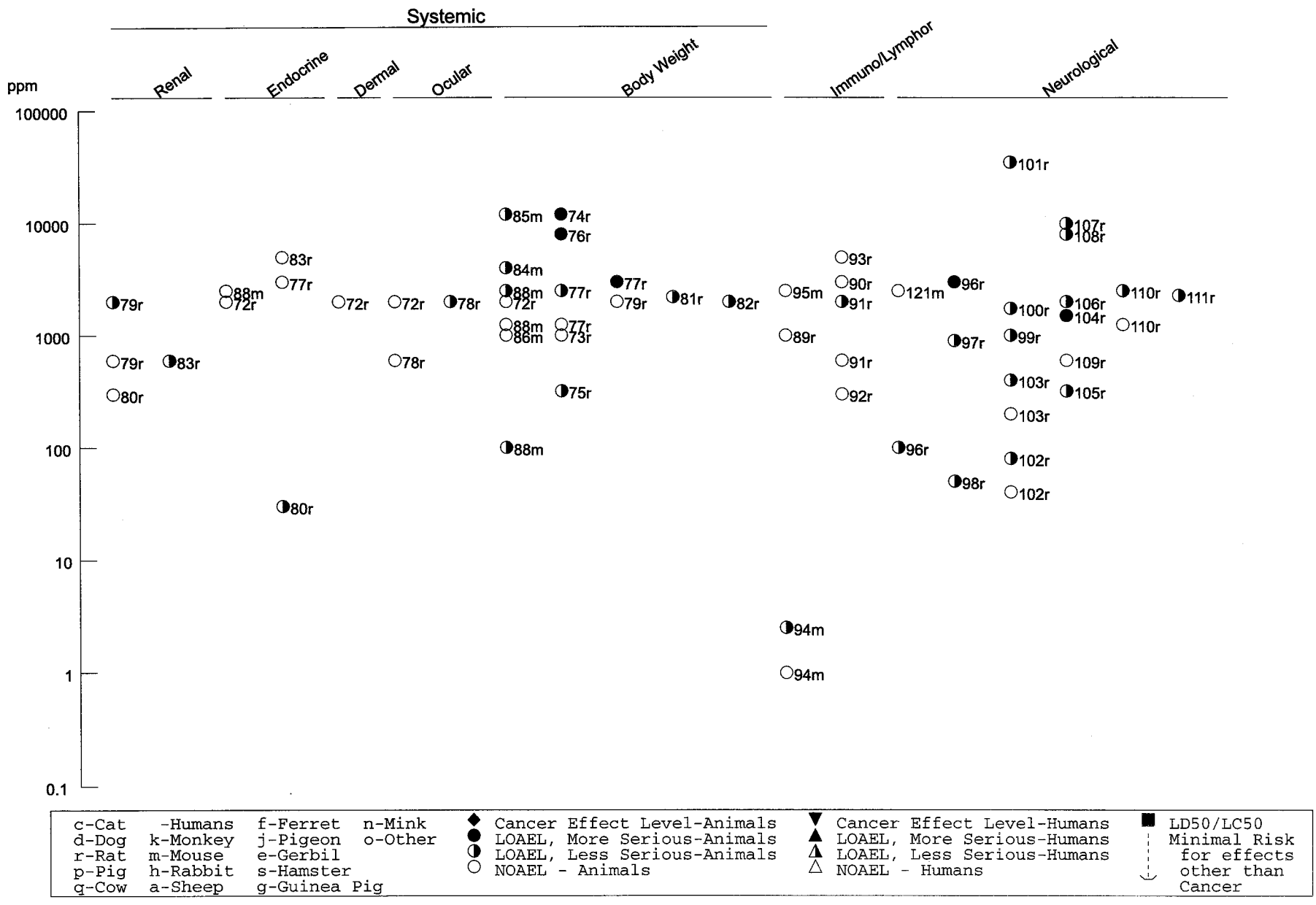


Figure 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Intermediate (15-364 days)

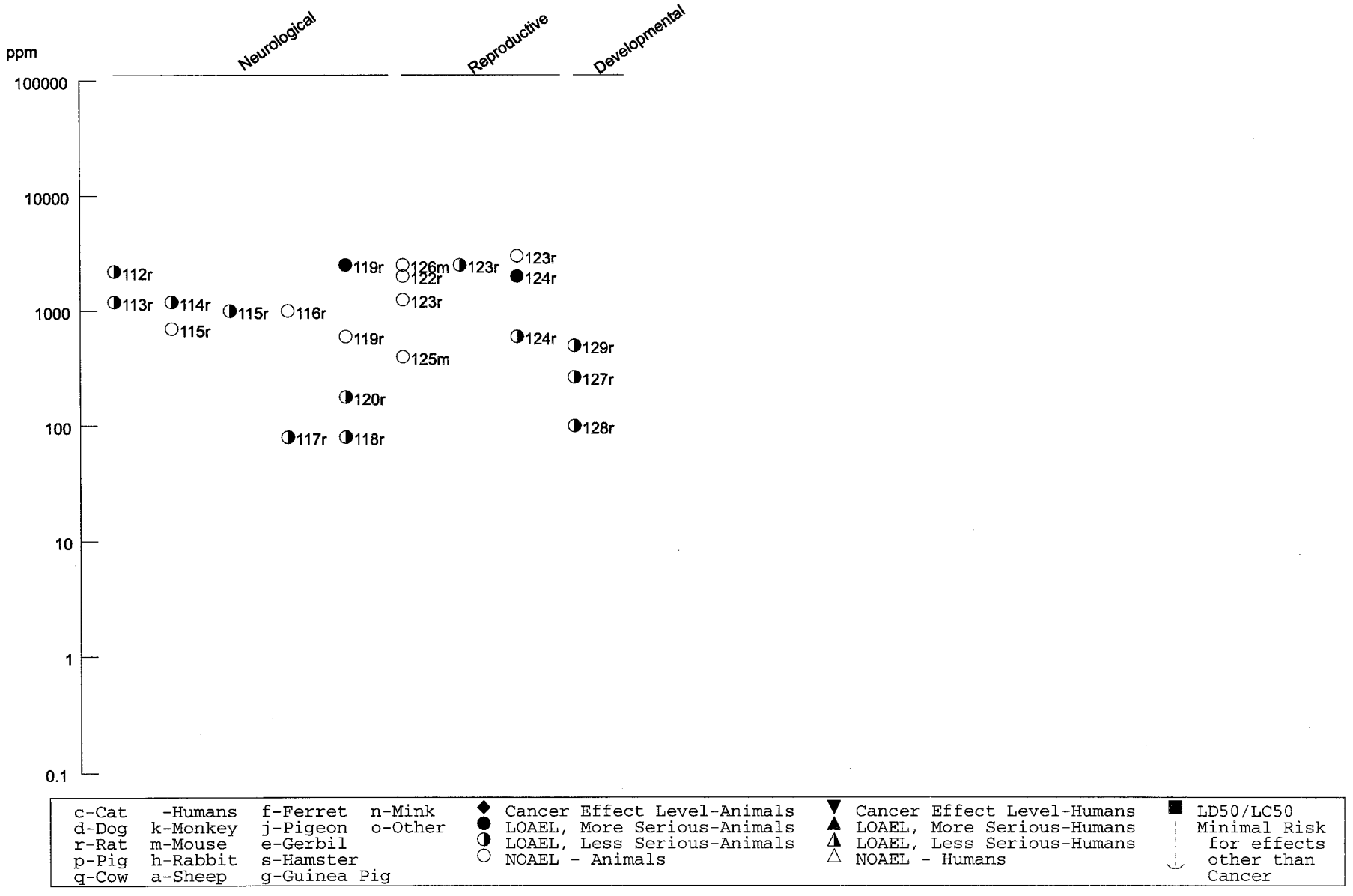


Figure 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Chronic (≥365 days)

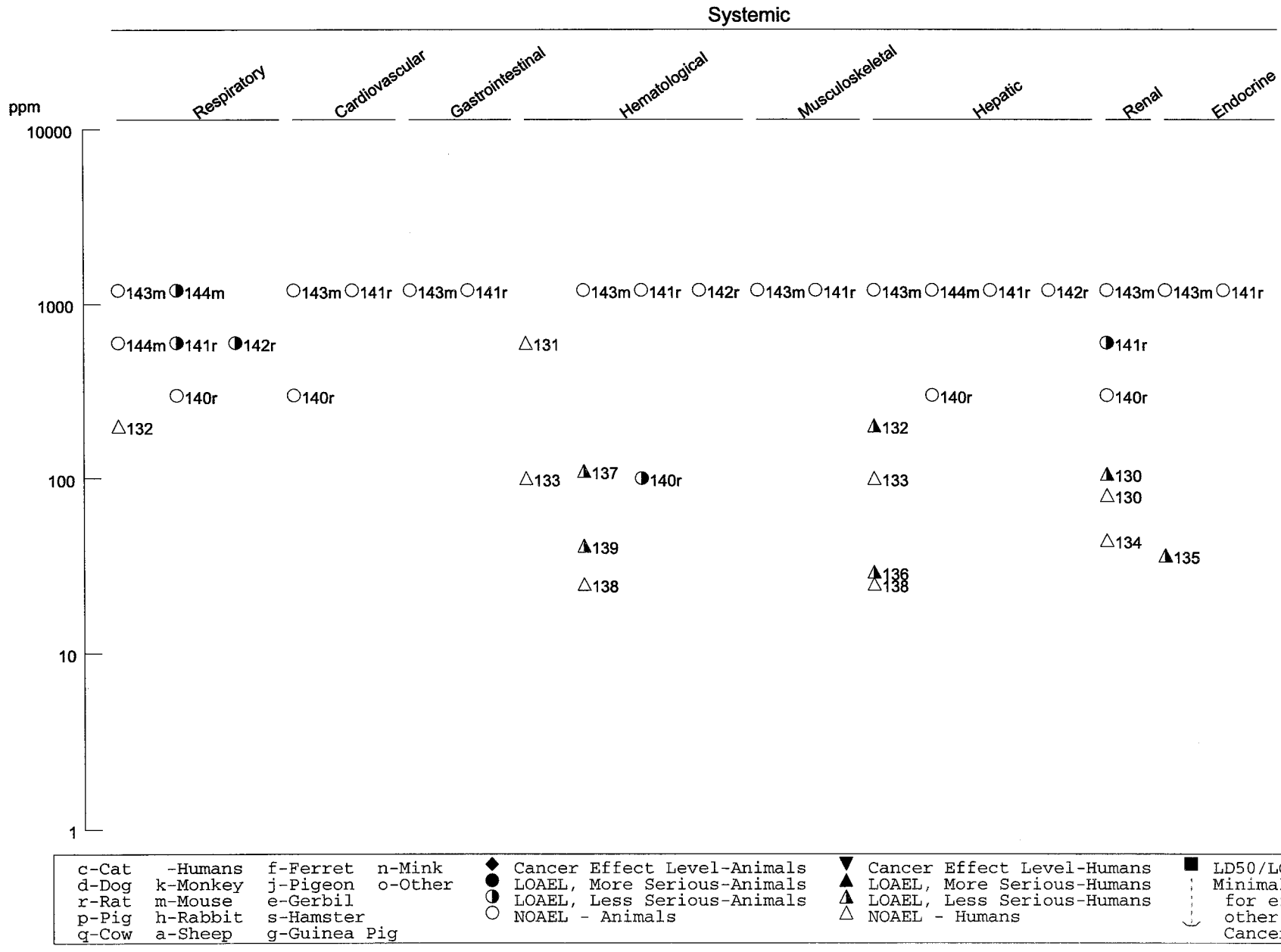
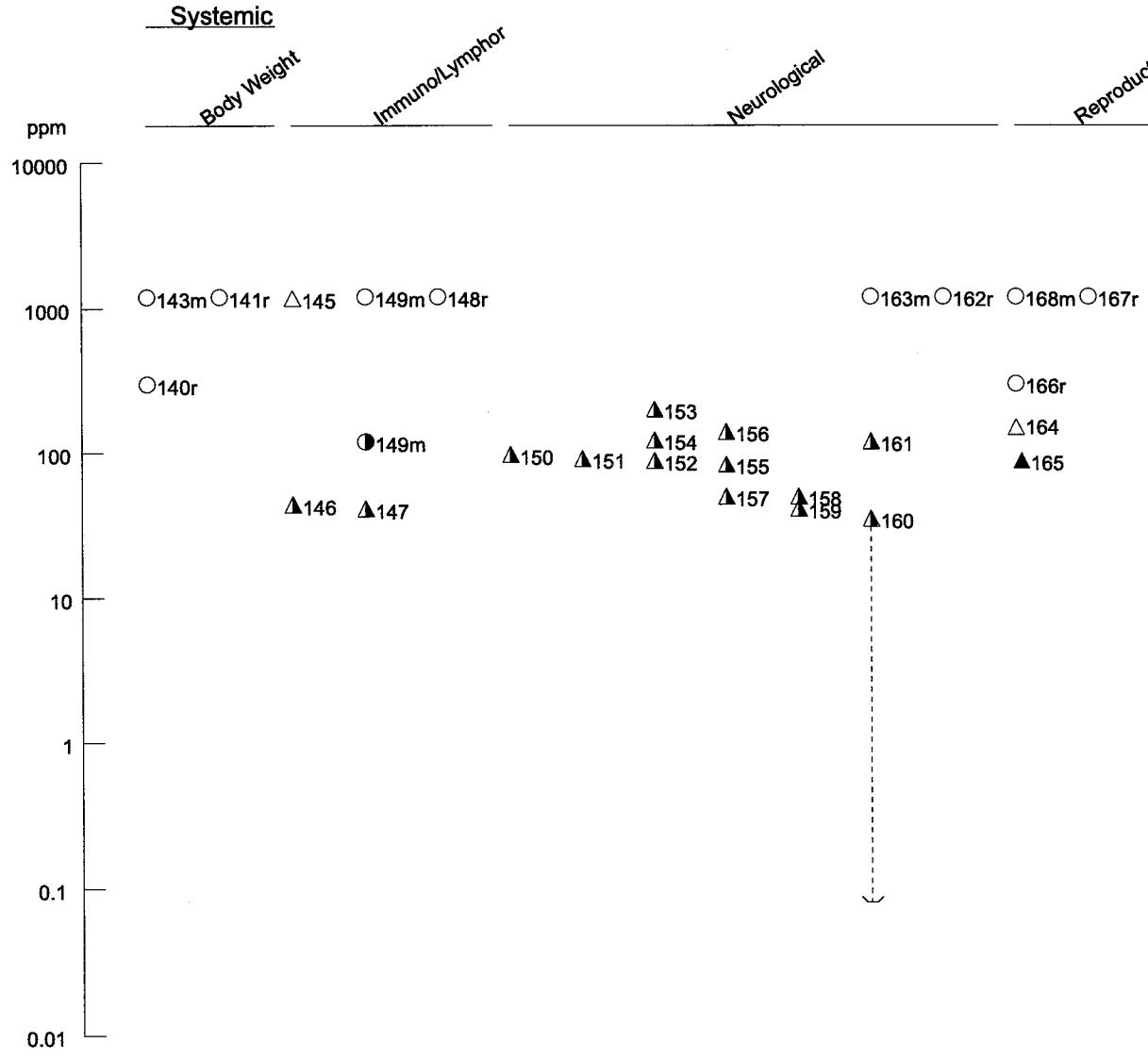


Figure 2-1. Levels of Significant Exposure to Toluene - Inhalation (continued)

Chronic (≥365 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 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				

more than 18 months had normal chest roentgenograms and did not report breathing difficulty (Guzelian et al. 1988).

Ten paint-sprayers exposed to 13 detected solvents (primarily 0.8–4.8 ppm toluene and isobutylacetate) and dusts had morphological changes in the nasal mucosa (Hellquist et al. 1983). However, there was no conclusive association between duration of exposure and mucosal abnormalities. Forty-two workers exposed to mixtures of solvents, of which toluene was generally a major component, reported symptoms of nasal irritation, in addition to eye irritation, nausea, skin conditions, dizziness, and headaches (Winchester and Madjar 1986). The concentrations of toluene to which the workers were exposed ranged from 1 to 80 ppm (mean of 15 ppm). However, concurrent exposure to a mixture of solvents and dusts in these studies precludes establishing an unequivocal causal relationship between exposure to toluene and mucosal irritation.

Rats exposed to 30 or 300 ppm toluene 6 hours/day, 5 days/week for 4 weeks showed histopathological changes in the tracheal epithelium (Poon et al. 1994). Rats exposed to 600 ppm for 5 weeks, 7 hours/day showed irritation of the lung and rats exposed to 2,500 and 5,000 ppm had pulmonary lesions (von Oettingen et al. 1942). No signs of respiratory distress or histological abnormalities were observed in the lungs of mice exposed to 4,000 ppm 3 hours/day, for 8 weeks, or in rats and mice exposed to12,000 ppm for seven 10-minute periods per day separated by a 20-minute recovery period (Bruckner and Peterson 1981b). However, this study did not include a histological examination of the upper respiratory tract and may therefore not have observed damage to this region. Alternatively, the different results reported by von Oettingen et al. (1942) and Bruckner and Peterson (1981b) may be explained by differences in the daily exposure duration with the shorter duration causing the less severe effects.

Significantly increased relative lung weights were reported in rats and male mice exposed to 2,500 and 3,000 ppm (6.5 hours/day, 5 days/week for 14–15 weeks) and in female mice exposed to >100 ppm toluene (NTP 1990). Mild-to-moderate degeneration of the olfactory and respiratory epithelium were observed in rats exposed to 600 ppm or 1,200 ppm 6.5 hours/day, 5 days/week for 15 months (NTP 1990). Minimal hyperplasia of the bronchial epithelium was seen in 4/10 mice exposed to 1,200 ppm, but no other treatment-related damage to the respiratory tract was observed (NTP 1990).

Inflammation of the nasal mucosa, erosion and metaplasia of the olfactory epithelium, and degeneration of the respiratory epithelium were reported in rats exposed to 600 or 1,200 ppm for 2 years (6.5 hours/day, 5 days/week) (NTP 1990). These effects were not observed in mice exposed to the same

concentrations for 2 years. No histopathological lesions were observed in the upper respiratory tract or lungs of rats exposed for 2 years to 300 ppm toluene (CIIT 1980).

Cardiovascular Effects. Cardiac arrhythmia is a cause of death that has been associated with some solvent abuse fatalities. However, studies in laboratory animals do not provide convincing support for a direct effect of toluene on the cardiovascular system (Bruckner and Peterson 1981b; CIIT 1980; NTP 1990). One study of acute exposure to a lethal concentration of toluene reported the induction of arrhythmia, but the authors suggest that this was due to a predisposing arrhythmia-producing heart abnormality (Ikeda et al. 1990). Other studies of acute exposure to near-lethal concentrations have reported a non significant increase in heart rate (Vidrio et al. 1986) or a reduction of experimentally-induced arrhythmia (Magos et al. 1990). Chronic exposure to toluene concentrations up to 1,200 ppm did not induce cardiovascular system lesions in two well-conducted animal studies (CIIT 1980; NTP 1990) and did not appear to be directly toxic to the cardiovascular system.

Cardiac arrhythmias were noted in two adult males who were found semi-conscious after suffering from toluene intoxication (>7,000 mg/m^3 toluene, 1,862 ppm) while removing glue from tiles in a swimming pool (Meulenbelt et al. 1990). Response seemed to be variable between these individuals. One man was exposed for 2 hours and exhibited a rapid heartbeat (sinus tachycardia), while the second man, exposed for 3 hours, exhibited a slow heartbeat (bradycardia) (Meulenbelt et al. 1990). Severe sinus bradycardia was also reported in a comatose man with severe toluene intoxication who had sniffed approximately 250 mL of thinner containing more than 50% toluene (Einav et al. 1997). No effects on systolic or diastolic blood pressure or pulse rate were reported in volunteers exposed to 800 ppm toluene for 3 hours (von Oettingen et al. 1942).

Cardiovascular response was assessed in 25 dogs killed by rebreathing 1 L of air containing 30,000 ppm toluene via an endotracheal tube (Ikeda et al. 1990). In most cases, death was due to hypoxia, but four of the dogs developed transient arrhythmia and in one case, death was due to ventricular fibrillation. The authors suggested that toluene had a direct effect on the septal and ventricular muscles of the heart, which permitted the development of fatal arrhythmias in sensitive dogs (Ikeda et al. 1990). Inhalation by anesthetized rats of 66,276 ppm toluene for 30 minutes (35 minutes inhalation of this concentration was fatal) produced a non significant increase in heart rate and changes in electrocardiographs indicative of depressed ventricular conduction (Vidrio et al. 1986). However, in rats with arrhythmias induced by aconitine injection or coronary ligation, a 15-minute exposure to 6,867 ppm toluene, 10 minutes before aconitine treatment significantly reduced the number of ventricular ectopic beats (Magos et al. 1990).

No histological abnormalities were observed in the hearts of mice exposed to 4,000 ppm for 3 hours/day, for 8 weeks or to mice and rats exposed to 12,000 ppm for 70 minutes/day for 8 weeks (Bruckner and Peterson 1981b). There were also no histopathological lesions of the heart that could be attributed to toluene in rats exposed to 300 ppm for 24 months (6 hours/day) (CIIT 1980) or in rats and mice exposed to up to 1,200 ppm for 24 months (6.5 hours/day) (NTP 1990). However, there were increased heart weights in rats and female mice exposed to 2,500 ppm toluene for 14–15 weeks (6.5 hours/day) (NTP 1990).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to toluene.

The incidence of ulcers of the forestomach was marginally, but not significantly, increased in male rats exposed to concentrations of 600–1,200 ppm toluene for 2 years (NTP 1990). These effects were not reported in mice or female rats exposed under the same conditions. There were no gastrointestinal effects in rats and mice exposed to up to 2,500–3,000 ppm toluene for 14–15 weeks(NTP 1990).

Hematological Effects. Hematological effects were not reported after inhalation exposure to toluene in the majority of recent human and animal studies. However, before the mid-1950s, chronic occupational exposure to toluene was associated with hematological effects in the same studies (Greenburg et al. 1942; Wilson 1943). These effects are now attributed to concurrent exposure to benzene, a common contaminant of toluene at that time (EPA 1985c). More recent studies of workers exposed to toluene or to mixed solvents containing toluene have not found consistent evidence for abnormal hematological parameters(Banfer 1961; Matsushita et al. 1975; Tahti et al. 1981; Ukai et al. 1993; Yin et al. 1987). Decreased leukocyte counts were observed in some animal studies (Hobara et al. 1984a; Horiguchi and Inoue 1977; NTP 1990; von Oettingen et al. 1942), but not in others (Ono et al. 1996; Poon et al. 1994). There is evidence, however, that the decrease is a reversible phenomenon (von Oettingen et al. 1942). The toxicological significance of transitory decreases in leukocyte counts is not clear. It appears that toluene affects the blood, but blood is probably not a critical target tissue following toluene exposure.

No effects on leukocyte counts were observed in volunteers exposed to 800 ppm toluene for 3 hours (von Oettingen et al. 1942). Two workers accidentally exposed to about 1,862 ppm for three hours had normal values for hematological and blood chemistry variables with the exception of an elevated union gap (Meulenbelt et al. 1990).

Ukai et al. (1993) reported no hematologic effects in 452 toluene-exposed shoemakers and printers (average exposure of 24.7 ppm) compared with unexposed controls from the same factories. Exposure was estimated from personal monitoring data, and at least 90% of total solvent exposure was due to toluene. Workers involved in printing, shoemaking, and audio equipment production, and exposed to 41 ppm toluene had significantly decreased lymphocyte counts when compared to controls (Yin et al. 1987). However, total leukocyte counts were not different from controls since the decrease in lymphocytes was counterbalanced by an increase in eosinophils. No significant hematological effects were observed in workers engaged in shoe-making (Matsushita et al. 1975) or printing (Banfer 1961) who were exposed to toluene for several years. The studies were limited by small cohort size and a lack of historical exposure data. Workers were exposed to atmospheric concentrations of toluene up to 600 ppm, but individual exposure monitoring was generally not performed. As a result, the studies had only limited power to detect adverse hematological effects in toluene-exposed workers.

In contrast, workers exposed for several years to toluene (benzene concentration <0.01%) in a tarpaulin factory had increased blood leukocyte counts (Tahti et al. 1981). Toluene exposure concentrations, which ranged from 20 to 200 ppm were similar to those reported by Banfer (1961). However, this study is limited by small cohort size, a lack of historical exposure monitoring, and the probability that workers were exposed to mixtures of chemicals.

Results of animal studies support the observation of decreased leukocyte counts following exposure to toluene. Decreased leukocyte counts were observed in dogs exposed acutely to 500 ppm of toluene (Hobara et al. 1984a). Throughout a 20-day exposure to 10, 100, and 1,000 ppm of toluene, mice exhibited a concentration-related decrease in thrombocyte counts (Horiguchi and Inoue 1977). The 100 and 1,000 ppm groups were reported to have decreased erythrocyte counts; but the authors did not provide analysis of the results or discuss the findings further. Both studies are limited by small numbers of animals in the treatment groups. Slight hypoplasia of the bone marrow was observed in mice exposed to 1,000 ppm (Horiguchi and Inoue 1977), but the effect was not statistically significant and was not found in mice or rats exposed to up to 1,200 ppm for 2 years (NTP 1990). Rats exposed to 2,500 and 5,000 ppm of toluene for 5 weeks had a daily, temporary decrease in leukocyte counts, but counts had normalized by the next day (von Oettingen et al. 1942). Decreased leukocyte counts were also reported in female rats exposed to 1,250 ppm toluene for 15 weeks, but not in mice or male rats exposed to up to 2,500 ppm (NTP 1990).

Increased hematocrit and blood glucose levels were observed in male rats exposed to 2,000 ppm toluene for 48 hours (Tahti et al. 1983). Erythrocyte membranes were stronger and less susceptible to lysis in rats exposed to 2,000 ppm of toluene than in controls (Korpela et al. 1983). This was demonstrated to be a reversible phenomenon since membrane strength returned to normal after toluene had dissipated from the system (Korpela and Tahti 1984). Other lipophilic agents such as anesthetics, tranquilizers, narcotics, and steroids have a similar effect on membrane strength (Magos et al. 1990).

No significant changes in hematological variables were observed in male rats exposed to 2,000 ppm 6 hours/day for 90 days (Ono et al. 1996), or in rats exposed to 300 ppm 6 hours/day, 5 days/week for 4 weeks (Poon et al. 1994).

In one chronic study, rats exposed to 100 or 300 ppm of toluene had significantly reduced hematocrit levels (CIIT 1980). However, in another study, no consistent effects on hematological variables were reported for mice or rats exposed to toluene at levels up to 1,200 ppm for 2 years (NTP 1990).

Musculoskeletal Effects. A 29-year-old man who had been sniffing glue containing toluene (concentration not specified) for 18 years and complained of severe muscle weakness was diagnosed with rhabdomyolysis (an acute disease of the skeletal muscles evidenced by myoglobin in the blood and urine) (Hong et al. 1996).

No histological effects on bone were reported in mice or rats exposed to toluene at concentrations up to 1,200 ppm for 2 years (NTP 1990).

Hepatic Effects. Studies of chronic toluene abusers or occupationally-exposed humans, have provided little evidence for serious liver damage due to inhaled toluene. Some studies of workers who were occupationally exposed to average concentrations between about 30 and 350 ppm toluene reported liver effects such as increased serum levels of enzymes (Guzelian et al. 1988; Svensson et al. 1992b), but others recorded no adverse effects (Lundberg and Hakansson 1985; Seijii et al. 1987; Ukai et al. 1993). A number of animal studies have reported increased liver sizes or minor ultrastructural changes in rats exposed to concentrations of toluene ranging from 150 ppm for 30 days to 4,000 ppm 3 hours/day for 8 weeks (Bruckner and Peterson 1981b; Kjellstrand et al. 1985; NTP 1990), but other studies have recorded no adverse effects in rats and mice exposed to concentrations of up to 1,200 ppm for 2 years (CIIT 1980; Kyrklund et al. 1987; NTP 1990).

No effects on blood levels of bilirubin, alkaline phosphatase activity, serum aspartate aminotransferase activity or serum alanine aminotransferase activity were reported for two workers accidentally exposed to 1,862 ppm toluene for three hours (Meulenbelt et al. 1990). Eight men from a printing factory employing 289 workers exposed to toluene at concentrations of less than 200 ppm, exceeded the upper end of the normal range for blood levels of bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) and had an ALT/AST ratio greater than 1 (Guzelian et al. 1988). Liver biopsies showed centrilobular and periportal fat accumulation and Kupffer cell hyperplasia. None of the men reported drinking alcohol to excess, but they may have had minimal occupational exposure to methyl alcohol, ethyl alcohol, diethyl ether, trichloroethylene, and lacquer thinners which could have confounded the results.

An early study of 106 painters exposed to toluene in an airplane factory reported enlargement of the liver in 30.2% of the exposed men, versus 7% of the control group (Greenburg et al. 1942). However, before the mid-1950s, chronic occupational exposure to toluene was associated with exposure to benzene, a common contaminant of toluene at that time (EPA 1985c), and this is a confounding factor for this study. Serum alkaline phosphatase values were significantly greater than controls in a group of 47 rotogravure workers occupationally exposed to a time-weighted-average (TWA) toluene concentration of 11–47 ppm (midpoint 29 ppm) for 3–39 years than in controls (Svensson et al. 1992b). The difference in alkaline phosphatase values remained significant even when the data were corrected to eliminate nine workers who reported consumption of alcoholic beverages.

In contrast, no significant elevations in serum liver enzymes were found in another group of 452 shoemakers and printers (exposed to average concentrations of 24.7 ppm toluene) compared with unexposed workers from the same factories (Ukai et al. 1993). Women working in a shoe factory for an average of more than 3 years and exposed to toluene concentrations which varied from 65 ppm (15-100 ppm) in winter and 100 ppm (10-200 ppm) in summer showed no changes in several serum variables indicative of liver damage compared with a control group of unexposed workers from the same factory (Matsushita et al. 1975).

A group of 157 female shoemakers exposed for 2–14 months to toluene (7–324 ppm) had decreased serum levels of lactate-dehydrogenase (LDH) as compared to controls, but levels of 8 other serum enzymes monitored as indices of liver damage were normal (Seiji et al. 1987). These workers were also exposed to *n*-hexane, cyclohexane, and methyl ethyl ketone at concentrations generally 1/10th of the toluene concentration. Because LDH is present in almost all body tissues, this finding cannot be

attributed to an effect in the liver with any certainty. A group of 47 Swedish paint industry workers exposed for more than 10 years to mixed organic solvents (xylene, toluene, isobutanol, n-butanol, ethanol, ethylacetate, n-butylacetate, mineral spirits, methylacetate, methylene chloride, methyl ethyl ketone, and isopropanol) did not have elevated serum concentrations of liver enzymes when compared to nonexposed controls (Lundberg and Hakansson 1985). Each of these studies is limited by small cohort size, exposure to multiple solvents, and by a lack of historical exposure monitoring data. As a result, the studies had only limited power to detect adverse effects caused by toluene.

Several case studies have reported effects on the liver from toluene exposure. Acute fatty liver during pregnancy was reported in a 26-year-old woman exposed for at least 2 months to toluene in glue. A liver biopsy done 9 days postpartum showed cytoplasmic change in the hepatocytes; however, there was no clinical or biochemical evidence of liver disease 1 month later (Paraf et al. 1993). A painter who had been exposed to toluene for 5 years exhibited hepatotoxicity, with fatty degeneration of hepatocytes and infiltration by lymphocytes (Shiomi et al. 1993).

Acute exposure to toluene has been reported to produce biochemical and ultrastructural changes in the livers of experimental animals. Mice, rats, and rabbits exposed to 795 ppm of toluene for 7 days showed increased liver weights and cytochrome P450 levels compared to unexposed controls (Ungvary et al. 1982). Electron microscopy revealed ultrastructural changes (increased rough or smooth endoplasmic reticulum) in the livers of all three species (Ungvary et al. 1982). Cytochrome b_5 levels were also increased in exposed rats and rabbits but were not measured in mice (Ungvary et al. 1982). Male rats exposed to 2,000 ppm toluene for 48 hours had increased serum levels of alanine aminotransferase and aspartate aminotransferase (Tahti et al. 1983). Exposure of rats to 4,000 ppm toluene for 6 hours resulted in a significant increases in hepatic levels of cytochrome P450 (CYP) 2E1, increased hepatic activities of nitrosodimethylamine demethylase and 7-pentoxyresorufin O-dependentylase and decreased levels of CYP2C11 (Wang et al. 1996).

Intermediate exposure of animals to toluene has generally produced liver responses similar to those reported for acute exposure. Increased liver weights were reported for male mice exposed to12,000 ppm toluene, 3 hours/day, 5 days/week for 8 weeks (Bruckner and Peterson 1981b), female mice exposed to 150 ppm continuously for 30 days (Kjellstrand et al. 1985), rats exposed to $1,200 ppm (males) or $2,500 ppm (females), or mice exposed to $625 ppm for 14 or 15 weeks (NTP 1990). However, male rats and mice exposed to 12,000 ppm toluene for 8 weeks (seven 10-minute exposures separated by 20-minute recovery periods) had decreased liver weights (Bruckner and Peterson 1981b), and no change

in liver weight was observed in rats exposed to 320 ppm (Kyrklund et al. 1987), male mice exposed to 150 ppm (Kjellstrand et al. 1985) continuously for 30 days, or rats exposed to 30 or 300 ppm toluene 6 hours/day, 5 days/week for 4 weeks (Poon et al. 1994). No effect on the liver was reported for rats exposed to 200–5,000 ppm toluene for 7 hours/day for 5 weeks (Von Oettingen et al. 1942).

Alkaline phosphatase activity was significantly elevated in male rats exposed to 300 ppm for 6 hours/day, 5 days/week for 4 weeks (Poon et al. 1994) and centrilobular hepatocellular hypertrophy was noted in male mice exposed to 2,500–3,000 ppm toluene for 14 weeks (NTP 1990).

No significant gross or histpathological liver changes or liver weight changes were found in rats exposed to toluene at 300 ppm (CIIT 1980) or rats or mice exposed to up to 1,200 ppm 6–6.5 hours/day, 5 days/week for up to 2 years (NTP 1990).

Renal Effects. Studies of chronic toluene abusers, occupationally exposed workers, and laboratory animals have provided little support for serious kidney damage due to inhaled toluene. Chronic abuse of toluene can produce acidosis, but in most cases, renal dysfunction is transient and normal function returns when exposure ceases (Goodwin 1988; Kamijo et al. 1998; Meulenbelt et al. 1990; Patel and Benjamin 1986). In general, studies of workers occupationally exposed to 100–200 ppm toluene, which assessed changes in tests of kidney function, have not shown significant effects (Askergren et al. 1981a; Nielsen et al. 1985; Stengel et al. 1998). Animal studies indicate that inhalation of toluene causes concentration-dependent kidney damage in rats, but only after chronic exposure to concentrations $600 ppm for at least 6 hours/day (Bruckner and Peterson 1981b; CIIT 1980; NTP 1990; Ono et al. 1996; Poon et al. 1994).

Several cases have been reported where occupational exposure to toluene or toluene abuse was associated with acidosis (Gerkin and LoVecchio 1998; Goodwin 1988; Jone and Wu 1988; Meulenbelt et al. 1990; Patel and Benjamin 1986). Acidosis generally reflects the inability of the kidneys to maintain the pH balance of the blood either due to saturation of kidney transport of hydrogen ion or a defect in tubular function. Severe renal tubular acidosis was observed in five pregnant women who were chronic abusers of paints containing toluene (Goodwin 1988). When paint-sniffing ended, normal acid-base balance returned within 72 hours, indicating that permanent damage to the tubules had not occurred. However, one 19-year-old male chronic solvent abuser was found, through a renal biopsy, to have severe tubular interstitial nephritis and focal tubular necrosis indicative of prolonged irritation of the kidney (Taverner et al. 1988). This patient required hemodialysis to correct hematuria and oliguria which was present at the time of his hospital admission. Hemodialysis was also required for a 22-year-old male chronic

solvent abuser with acidosis and hypokalemia (Gerkin and LoVecchio 1998). A 22-year-old woman, who had sniffed approximately 6 L of toluene during the previous month, was found to have metabolic acidosis and histological evidence of tubular injury. The acidosis normalized, but both proximal and distal tubular dysfunction persisted (Kamijima et al. 1994). Proteinuria, hematuria, and urinary calculi were reported in three solvent abuse case studies (Kaneko et al. 1992); the abused product was primarily toluene in one case. Autopsy of a 19-year-old woman, who had sniffed thinner containing 67% toluene for 5 years, revealed severe renal tubular degeneration and necrosis (Kamijo et al. 1998).

A group of 43 printing trade workers exposed to inks containing toluene, alcohols, and ethyl acetate for 9–25 years were experimentally exposed to 382 mg/m^3 (102 ppm) of toluene for 6.5 hours (Nielsen et al. 1985). No significant differences in excretion of albumin and β-2-microglobulin were observed either before or after exposure when the workers were compared to controls matched by age, educational level, and smoking habits (Nielsen et al. 1985).

In a longitudinal study of 92 printers exposed to 97–232 mg/m^3 (26–62 ppm) toluene, markers of early renal damage (microalbumin, N-acetyl-b-D-glucosaminidase, and alanine-aminopeptidase) were not significantly elevated in urine, but creatinine clearance was higher among exposed workers than unexposed controls (Stengel et al. 1998). Comparison of a group of 42 printers, occupationally exposed to 300–400 mg/m^3 toluene (80–107 ppm), with a group of age-matched, unexposed controls showed that printers excreted significantly more albumin than controls, but no increase in the excretion of β-2-microglobulin was observed (Askergren et al. 1981a). Glomerular filtration rate in a group of 34 printers (toluene exposure level not stated) was slightly increased compared with unexposed controls, but the difference was not significant (Askergren et al. 1981b).

In an early animal study, toluene produced pathological changes in the kidneys of rats. Inhalation of 600–5,000 ppm of toluene 7 hours per day for 5 weeks caused the formation of renal casts within the collecting tubules of exposed rats (von Oettingen et al. 1942). In a recent study (Ono et al. 1996), an increase in kidney weights and necrosis of kidney tubules were seen in male rats exposed to 2,000 ppm toluene for 90 days. No histological abnormalities were observed in the kidneys of mice exposed to 4,000 ppm for daily 3-hour periods or mice and rats exposed to 12,000 ppm for 70 minutes/day, 5 days/week for 8 weeks, but kidney weights were significantly decreased in rats and mice exposed to 12,000 ppm (Bruckner and Peterson 1981b). Increased relative kidney weights, but no histological lesions were seen in rats exposed for 15 weeks and female mice exposed to toluene for 14 weeks at

1,250 ppm (6.5 hours per day) (NTP 1990). No effects on the kidneys were observed in rats exposed to 30 or 300 ppm toluene for 4 weeks (Poon et al. 1994).

Gross and microscopic pathological examination of rats chronically exposed to 300 ppm of toluene for 24 months found no treatment-related renal effects (CIIT 1980). Nephropathy was observed in most (96–98%) of the rats (including controls) from a 2-year inhalation study; the severity increased with concentration (600–1,200 ppm) (NTP 1990). The incidence of renal tubular cysts increased with concentration level in males. No renal lesions were reported in mice exposed under the same conditions (NTP 1990). Since the only essential difference between the CIIT and NTP studies was the concentration level used, it appears that the occurrence of renal tubular cysts was concentration-related.

Endocrine Effects. A 29-year-old man who had been sniffing glue containing toluene (concentration not specified) for 18 years was diagnosed with hypothyroidism (Hong et al. 1996). Autopsy of a 19-year-old woman who had been sniffing thinner (67% toluene) for 5 years revealed histological evidence of massive bilateral adrenal hemorrhage with severe degeneration and necrosis of the adrenal cortex (Kamijo et al. 1998). Plasma levels of follicle stimulating hormone, lutenizing hormone, and testosterone were reduced in printers exposed to median toluene levels of 36 ppm for an average of 25 years compared with unexposed controls (Svensson et al. 1992a).

Female rats exposed to 30 or 300 ppm toluene for 6 hours/day, 5 days/week for 4 weeks showed a treatment-related reduction in follicle size of the thyroid (Poon et al. 1994). No effect on the adrenal glands was reported for rats exposed to 200–5,000 ppm toluene for 7 hours/day for 5 weeks (Von Oettingen et al. 1942). No gross morphological abnormalities on the pancreas, adrenal, or thyroid glands were observed in rats exposed to 100–2,000 ppm toluene for 95 days (API 1985). Mice exposed to up to 2,500 ppm for 14 weeks (NTP 1990), rats exposed to up to 3,000 ppm for 15 weeks, and mice and rats exposed to up to 1,200 ppm for 2 years (NTP 1990) showed no histological abnormalities in the pancreas, adrenal, or thyroid glands.

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

No effects on the skin were observed in rats exposed to 100–2,000 ppm toluene for 95 days (API 1985).

Ocular Effects. Humans exposed for 6–8 hours to toluene concentrations of 100 ppm and greater developed irritation of the eyes (Andersen et al. 1983; Baelum et al. 1985; Carpenter et al. 1944; Meulenbelt et al. 1990). No irritation was reported with 6 hours of exposure to 40 ppm toluene (Andersen et al. 1983). Reports of color vision deficits in occupationally exposed workers have linked increased color confusion with chronic exposure to <100 ppm toluene (Zavalic et al. 1998a, 1998b, 1998c). These studies are discussed in the Section 2.2.1.4, Neurological Effects.

Pregnant rats exposed to 2,000 ppm 6 hours/day for 21 days showed lacrimation (Ono et al. 1996), but no lacrimation or discharge was reported for male, female, or pregnant female rats exposed to 100–2,000 ppm, 6 hours/day for 95 days (API 1985).

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

Body weights in rats decreased compared with controls following inhalation exposure to toluene concentrations of 2,000 ppm for 48 hours (Tahti et al. 1983), 2,000 ppm, 8 hours/day, 7 days/week for 11 weeks (Pryor 1991), 320 ppm, 24 hours/day for 30 days (Kyrklund et al. 1987), 8,000 ppm 2–2.5 hours/day, 5 days/week for 13 weeks (Mattsson et al. 1990), 12,000 ppm 70 minutes/day, 5 days/week for 8 weeks (Bruckner and Peterson 1981b), 2,500 ppm, or 6.5 hours/day for 15 weeks (NTP 1990). In contrast, no effects on body weights were observed in rats or mice exposed to 1,000 ppm toluene for 6 hours/day, 5 days/week, for 20 or 42 days (API 1997; Horiguchi and Inoue 1977), in rats exposed to up to 2,000 ppm toluene 6 hours/day for 90 or 95 days (API 1985; Ono et al. 1996), or in rats or mice exposed to up to 1,200 ppm toluene 6–6.5 hours/day for 2 years (CIIT 1980; NTP 1990). Decreased body weights were seen in female and male mice exposed 6.5 hours/day to concentrations $100 and 2,500 ppm, respectively (NTP 1990), and in male mice exposed to 4,000 ppm, 3 hours/day or 12,000 ppm 70 minutes/day for 8 weeks (Bruckner and Peterson 1981b).

Other Systemic Effects. No studies were located regarding other systemic effects in humans after inhalation exposure to toluene.

2.2.1.3 Immunological and Lymphoreticular Effects

Only limited data are available on the immunological or lymphoreticular effects of inhalation exposure to toluene. Studies in exposed workers are confounded to varying degrees by exposure to multiple solvents,

but indicate that there may be slight effects of toluene on immunoglobins, leukocytes, and lymphocytes, but the significance of these effects in humans is uncertain. A single mouse study reported that exposure to concentrations >100 ppm, 3 hours/day for 4 weeks decreased resistance to respiratory infection (Aranyi et al. 1985).

No differences in serum IgG, IgA and IgM values were noted when rotogravure printers exposed to concentrations of 104–1,170 ppm for an average of 13 years were compared to office workers at the same facility (Pelclova et al. 1990). Blood IgE levels in 92 printers exposed to 97–232 mg/m^3 (26–62 ppm) toluene for an average of 16 years were significantly elevated compared to unexposed controls, and a dose-response relation was observed between cumulative toluene exposure and IgE levels (Stengel et al. 1998). Total lymphocytes were significantly decreased in workers involved in shoemaking, printing, and audio equipment production (Yin et al. 1987). Mean toluene exposures were 41 ppm for females and 46 ppm for males over an average of 82 months.

A decrease in the T lymphocyte count of workers occupationally exposed to a mixture of benzene (0–116 ppm), toluene (0–160 ppm), and xylene (0–85 ppm) was observed (Moszczynsky and Lisiewicz 1984). However, no signs of diminished immunological function or disturbances in immune skin reactions against such antigens as tuberculin or distreptase were observed in the subjects studied. The reduction of T lymphocytes may have been the result of the depressive effect of benzene on the lymphocyte system. Workers exposed to a mixture of 0.8–40 ppm toluene (0.003–0.16 mg/L), 56–940 ppm benzene (0.18–3.0 mg/L), and 40–609 ppm xylene (0.18–3.0 mg/L) had significantly lower serum IgG and IgA levels than unexposed controls (Lange et al. 1973). Leukocyte agglutinins for autologous leukocytes and increased leukoagglutination titer in human sera after incubation with the solvents were also observed (Lange et al. 1973). The results of these studies are confounded by mixed exposure and their significance is therefore uncertain.

A single 3-hour exposure of mice to 2.5–500 ppm toluene produced a significant increase in susceptibility to respiratory infections compared to unexposed controls when mice were challenged by *Streptococcus zooepidemicus* (Aranyi et al. 1985). Exposure to 1 ppm for 3 hours, 5 days (3 hours/day), or 4 weeks (3 hours/day) produced no significant difference in susceptibility compared to controls. Pulmonary bactericidal activity was decreased at concentrations of 2.5 ppm and 100–500 ppm, but not at concentrations of 5–50 ppm. The bactericidal activity of the lung was decreased during the 5-day treatment but not with the 4-week treatment. The authors hypothesized that toluene exerted an adverse effect on alveolar macrophage function, thereby decreasing disease resistance.

No changes in weight or histology of the spleen were recorded for rats exposed to 30–5,000 ppm toluene, 6–7 hours/day for 4–5 weeks (Poon et al. 1994; von Oettingen et al. 1942), or for the spleen or thymus of rats and mice exposed to toluene concentrations up to 3,000 ppm for 14–15 weeks (NTP 1990). Exposure of mice and rats to up to 1,200 ppm, 6.5 hours/day for 2 years produced no histological changes in the thymus, but there was an increased incidence of pigmentation of the spleen in male mice exposed to concentrations $120 ppm (NTP 1990).

Decreased thymus weights were observed in male rats exposed to 2,000 ppm 6 hours/day for 90 days (Ono et al. 1996) and in dams exposed to 600 ppm 6 hours/day during gestation days 7–17 (Ono et al. 1995). However, no effects on the thymus were reported in rats and mice exposed 6 hours/day to up to 1,200 ppm for 2 years or up to 3,000 ppm toluene for 14–15 weeks (NTP 1990) or in male rats exposed to 1,000 ppm toluene for 6 hours/day for up to 42 days (API 1997).

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

2.2.1.4 Neurological Effects

Dysfunction of the central nervous system is a critical human health concern following acute, intermediate, or chronic inhalation exposure to toluene. Chronic toluene abuse in humans has been associated with neurotoxic symptoms, narcosis, and death (Byrne et al. 1991; Caldemeyer et al. 1996; Devathasan et al. 1984). Case reports from chronic abusers indicate that prolonged exposure to toluene results in permanent damage to the central nervous system (Byrne et al. 1991; King et al. 1981; Rosenberg et al. 1988b). Neurotoxic symptoms and reduced ability in tests of cognitive and neuromuscular function have been observed in humans occupationally exposed to average concentrations as low as 80–150 ppm (Boey et al. 1997; Murata et al. 1993; Orbaek and Nise 1989; Vrca et al. 1995, 1997b; Yin et al. 1987). Performance deficits in tests of neurobehavior have also been observed in volunteers acutely exposed to controlled concentrations >50 ppm (Andersen et al. 1983; Baelum et al. 1985; Echeverria et al. 1991; EPA 1985c; Iregren 1986; Rahill et al. 1996; von Oettingen et al. 1942) and in laboratory animals repeatedly exposed to >500 ppm toluene (Larsby et al. 1986; Lorenzana-Jimenez and Salas 1990; Miyagawa et al. 1998; Pryor 1991). Studies of occupationally exposed workers also indicate that chronic exposure to average concentrations as low as 30–130 ppm damages hearing and color vision presumably involving, at least in part, effects on neurological components of these systems (Abbate et al. 1993; Morata et al. 1997; Zavalic et al. 1998a, 1988b, 1988c). Hearing loss has also been

reported in laboratory animals exposed to 700–1,500 ppm toluene (Campo et al. 1997; Johnson and Canlon 1994; Lataye and Campo 1997; Pryor et al. 1984b).

Experimental studies in volunteers show that acute exposure to toluene concentrations below 50 ppm results in few, if any, observable effects, but signs of neurological impairment have been observed with acute exposure to concentrations greater than 50 ppm. For example, exposure to 40 ppm of toluene for 6 hours did not produce statistically significant differences in the results of tests measuring psychometric performance and subjective evaluations of well being when compared to controls (Andersen et al. 1983). In contrast, 6–6.5-hour exposures to 100 ppm toluene caused fatigue, sleepiness, headaches, nausea, decreased manual dexterity, decreased color discrimination, decreased accuracy in visual perception, and decreased accuracy in multiplication tests (Andersen et al. 1983; Baelum et al. 1985). An acute inhalation MRL of 1 ppm was calculated as described in the footnote in Table 2-1 and Appendix A, based on the NOAEL (40 ppm) from the study by Andersen et al. (1983).

Several other human studies support the derivation of the acute inhalation MRL and the hypothesis that subtle neurological effects can occur with acute exposure to concentrations in the 75–150 ppm range. Exposure of volunteers to 0, 75, or 150 ppm toluene for 7 hours caused a concentration-related impairment of function on digit span, pattern recognition, the one-hole test, and pattern memory (Echeverria et al. 1991). There was an effect on the results of the symbol digit test, but the effect was not concentration-related. Tests were administered to each subject before exposure and at the end of the exposure period. The treatment effect between groups was smaller than the variation of subjects within the group, thus each subject was used as their own control to more accurately assess the changes in performance due to exposure. There were no differences in the results on simple reaction time, mood (profile on mood scale), visual memory, hand-eye coordination, verbal short-term memory (Sternberg test), finger tapping, reaction time, continuous performance test, and critical tracking test. Six volunteers exposed to 100 ppm toluene for 6 hours, followed by exercise, showed significantly lower results on neuropsychological tests than volunteers exposed to clean air only (Rahill et al. 1996). Exposure of 26 painters to controlled amounts of toluene (5 or 80 ppm) for 4 hours did not change their performance in tests of reaction time, color-word vigilance, or memory reproduction (Iregren 1986). Workers in a printing factory (exposed to <200 ppm toluene) returning to work after a 4-day vacation reported a feeling of mild intoxication to which they became tolerant within 1 or 2 days (Guzelian et al. 1988). At concentrations of 200–800 ppm, acute exposures initially resulted in excitatory effects such as exhilaration and lightheadedness. These effects were followed by the development of narcosis,

characterized by impaired intellectual, psychomotor, and neuromuscular effects with increased duration of exposure (EPA 1985c; von Oettingen et al. 1942).

Humans exposed to high levels of toluene as a result of solvent abuse or industrial accidents have displayed serious central nervous system dysfunction. Accurate exposure data are not available for these individuals, but the concentrations inhaled by chronic abusers have been estimated to range from 4,000 to 12,000 ppm (Gospe et al. 1994). In some cases, the degree of central nervous system depression was sufficient to result in death. Prolonged abuse has been reported to cause permanent damage resulting in abnormal electroencephalogram (EEG) activity, ataxia, tremors, temporal lobe epilepsy, paranoid psychosis, hallucinations, nystagmus (involuntary eye movement), cerebral atrophy, and impaired speech, hearing, and vision (Byrne et al. 1991; Devathasan et al. 1984; Hunnewell and Miller 1998; King et al. 1981; Maas et al. 1991; Meulenbelt et al. 1990; Miyagi et al. 1999; Ryu et al. 1998; Suzuki et al. 1983).

In two hospitalized patients with a history of solvent abuse, there was a decrease in intelligence quotient when the results of tests administered before solvent abuse began were compared to those measured during hospitalization for long-term abuse (Byrne et al. 1991). Examination of 19 children (ages 8–14 years) hospitalized with acute encephalopathy due to toluene exposure indicated that 5 of the children retained psychological impairment and personality change when discharged from the hospital, while one child had a persistent cerebellar ataxia 1 year after cessation of toluene abuse (King et al. 1981).

In general, results from case studies of toluene abusers suggest that some of the neurological symptoms associated with chronic toluene abuse may be the result of permanent structural changes in the brain. Evaluation of chronic toluene abusers by magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT) has shown an increase in the white matter signal, a loss of gray and white matter differentiation, and decreased perfusion in the cerebral cortex, basal ganglia, and thalami (Caldemeyer et al. 1996; Filley et al. 1990; Ikeda and Tsukagoshi 1990; Kamran and Bakshi 1998; Rosenberg et al. 1988a; Ryu et al. 1998; Yamanouchi et al. 1995). Cerebral, cerebellar, and brainstem atrophy were also present (Kamran and Bakshi 1998; Rosenberg et al. 1988b). Correlations between clinical signs of neurological impairment and damage visible in MRI images have also been reported (Caldemeyer et al. 1996; Hormes et al. 1986; Rosenberg et al. 1988b). Abnormalities in MRI and brainstem auditory evoked response (BAER) results were still present in chronic abusers who had refrained from toluene exposure for two to nine months (Rosenberg et al. 1988b).

Toluene had an effect on pattern-visual evoked potentials in 32 males and 2 females who abused thinner containing toluene for 5–10 years. Statistical differences between controls and toluene abusers were seen on latencies of waves P-100 and N-145 (Poblano et al. 1996).

Murata et al. (1993) compared cardiac autonomic function in printers exposed to 83 ppm airborne toluene for 1–36 years with matched controls. Autonomic function was evaluated from measurements of heart rate, the coefficient of variation in electrocardiographic R-R intervals, the distribution of nerve conduction velocities, and the maximal motor and sensory nerve conduction velocities in the median nerve. Some printers reported subjective symptoms such as fatigue, headache, and irritation. Heart rate was not significantly different in exposed individuals and controls. However, there were statistically significant reductions in electrocardiographic R-R intervals, indicating possible dysfunction of the autonomic nervous system. There was a significant decrease in the motor and sensory conduction velocity in the palm segment of the median nerve in toluene-exposed workers, but there was no significant difference in the distribution of the nerve conductance velocities between exposed and control subjects (Murata et al. 1993).

Several studies of workers repeatedly exposed predominantly to toluene in workplace air have found evidence for increased incidence of self-reported neurological symptoms (Orbaek and Nise 1989; Yin et al. 1987); performance deficits in neurobehavioral tests (Boey et al. 1997; Foo et al. 1990; Orbaek and Nise 1989); hearing loss (Abbate et al. 1993; Morata et al. 1997); changes in visual evoked potentials (Vrca et al. 1995, 1997a, 1997b), and color vision loss (Zavalic et al. 1998a, 1998b, 1998c).

A group of 95 workers exposed to TWA of 41–46 ppm toluene during shoemaking, printing, and audio equipment production were evaluated for symptoms and signs of exposure when compared to 130 control subjects (Yin et al. 1987). The incidence of health-related complaints among the toluene exposed workers was 2–3 times that of the controls. Dizziness was reported by about two-thirds of the toluene exposed respondents. These subjects also complained of headaches, sore throats, eye irritation, and difficulty with sleep. When the exposed subjects were divided into 2 groups, one with TWA exposures of less than 40 ppm and the other with exposures greater than or equal to 40 ppm, the incidence of headache and sore throat, but not dizziness, showed a concentration-response pattern (Yin et al. 1987). Tests of postural sway carried out on 27 United States Air Force workers exposed to jet fuel (mean cumulative exposure 23.8±6.1 ppm toluene) found a significant association between toluene exposure and increased postural sway (Smith et al. 1997). However, the results of this study are confounded by concurrent exposure to other chemicals, including benzene and xylene.

Thirty rotogravure printers from two plants and 72 unexposed workers completed a questionnaire designed to record their neurasthenic complaints and were given a series of tests designed to evaluate psychometric function (Orbaek and Nise 1989). At the time of the study (1985), 19 of the printers were exposed to TWA toluene levels of 11.6 ppm, and the remainder were exposed to 42.4 ppm. However, the printers had been exposed to solvents for at least 10 years (employment ranged 4–43 years), and estimated air concentrations at earlier times were much higher (as high as 453 ppm prior to 1970). Taking the midpoints in the ranges of concentration estimates for 1970–1985 for the two factories and calculating their mean yields, a representative exposure concentration of 140 ppm was determined. Significantly more printers reported neurasthenic symptoms than controls, but no significant differences were found between printers and controls for 10 of 11 psychometric tests and in the remaining test (Cylinder Board test of motor skill), printers performed better than controls.

A stronger correlation between impaired neurobehavioral performance and toluene exposure was seen in 30 female workers exposed to 88 ppm toluene as compared to 30 workers in the same facility exposed to only 13 ppm (Foo et al. 1990). The higher exposure group received poorer test scores in tests of visual retention, visual reproduction, trail making, grooved peg board, digit span, and digit symbol, but not on tests of simple reaction time and finger tapping.

Another group of 29 exposed workers in Singapore (average TWA toluene exposure of 90.9 ppm) performed more poorly than a control group (average TWA exposure of 12.2 ppm) on 8 neurobehavioral tests. The exposed group performed significantly more poorly in verbal and nonverbal memory as measured by the digit span and visual reproduction tests (Boey et al. 1997).

Low-level occupational exposure to an average of 97 ppm toluene for 12–14 years had an apparent effect on hearing in 40 rotogravure workers when brainstem auditory evoked potential (BAEP) results were compared to a group of 40 workers who were of comparable age but were not exposed to toluene (Abbate et al. 1993). Workers were carefully screened to eliminate slight hearing abnormalities or exposure to other chemicals. Two series of stimuli were used, one with 11 repetitions/second and one with 90 repetitions/second. In both cases the intensity was 80 dB/nHL. Mean latencies were significantly higher for the exposed group than the control group for each BAEP wave evaluated (I, III, and V). Discernment mean values for the exposed and control groups were distributed homogeneously with very little overlap of exposed and control responses for both the 11-repetition and 90-repetition cycles. Wave I showed the most pronounced increase in latency. According to the authors, the effects on Wave I could

be due to either a change in the membrane of the peripheral receptor, a modification of the structure of the junction, or a change in the stimulus transduction mechanism.

In two other studies, BAEPs in workers exposed to average concentrations of about 50 ppm for an average of 21.4 years were found to be affected, with a significant decrease in all wave amplitudes and a significant increase in all wave latencies except P2 (Vrca et al. 1995, 1997a).

In a cross-sectional examination of 124 Brazilian workers exposed to various levels of noise and a variety of organic solvents, including toluene at TWA concentrations ranging from 0.037 to 244 ppm, (midpoint=122 ppm), 49% of workers experienced hearing loss (Morata et al. 1997). Toluene exposure (and exposure to a number of other solvents including ethanol and ethyl acetate) was estimated by personal monitoring and measurement of hippuric acid in urine samples. Confidence in the study is limited because of exposure to multiple solvents and possible confounding from noise exposure. However, logistic regression analysis showed hippuric acid concentration to be significantly associated with hearing loss and the odds ratio estimates for hearing loss were 1.76 times greater for each gram of hippuric acid per gram creatinine (95% CI 1.00–2.98).

Occupational exposure to toluene may also affect other sensory-evoked potentials. Visual evoked potentials (P300, N75, N145, and P100 waves) in printers occupationally exposed to average concentrations of 50 ppm toluene for an average of 21 years were compared to those of unexposed controls (matched for alcohol and coffee consumption, smoking, age, years of work, education, and head injuries) (Vrca et al. 1995, 1997a, 1997b). Individual exposure was estimated by measuring toluene levels in blood (0.036 mg/L in exposed workers, 0.0096 mg/L in controls) and hippuric acid levels in urine (0.426 g/g creatine in exposed workers, 0.338 g/g creatine in controls). There was a significant increase in the number of exposed individuals displaying reduced amplitude of P300R waves and prolonged latency of the accompanying spontaneous wave P300F (Vrca et al. 1997b). The amplitudes of the N75, P100, and N145 waves (Vrca et al. 1995, 1997a), and the latency of the P100 wave, were significantly increased in exposed subjects compared with controls (Vrca et al. 1995).

Chronic exposure to toluene may also cause color vision loss. Zavalic et al. (1998a) examined color vision in 83 controls, 41 shoemakers, and 32 printers exposed respectively to geometric mean toluene concentrations of 0, 35, or 156 ppm. Toluene exposure was estimated by measuring toluene levels in the air and in the blood of workers, and by measuring the amount of hippuric acid and orthocresol in their urine at the end of the work shift. The technology, ventilation, and types of workplaces included in the study had not changed in the preceding 30 years. Color confusion was significantly higher in printers

compared with both shoemakers and controls. Color confusion index was increased in shoemakers compared with controls, but the difference was not significant (Zavalic et al. 1998a). Regression analysis established a significant correlation between color confusion as a dependent and alcohol intake and age as independent variables for the control group. Age- and alcohol-adjusted color confusion index was significantly increased in printers compared with shoemakers and controls, and in shoemakers compared with controls. After age and alcohol adjustments, individual color confusion indices were significantly correlated with individual exposure estimates (air, blood, or urine) in printers, but in shoemakers, the correlation was not statistically significant. The significantly increased color confusion index for the shoemakers in this study was assessed as a less-serious adverse effect and the LOAEL of 32 ppm served as the basis for the chronic-duration inhalation MRL, 1 ppm, for toluene (see footnote of Table 2.1, Section 2.5 and Appendix A).

Further analysis of color vision loss in the same groups of workers described above (Zavalic et al. 1998a) was carried out to compare loss in the blue-yellow and red-green ranges (Zavalic et al. 1998c). Both blue-yellow and red-green color confusion were significantly increased in printers, but there was no significant difference in the prevalence of either type of color confusion between exposed and unexposed workers (Zavalic et al. 1998c).

Color vision impairment was also evaluated in another group of 45 male workers exposed to mean concentrations of about 120 ppm toluene (Zavalic et al. 1998b). Color vision was significantly impaired in exposed workers compared with unexposed controls. A comparison of color vision assessments made on Monday and Wednesday mornings showed no significant difference. This suggests that color vision impairment results from chronic rather than acute exposure to toluene.

Muttray et al. (1995, 1999) also attempted to distinguish between effects on vision due to chronic and acute exposure to toluene. Color vision was assessed in 59 male rotogravure workers occupationally exposed to unspecified levels of toluene for periods of 1 month to 36 years (mean of 10 years) (Muttray et al. 1995). Results of vision testing at the beginning and end of the work week were compared and no difference was recorded. A second study compared color vision in eight printers (occupationally exposed to toluene) and eight workers previously unexposed to toluene, before and after cleaning a print machine with toluene (Muttray et al. 1999). The task took 28–41 minutes and involved exposure to 300–362 ppm toluene (1,115–1,358 mg/m^3). No impairment in color vision was recorded for either group. However, a comparison of the precleaning performance of the printers with that of a group of matched controls

showed a non significant decrease in color vision for the printers, which may indicate a chronic effect of toluene exposure on color vision (Muttray et al. 1999).

Studies of human color vision impairment suggest that vision impairment results from chronic, rather than acute, exposure to toluene (Muttray et al. 1995, 1999; Zavalic et al. 1998a, 1998b, 1998c). The mechanism by which toluene exposure influences color vision is not known. Visual evoked potentials are affected in chronically exposed individuals and show exposure-related changes in amplitude and latency (Poblano et al. 1996; Vrca et al. 1995, 1997a, 1997b). However, it is not clear whether the impairment of color vision produced by toluene exposure is due solely to neurological damage or also involves damage to the eyes. Toluene exposure causes eye irritation in humans (Andersen et al. 1983; Baelum 1990; Carpenter et al. 1944; Meulenbelt et al. 1990) and animals (Ono et al. 1976), but no studies were located that examined eyes for structural damage due to chronic toluene exposure.

A number of studies of humans chronically exposed to mixtures of solvents containing toluene provide supporting evidence for the neurotoxicity of toluene, but concurrent exposure to other solvents limits the conclusions that can be drawn from the results. Painters (100 individuals) exposed to toluene and other solvents for 1–40 years had poorer performance on the block design of visual cognitive ability (Hanninen et al. 1976). Another study of 325 painters exposed to mixed solvents (including toluene) for an average of 5 years found that reduced ability in tests of pattern comparison and memory was correlated with solvent-exposure (Tsai et al. 1997). Shoemakers exposed to toluene (20 or 71 ppm) and other solvents for more than 10 years showed a significant reduction in the Santa Ana dexterity test and a non significant reduction in visual retention (Lee et al. 1998a). Workers exposed to mixed solvents (including toluene) during a spraying process showed a significant impairment of color vision with errors of the blue-yellow type (Muttray et al. 1997). A study of chronic petrol sniffers found that petrol sniffing was associated with neurological and cognitive abnormalities such as tremors, abnormal reflexes, and deficits of visual attention and memory (Maruff et al. 1998). However, significant correlations were recorded for the magnitude of neurological and cognitive defects and blood lead levels, but not for neurological and cognitive defects and blood hydrocarbon levels (Maruff et al. 1998). A group of workers exposed to mixed solvents and admitted to the hospital due to suspicion of solvent-induced chronic toxic encephalopathy had reduced scores in tests of distorted speech and cortical response audiometry compared to unexposed controls (Niklasson et al. 1998).

In laboratory animals, acute exposure to toluene has both excitatory and depressant effects on the central nervous system. A high correlation between the extent of central nervous system depression and brain

toluene levels was observed in both mice and rats exposed to 2,600–12,000 ppm toluene for varying periods of time (Bruckner and Peterson 1981a, 1981b). Acute exposure to toluene concentrations between 500 and 15,000 ppm produced an initial increase in locomotor activity in rats and mice followed by a decrease in activity with longer exposure (Bowen and Balster 1998; Bushnell et al. 1985; Hinman 1987). Exposure of mice to 300 ppm for 6, 1-hour exposures with 3–4 days between exposures, had no effect on animal movements. Movements were increased at concentrations of 560–1,780 ppm, but were decreased at concentrations of 3,000 ppm (Wood and Colotla 1990). This study clearly indicates a biphasic response with low-concentration stimulation and high-concentration depression of motor activity.

Monkeys exposed to concentrations of 2,000–4,500 ppm toluene (head only) for 50 minutes on 2 days separated by 3 days without exposure, showed significantly increased response time and decreased accuracy on a test of conditioned response to a reward stimulus for concentrations (Taylor and Evans 1985). Exposure to toluene concentrations of 2,000 ppm or less did not cause overt signs of neurological impairment such as ataxia and tremors. Exposure to 100 and 200 ppm toluene had no effect on performance, but exposure to 500 and 1,000 ppm toluene caused nonsignificant decreases in response time and accuracy.

Exposure to 1,500 ppm toluene 1 hour/day for 14 days produced nystagmus (involuntary movement of the eyeballs) in rats with disturbances in the vestibular and opto-oculomotor systems (Larsby et al. 1986). These findings suggest that the cerebellum is a target site for toluene, and confirm an earlier report that toluene caused nystagmus in rats when arterial blood levels were greater than 75 ppm (Tham et al. 1982).

In several animal studies, acute toluene exposure diminished the ability of rats to perform trained neuromuscular responses. Exposure of rats to 125, 250, or 500 ppm toluene for 4 hours caused a decline in lever-press shock avoidance performance 20 minutes after exposure, but recovery was complete 2 hours later (Kishi et al. 1988). A single 4-hour exposure to concentrations of 810–6,250 ppm toluene caused a concentration-related impairment of performance by rats in tests designed to measure neuromuscular performance (Mullin and Krivanek 1982). Exposure to $480 ppm toluene for 4 hours decreased the ability of trained rats to perform a sequence of lever press actions associated with a reward (milk) (Wood et al. 1983).

In animals, changes in the levels of brain neurotransmitters in rodents exposed to toluene have been observed. Significant localized changes in dopamine (DA) or norepinephrine (NE) brain levels were

noted in rats exposed to 400 ppm toluene 24 hours/day for 30 days (Ikeda et al. 1986) and in newborn male rats 7 weeks after a 10-day exposure to 80 ppm toluene for 6 hours per day (von Euler et al. 1989b). Neurotransmitter levels in some areas of the brain were increased, in some areas were decreased, and in other areas remained the same. Toluene exposure at 80 ppm for 4 weeks was found to affect dopamine D_2 agonist binding in the rat caudate-putamen (Hillefors-Berglund et al. 1995; von Euler et al. 1993). Dopamine levels were increased in the cerebellum and striatum of rats exposed to 1,000– 4,000 ppm toluene for 20 minutes, while norepinephrine and 5-hydroxytryptamine were significantly increased in the cerebellum and cortex (Kim et al. 1998). Several significant changes in the activities of enzymes responsible for neurotransmitter synthesis (glutamic acid decarboxylase, choline acetyltransferase and aromatic amino acid decarboxylase) in different areas of the brain were seen in male rats exposed to toluene at concentrations of 50–1,000 ppm for 4 weeks or 500 ppm for 12 weeks (Bjornaes and Naalsund 1988). Concentration-response trends were not apparent in the data and there were variant responses by different areas of the brain. There was also some evidence of change in glutamate and gamma amino butyric acid (GABA) binding. Binding increased in most of the brain areas studied, but decreased in some areas. Because of the variability in response, these data are difficult to evaluate. Von Euler et al. (1994) reported that rats exposed to 80 ppm for 6 hours per day for 4 weeks, had increased serum prolactin levels. However, no significant changes in serum prolactin levels were reported at concentrations up to 320 ppm, also for 4 weeks exposure (Hillefors-Berglund et al. 1995). Exposure to 400 ppm toluene 7 hour/day for 10 days produced a statistically significant increase in the total dehydrogenase activity in the brains of female mice (Courtney et al. 1986).

Changes in brain levels of glial fibrillary acidic protein (GFAP), a structural marker for astrocytes, have been found in toluene-exposed rats. Rats exposed to 1,000 ppm toluene for 3 or 7 days exhibited a significant decrease in GFAP levels in the thalamus (Little et al. 1998). Rats exposed to 100–3,000 ppm toluene 6 hours/day, 5 days/week for up to 42 days exhibited changes in the concentration of GFAP in the cerebellum, hippocampus, and thalamus (API 1997). For the first week of exposure, GFAP concentration of exposed animals was significantly increased in the cerebellum and hippocampus, and decreased in the thalamus compared with unexposed controls (API 1997). After 21 days, the concentration of GFAP in the hippocampus was significantly decreased in rats exposed to 1,000 ppm compared with controls, while at 42 days, rats exposed to 300 ppm had significantly higher concentrations of GFAP in the cerebellum compared with controls, but rats exposed to 1,000 ppm did not (API 1997). In mice exposed to 500–2,000 ppm for 8 hours, no significant alterations in *c-fos*, *c-jun*, or GFAP mRNA in the cerebrum were found (Matsuoka et al. 1997).

Rats continuously exposed to toluene at 320 ppm for 30 days had decreased total brain weight and decreased weight of the cerebral cortex (Kyrklund et al. 1987). There was a decrease in the total phospholipid content of the cerebral cortex accompanied by a small increase in phosphatidic acid levels. These data suggest a breakdown of phospholipids resulting in a loss of gray matter (Kyrklund et al. 1987). The mechanism of action for this effect is uncertain. Increased relative brain weights and ataxia in rats were reported after 15-week exposures to toluene at 2,500 and 3,000 ppm 6.5 hours/day, 5 days/week (NTP 1990). No gross or microscopic tissue changes or changes in brain weights were observed in mice exposed to concentrations up to 2,500 ppm for 14 weeks or in rats or mice exposed by the same protocol to toluene at concentrations up to 1,200 ppm for 2 years (NTP 1990).

Animal studies have demonstrated that intermediate exposure to toluene can produce subtle changes in the auditory system. Intermediate exposure to toluene produced a permanent loss of hearing in the high frequency range (approximately 16 kHz) in rats exposed 14 hours/day to 1,200 ppm for 5–9 weeks or 1,000 ppm for 2 weeks (Pryor and Rebert 1992; Pryor et al. 1984a, 1984b). The threshold concentration for hearing loss was between 700 and 1,000 ppm (Johnson et al. 1988; Pryor et al. 1984b). Hearing loss occurred independent of whether or not exposure was continuous or episodic with many short exposures during the day (Pryor 1991); it was compounded by postexposure high noise levels (Johnson et al. 1988). Lataye and Campo (1997) demonstrated that combined exposure to toluene and noise produced a greater loss of hearing function in rats than exposure to toluene alone or noise alone. In another study, hearing loss was produced in rats after exposure to 1,750 ppm toluene for 6 hours/day, 5 days/week for 4 weeks and loss of hair cells in the organ of corti was observed after exposure to 1,000 ppm (Campo et al. 1997, 1998). Johnson and Canlon (1994) showed loss of outer hair cells in the cochleae of rats exposed to 1,400 ppm 14 hours/day for 8 days. Outer hair cell loss was observed after 5 days of exposure, and loss progressed to the inner hair cells after 6 weeks postexposure. Pryor et al. (1984a) demonstrated that high frequency hearing loss is more severe in weanling rats than in young adult rats, after exposure to 1,200 ppm for 14 hours/day for 5 weeks. Hearing loss due to toluene exposure was also exacerbated by simultaneous treatment with ethanol (Campo et al. 1998) or high doses of acilyl solicylicacid (aspirin) (Johnson 1992).

When rats were preexposed to phenobarbital to stimulate liver metabolism of toluene and then exposed to levels of 1,929 ppm 8 hours/day for 1 week, hearing was not affected as measured by the BAER test (Pryor et al. 1991). However, rats not pretreated with phenobarbital did experience hearing loss. This observation is consistent with the idea that toluene itself, and not one of its metabolites, is responsible for the hearing loss.

Toluene had more of an effect on hearing loss in mice that had a genetic predisposition for early onset spontaneous auditory degeneration than on mice that were predisposed to late onset moderate hearing loss (Li et al. 1992). Thus, the severity of toluene-induced hearing loss appears to be influenced by genetic susceptibility.

Impaired motor functions have been observed in animals following repeated exposure to toluene. Daily exposure of neonate rats to 10,000, 20,000, or 40,000 ppm toluene for 15 minutes from postpartum days 2–30 resulted in a concentration-related increase in the time taken for the righting-reflex to occur (Lorenzana-Jimenez and Salas 1990). At each concentration, the time taken for the righting-reflex to occur decreased over the first 4 weeks of exposure then increased over the last 4 weeks of exposure, but did not regain the level of latency observed in the first week of the experiment. These data suggest that these animals were developing tolerance to some neurobehavioral effects of toluene. However, it is not clear whether this is due to metabolic induction by continued exposure or an age related effect.

Tilting plane and rotarod test performances did not differ significantly between control rats and rats exposed to 1,000 ppm toluene for 8 hours/day, 7 days/week for 13 weeks (Tahti et al. 1983). Rats exposed to 2,500 ppm toluene 7 hours/day for 5 weeks showed a lack of coordination (Von Oettingen et al. 1942). Exposure of rats to concentrations of 2,273 ppm 8 hours per day for 16 weeks or to 2,200–6,200 ppm intermittently (8 hours/day, 15–60 minutes/hour) for 23 weeks caused a shortening and widening of the gait (Pryor 1991). Increased nose-poking was reported in rats exposed to 178–300 ppm toluene for 3 weeks (2 times/week, 2 hours/day) (Wood and Cox 1995). Latency of escape from an electric shock was reduced in both young rats (50 days old) and older rats (120 days old) exposed to 30,000–40,000 ppm toluene for 15 minutes/day for 30 days (Castilla-Serna et al. 1991). Exposure of male weanling rats (23 days old) to 1,200 ppm 14 hours/day for 9 weeks produced a significant reduction in a tone-induced multisensory conditioned avoidance response (Pryor and Rebert 1992).

Circadian rhythms apparently have an effect on toluene metabolism and, thus, on its neurological effects. Rats that were exposed to 4,000 ppm toluene for 4 hours during daylight demonstrated poorer shock avoidance than animals exposed during the dark (Harabuchi et al. 1993). There was a correlation of shock avoidance with toluene levels in the brain; the higher the brain toluene level, the poorer the ability of the animal to properly respond to stimuli and press the lever that prevented the electrical shock.

Flash evoked potential (FEP) responses were abnormal in rats exposed to single 30-minute exposures to 500–16,000 ppm toluene (Rebert et al. 1989a, 1989b) or 15 to 35 minute exposures to 8,000 ppm,

4–9 times/day, 5 days/week for 13 weeks (Mattsson et al. 1990). This technique measures the electrical response of the visual components of the nervous system to a high intensity flashing strobe light. Distortion of the FEP waveform is indicative of impaired visual response to light. In both the acute and subchronic testing situations, the toluene caused changes in the amplitude of several components of the FEP waveform, suggesting that impaired visual response can result from both short and long term exposure to high concentrations of toluene.

Toluene exposure also changes sleep patterns in animals. Both single 4- or 8-hour episodes of toluene exposure (900–4,000 ppm) and repeated exposures, 8 hour/day for 3 weeks (900 and 2,700 ppm), changed patterns of sleep and wakefulness in rats (Arito et al. 1988; Takeuchi and Hisanaga 1977). After the single exposures, there was a decrease in wakefulness and an increase in slow-wave sleep; a prolonged sleep latency was apparent for the 2 days following exposure. Latency was defined as the time interval between the end of the exposure period and the beginning of a particular phase of the sleep cycle. Following the 3-week exposures, there was an increase in wakefulness during the dark period on the 2 days after exposure and a decrease in slow wave sleep on the first day. Exposure to concentrations of 100–700 ppm for 2 hours increased the duration of the wake cycle and decreased both rapid-eye movement and nonrapid eye movement sleep in a concentration-related fashion in young and adult male rats (Ghosh et al. 1989, 1990).

Toluene may affect memory in rats. Rats exposed to 600 ppm toluene for 50 days, starting at 23 days old, were trained in a radial-arm maze and their performance was compared with air-exposed control animals. No significant effects were observed for working memory errors (reentries into “already-entered” areas) (Miyagawa et al. 1995). However, rats exposed to 1,600 or 3,200 ppm toluene for 4 hours showed a concentration-related decrease in accuracy in a neurological test of short-term memory (Miyagawa et al. 1998). Exposure to 1,500 ppm toluene 6 hours/day, 5 days/week for 6 months followed by a 4-month exposure-free period was found to reduce the number of neurons in the rat hippocampus, an important area in the brain for memory and learning processes (Korbo et al. 1996).

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

2.2.1.5 Reproductive Effects

Current data do not provide convincing evidence that toluene causes reproductive effects in humans. Reports that occupational exposure to toluene may lead to an increased incidence of spontaneous abortion (Ng et al. 1992b; Taskinen et al. 1989) have not been supported by the results of animal testing. Studies in rats have shown no evidence of adverse effects on mating or fertility (API 1985; NTP 1990; Ono et al. 1996). Toluene exposure produced microscopic changes in ovarian structure and a reduction in sperm count and the weight of epididymides in rats (Ono et al. 1996; Tap et al. 1996). However, changes in sperm count and epididymus weight were not accompanied by any change in indices of reproductive performance (Ono et al. 1996).

Ng et al. (1992b) reported a significant increase in spontaneous abortion for women employed in an audio speaker factory and exposed to 50–150 ppm (mean of 88 ppm) for 10 years (12.4%), compared with controls exposed to 0–25 ppm toluene from the same factory (2.9%) and unexposed controls from the general population (4.5%). The majority of women examined did not smoke or drink and were of similar socioeconomic status (Ng et al. 1992b). Exposed workers did not report increased incidence for menstrual cycle irregularities, altered extent of uterine bleeding, or occurrence of dysmenorrhea (Ng et al. 1992a). Other possible confounding factors such as exposure to chemicals other than toluene were minimized by inclusion of controls who carried out similar types of work, but did not use toluene-based adhesives (Ng et al. 1992b).

The incidence of spontaneous abortions exceeded population norms among 5 female workers (Lindbohm et al. 1992) and among the wives of 28 or 48 male workers (Lindbohm et al. 1992; Taskinen et al. 1989) exposed to toluene; however, exposure levels were not reported in these studies and only a small number of cases were included. A study of time to pregnancy among the wives of 316 men occupationally exposed to mixed organic solvents found that paternal exposure to organic solvents was significantly correlated with decreased fecundability of primagravida, but not among couples with at least one previous pregnancy (Sallmen et al. 1998). The significance of these studies is limited by the failure to account for the large number of possible confounding factors such as smoking, alcohol consumption, and exposure to mixed chemicals.

Changes in human gonadotropic hormone levels have been associated with toluene exposure. A single case report of testicular atrophy involving chronic solvent abuse was located (Suzuki et al. 1983). Exposure to increasing concentrations of toluene (8–<111 ppm) was associated with decreased plasma

levels of the luteinizing hormone, follicular stimulating hormone, and testosterone levels in 20 or 47 males occupationally exposed for 0.5–39 years to average toluene concentrations of 36 or 29 ppm (Svensson et al. 1992a, 1992b). It is not clear how changes of this sort would affect reproductive success. Interpretation of these data is complicated by the lack of data on male reproductive function. It is generally considered that effects of this sort may be the result of effects of toluene on the catecholamine hormones of the hypothalamus or a consequence of toluene or a metabolite having a dopamine-like activity. Thus, it appears that any potential toluene-induced hormonal effects on reproductive success which may occur may be secondary to effects on the central nervous system.

Significantly decreased sperm counts (26%) and decreased weights of the epididymides (15%) were reported in male rats exposed to 2,000 ppm 6 hours/day for a total of 90 days, including 60 days before mating to females that were exposed for 14 days before and 7 days after mating (Ono et al. 1996). A slight decrease in sperm count (13%) was also observed at 600 ppm, but histological examination of the testes and epididymes found no abnormalities at either concentration. No significant exposure-related effects on mating behavior or fertility indices were found in this study (Ono et al. 1996). Exposure of female rats to 3,000 ppm toluene for 7 days produced abundant vacuoles, lytic areas, and mitochondrial degeneration in the antral follicles of the ovaries (Tap et al. 1996).

The administration of toluene to male mice at concentrations of 100 or 400 ppm for 8 weeks did not induce dominant lethal mutations or cause pre- and postimplantation losses (API 1981). There were no treatment-related histopathological lesions in the testes of rats and mice exposed to up to 3,000 ppm toluene for 14–15 weeks although rats showed a 15% increase in testis weight (NTP 1990). Similarly, toluene did not cause histopathological lesions of the ovaries or testes in rats exposed to toluene (300 ppm) for 24 months (CIIT 1980) or in rats and mice at concentrations of 1,200 ppm for 2 years (NTP 1990). Continuous exposure of pregnant rabbits to 267 ppm during days 7–20 of pregnancy produced maternal toxicity (decreased weight gain) and abortions in 4/8 does, but no effect was observed with exposure to 133 ppm (Ungvary and Tatrai 1985). Exposure of mice to 267 ppm, 3–4 hours/day on days 6–15 of gestation produced no increase in fetal mortality (Ungvary and Tatrai 1985).

In 2-generation reproduction studies in rats, exposure to 2,000 ppm 6 hours/day for up to 95 days did not adversely affect reproductive parameters or offspring survival compared with unexposed controls (API 1985). Another rat study found no effects on mating, fertility, or pregnancy indices for F_1 rats that had been exposed *in utero* to 1,200 ppm toluene for 6 hours/day during gestation days 9–21 (Thiel and Chahoud 1997).

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

2.2.1.6 Developmental Effects

Case reports of birth defects in children of mothers who abused toluene during pregnancy and a report of nervous system defects in Finnish women exposed to mixed solvents in their workplace suggest that exposure to high levels of toluene may be toxic to the developing fetus, but cannot support definitive conclusions because of the lack of exposure data and the inability to adjust for confounding exposures to other potential developmental toxicants.

A retrospective study of 14 women in Finland occupationally exposed to mixed solvents, some of which included toluene, and also exposed to various drugs including aspirin, vasodilators, and diuretics suggested that solvent exposure may increase the risk of central nervous system anomalies and defects of neural tube closure in children exposed *in utero* (Holmberg 1979). However, the sample size was too small to be truly meaningful.

Microcephaly, central nervous system dysfunction, attentional deficits, minor craniofacial and limb anomalies, developmental delay, and variable growth have been described in case reports of children who were exposed to toluene *in utero* as a result of maternal solvent abuse during pregnancy (Arnold and Wilkins-Haug 1990; Arnold et al. 1994; Hersh 1988; Hersh et al. 1985; Lindemann 1991; Pearson et al. 1994; Wilkins-Haug and Gabow 1991a). Growth retardation and dysmorphism were reported in five infants born to women who were chronic paint sniffers (Goodwin 1988).

Children born to toluene abusers have exhibited renal tubular acidosis immediately after birth that is thought to be due to alterations in ion gradient maintenance in the renal tubules. The kidney effects are often associated with hyperchloremia (Erramouspe et al. 1996; Goodwin 1988; Lindemann 1991). In one report (Goodwin 1988) the acidosis was resolved within 3 days of birth, while in the other two reports, it took about 2 weeks for the resolution of the metabolic acidosis. There were no abnormalities in the urinary tract of two children born to chronic toluene abusers based on results of a renal ultrasound evaluation (Hersh 1988).

A number of developmental toxicity studies with rats, mice, and rabbits involving toluene exposure during gestation have been conducted to further describe developmentally toxic effects from toluene and

exposure-response relationships. In general, the results indicate that toluene is not a potent teratogenic agent at exposure levels below those inducing maternal toxicity, but can retard fetal growth and skeletal development and alter development of behavior in offspring.

In pregnant rats exposed to 399 ppm, 24 hours/day on gestation days 1–8 or 9–14, no statistically significantly increased incidences of fetuses with visceral or skeletal malformations were found (Hudak and Ungvary 1978). However, 5/14 dams died, fetal body weight was decreased, and retardation of fetal skeletal development occurred with the days 1–8 exposure protocol, and 2/21 dams died and increased incidence of skeletal anomalies (extra ribs, fused sternebrae) were found with the days 9–14 protocol (Hudak and Ungvary 1978). No maternal mortality, fetal weight loss, or fetal malformations were found in another group of rats exposed to 266 ppm, 8 hours/day on gestation days 1–21, but significantly increased incidence of fetuses with skeletal retardation occurred (Hudak and Ungvary 1978). In other groups of rats exposed to 250, 750, 1,500, or 3,000 ppm, 6 hours/day on gestation days 6–15, no effects were found on maternal or fetal survival, but mean fetal body weights were significantly decreased by about 8–14% at 1,500 and 3,000 ppm and the percentage of fetuses with unossified sternebrae was significantly increased at 3000 ppm (60 versus 37% in controls) (Huntingdon Research Centre 1992b). Incidences of fetuses or litters with skeletal or visceral malformations were slightly increased to a statistically significant extent in the 250-, 1,500-, and 3,000-ppm groups, but the response did not increase with exposure level, indicating that it was not exposure-related (Huntingdon Research Centre 1992b). A preliminary gestational exposure study that did not include comprehensive examination for skeletal and visceral fetal variations and malformations found extreme maternal toxicity and marked resorption of fetuses in pregnant rats exposed to 5,000 ppm 6 hours/day on gestation days 6–15, and significantly decreased mean fetal body weight in a 3,500-ppm exposed group (by about 20% compared with controls) (Huntingdon Research Centre 1992a). No statistically significant effects on maternal or fetal survival, implantation, numbers or incidences of fetuses or litters with skeletal or visceral malformations, anomalies, or variations were found in pregnant rats exposed to 600 or 2,000 ppm, 6 hours/day on gestation days 7–17, but maternal and fetal body weights were reduced in the 2,000 ppm group compared with controls (Ono et al. 1995). Offspring of rats exposed to 1,200 ppm toluene, 6 hours/day on gestation days 9–21 showed a significant reduction in fetal weight, a delay in physical development (vaginal opening) and higher mortality until weaning compared to unexposed controls (Thiel and Chahoud 1997). Rats exposed to 1,000 ppm, 6 hours/day on gestation days 9–21 also had significantly reduced body weights at birth and developmental delay, but no effect on fetal weight or development was recorded for rats exposed to 300 or 600 ppm.

In mice exposed to 133 ppm, 24 hours/day on gestation days 6–13, no effects on maternal survival or incidences of fetuses with malformations were found, but fetal body weight was significantly decreased (Hudak and Ungvary 1978). All 15 pregnant mice died that were exposed to 399 ppm, 24 hours/day on gestation days 6–13 (Hudak and Ungvary 1978). Exposure of pregnant mice to 133 or 266 ppm, 3–4 hours/day on gestation days 6–15, did not significantly affect maternal or fetal survival, or incidences of fetuses with visceral or skeletal anomalies or malformations, but, in the group exposed to 266 ppm, incidences of fetuses with decreased body weight and skeletal retardations were significantly increased (Ungvary and Tatrai 1985). Other groups of pregnant mice exposed to 200 or 400 ppm, 7 hours/day on gestation days 7–16 showed no statistically significant differences from controls in maternal or fetal survival, maternal or fetal body weights, and the number of implantation sites or live fetuses (Courtney et al. 1986). Significantly increased incidence of fetuses or litters with visceral or skeletal anomalies or malformations were restricted to increased litters with fetuses with enlarged renal pelves in the 200-ppm group (but not in the 400-ppm group) and a difference in the distribution of fetuses with varying numbers of ribs in the 400-ppm group compared with the control group. Courtney et al. (1986) suggested that these effects may be due to toluene-induced "desynchronization of growth and maturation" of the developing fetus. Offspring, evaluated 21 days after birth, of a separate group of pregnant mice exposed to 400 ppm toluene by the same protocol showed no significant differences from control offspring in body or organ weights or activities of lactate dehydrogenase in several tissues, except that brain activities of this enzyme were elevated (Courtney et al. 1986).

In rabbits exposed to 133 ppm, 24 hours/day on gestation days 7–20, no significant effects were found on maternal or fetal survival, fetal body weight, or incidences of fetuses with skeletal retardation, minor anomalies, or skeletal or visceral malformations (Ungvary and Tatrai 1985). Following exposure to 266 ppm by the same protocol, 2/8 dams died, 4/8 dams aborted, and no live fetuses were found at sacrifice (Ungvary and Tatrai 1985). In other groups of rabbits exposed to 30, 100, 300, or 500 ppm, 6 hours/day on gestation day 6–18, no signs of maternal toxicity occurred and no significant effects, compared with controls, were found on fetal weight or survival, pre- or postimplantation losses, or incidences of fetuses with external, soft-tissue, or skeletal variations or malformations (Klimisch et al. 1992).

Results from studies of neurobehavioral end points in rats following *in utero* exposure to toluene suggest that maternal exposure to concentrations above 1,200 ppm, 6 hours/day during late embryonic and fetal development can impair behavioral development of rat offspring. Rat pups, that were evaluated on postnatal days 1–20 and whose mothers were exposed to 2,000 ppm for 60 minutes, 3 times/day on

gestation days 12–17, gained less weight and displayed significant performance deficits in neurobehavioral tests of reflex development, muscle strength, and motor coordination (Jones and Balster 1997). These effects were not observed in offspring of dams exposed to 200 or 400 ppm by the same protocol (Jones and Balster 1997). Rat offspring of dams exposed to 600 or 2,000 ppm, 6 hours/day on gestation days 7–17 showed no significant differences from control rats in tests of reflexes, locomotor activity, balance on a rotating rod, learning ability, or in physical development (e.g., eye opening) during the first 5 days after birth (Ono et al. 1995). No consistent and concentration-dependent performance deficits were found in tests of reflexes, balance on a rotating rod, locomotor activity, or discrimination learning in rat offspring, evaluated at several ages, of dams exposed to 300, 600, 1,000, or 1,200 ppm, 6 hours/day on gestation days 9–21 compared with controls (Thiel and Chahoud 1997).

In groups of rat pups exposed to toluene (100 or 500 ppm, 12 hours/day) from postnatal days 1–28, the volumes of the granular cell layer of the area dentate of the hippocampus were smaller (6 and 13%, respectively) compared to unexposed controls (Slomianka et al. 1990). However, when animals in the 500 ppm group were allowed to recover for 92 days after exposure ceased, these effects were reversible (Slomianka et al. 1992). The authors attributed this response to desynchronization in growth and maturation.

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

2.2.1.7 Genotoxic Effects

Human data are inconclusive with regard to the genotoxicity of toluene. Studies of exposed workers are limited by concurrent exposure to other chemicals, small cohort size, and a lack of historical exposure monitoring, and it is likely that they are not sufficiently sensitive to detect small, but significant, manifestations of genetic toxicity in toluene exposed workers. Genotoxicity testing of laboratory animals *in vivo* has been limited and has produced mostly negative results. Negative results were also reported in the *in vitro* studies discussed in Section 2.5.

An analysis of chromosome abnormalities in peripheral lymphocytes of printers exposed to 104 to 1,170 ppm toluene and unexposed workers from the same and nearby sites found an increase in chromosome breaks and aberrant cells in lymphocytes of exposed workers (Pelclová et al. 1990). Hammer et al. (1998) reported a concentration-related increase in sister chromatid exchange in

lymphocytes of printers exposed to 141–328 mg/m^3 (38–87 ppm) airborne toluene (measured by personal monitor). Chromosome analysis of lymphocytes of workers exposed to 200–300 ppm toluene in a rotogravure printing plant found a significantly increased frequency of sister chromatid exchanges and chromatid breaks compared to unexposed controls (Bauchinger et al. 1982). Re-examination of workers from the same plant after cessation of exposure to toluene found that more than 2 years without exposure was necessary to remove a significantly higher incidence of chromatid-aberrations in workers than in never-exposed controls (Schmid et al. 1985). Lymphocytes from printers exposed to a median time-weighted air level of 150 mg/m^3 (40 ppm) toluene per week were found to be significantly more sensitive to the production of micronuclei after stimulation with pokeweed mitogen than lymphocytes from unexposed controls (Nise et al. 1991).

A study of DNA damage in Bulgarian shoe workers exposed to factory air containing 96.0–412.3 mg/m^3 toluene (28–121 ppm) found no exposure related differences in DNA damage in leukocytes as assessed by the Comet assay (Pitarque et al. 1999). Toluene did not induce sister chromatid exchanges in lymphocytes of volunteers exposed to 50 ppm airborne toluene for 7 hours/day for 3 days on 3 occasions at 2 week intervals (Richer et al. 1993). Other investigators have also found no correlation between chronic occupational exposure to toluene and increased frequencies of either chromosome aberrations (Haglund et al. 1980; Maki-Paakkanen et al. 1980) or sister chromatid exchanges (Haglund et al. 1980).

In vivo tests of toluene in laboratory animals have produced mixed results. Exposure to toluene vapor induced mitotic arrest (C-mitosis) in embryos of the grasshopper, *Melanoplus sanguinipes* (Liang et al. 1983). Toluene was reported to induce chromosomal aberrations in the bone marrow cells of rats following exposure by inhalation (Dobrokhotov and Enikeev 1977). Toluene did not induce DNA damage in the blood, bone marrow, or liver of mice exposed to 500 ppm toluene for 6 hours/day, 5 days/week for eight weeks (Plappert et al. 1994). Toluene did not induce dominant lethal mutations in sperm cell of mice exposed to 400 ppm for 6 hours/day, 5 days/week for 8 weeks, but female mice were not assessed for genotoxic effects (API 1981). Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

Eleven human epidemiology studies were located that assessed toluene exposure as a possible risk factor for cancer. Cancers of most sites were not significantly associated with toluene exposure in any study and there was weak consistency in the findings of those studies that did find association of a particular cancer type with toluene exposure. Three cohort studies involved occupationally exposed workers

exposed predominantly to toluene (Antilla et al. 1998; Svensson et al. 1990; Walker et al. 1993), whereas the remainder of the human studies primarily involved subjects exposed to mixtures of solvents including toluene (Austin and Schnatter 1983; Blair et al. 1998; Carpenter et al. 1988; Gérin et al. 1998; Lundberg and Milatou-Smith 1998; Olsson and Brand 1980; Wen et al. 1985; Wilcosky et al. 1984). The information from these studies is inadequate to assess the carcinogenic potential of toluene, predominantly because of the lack of consistent findings across the studies and the likelihood that many of the studied groups were exposed to multiple chemicals.

Svensson et al. (1990) compared cancer incidence and mortality in a cohort of Swedish printers, exposed primarily to toluene and employed for at least 3 months between 1925 and 1985, to mortality and cancer incidence for the region. Current and historical monitoring data were used to estimate yearly average concentrations of toluene in the air. Concentrations had declined from about 450 ppm in the 1940s to 30 ppm by the mid-1980s. There were indications of excess risk of morbidity (standardized incidence ratio, SIR) and mortality (standardized morbidity ratios, SMR) for respiratory tract cancer (SMR, 1.4; 95% CI, 0.7–2.5; n=11; SIR, 1.8; 95% CI, 1.0–2.9; n=16), stomach cancer (SMR, 2.7; 95% CI, 1.1–5.6; n=7; SIR, 2.3; 95% CI, 0.9–4.8; n=7) and colo-rectal cancer (SMR, 2.2; 95% CI, 0.9–4.5; n=7; SIR, 1.5; 95% CI, 0.7–2.8; n=9), but there was no significant association between increased risk and cumulative exposure.

Walker et al. (1993) conducted a cohort mortality study among 7,814 shoe-manufacturing workers (2,529 men and 5,285 women) from two plants in Ohio in operation since the 1930s. Workers were exposed to solvents and solvent-based adhesives. Based on results of a hygiene survey (1977–1979), exposure was thought to be primarily to toluene (10–72 ppm), but other chemicals (e.g., 2-butanone, acetone, and hexane) were also recorded at similar concentrations. IARC (1999) noted that benzene may have been present as an impurity of toluene. Mortality follow up was from 1940 to 1982 and relative risk estimates (SMRs) were derived using the general population of the United States as controls. There were excess risks of lung cancer for both men (SMR, 1.6; 95% CI, 1.2–2.0; n=68) and women (SMR, 1.3; 95% CI, 0.9–1.9; n=31), but smoking may have been a confounding factor and relative risk of lung cancer did not increase with increasing duration of employment. There was a slight excess risk for colon cancer among men (SMR, 1.3; 95% CI, 0.8–2.1; n=18) and women (SMR, 1.2; 95% CI, 0.8–1.8; n=28). Other cancers showed no excess risk.

Antilla et al. (1998) carried out a retrospective cohort analysis of 5,301 workers (3,922 male and 1,379 female) monitored for biological markers of occupational exposure to styrene, toluene or xylene

over the period 1973–1992. No increase in overall cancer risk or risk for cancers at specific tissue sites was associated with exposure to toluene, except for a non significant increase in the incidence of lung cancer in individuals exposed to toluene for more than 10 years (SIR, 1.62; 95% CI, 0.33–4.73; 3 cases). Antilla et al. (1998) noted, however, that these workers may also have been exposed to benzene.

Many of the other human epidemiological cancer studies showed positive associations between exposure to toluene and cancer at one or more tissue site, but individuals were exposed to multiple chemicals in all of these studies (Austin and Schnatter 1983; Blair et al. 1998; Carpenter et al. 1988; Gérin et al. 1998; Lundberg and Milatou-Smith 1998; Olsson and Brandt 1980; Wen et al. 1985; Wilcosky et al. 1984). Nested case-control studies included studies of prostate and brain cancer within cohorts of Texas petrochemical plant workers (Austin and Schnatter 1983; Wen et al. 1985), of lung cancer, stomach cancer, and leukemia among U.S. rubber workers (Wilcosky et al. 1984), cancer of the central nervous system among a group of Tennessee nuclear facility workers (Carpenter et al. 1988), prostate cancer and multiple myeloma among Swedish paint industry workers (Lundberg and Milatou-Smith 1998), and multiple myeloma, nonHodgkin's lymphoma, and breast cancer among aircraft maintenance facility workers (Blair et al. 1998). Community-based case-control studies examined possible associations between Hodgkin's disease in Swedish patients and controls (Olsson and Brandt 1980) and several cancer types in Canadian patients and controls (Gérin et al. 1998).

Inhalation cancer bioassays carried out in experimental animals have produced no evidence to support toluene as a potential carcinogen. No increased incidences of treatment-related neoplastic lesions were observed in Fischer 344 rats or $B_6C_3F_1$ mice exposed to toluene concentrations up to 1,200 ppm for 6.5 hours/day, 5 days/week for 2 years (NTP 1990). Similar results were reported for another study in which Fischer 344 rats were exposed to toluene concentrations up to 300 ppm 6 hours/day, 5 days/week for 2 years, but the maximum exposure concentration in this study was likely below that necessary to approach a maximum tolerated dose (CIIT 1980). The NTP (1990) study was well conducted, achieved the maximum tolerated dose, and provides evidence suggesting a lack of carcinogenicity of toluene in experimental animals.

2.2.2 Oral Exposure

Studies of the effects of oral exposure to toluene are limited. Only one study was located regarding health effects in humans after oral exposure to toluene and there are only a minimal number of animal studies.

2.2.2.1 Death

Ingestion of approximately 60 mL (625 mg/kg) of toluene proved fatal for a 51-year old male (Ameno et al. 1989). Death occurred within 30 minutes of ingestion. The autopsy results revealed constriction and necrosis of the myocardial fibers, a markedly swollen liver, congestion and hemorrhage of the lungs, and acute tubular kidney necrosis. The probable cause of death was determined to be severe depression of central nervous system function.

The limited number of studies on the acute oral toxicity of toluene in animals have focused on lethal effects. The acute oral LD_{50} of toluene in adult rats ranged from 5.5 to 7.4 g/kg (Kimura et al. 1971; Smyth et al. 1969; Withey and Hall 1975; Wolf et al. 1956). Age may play a role in determining the lethal dose for toluene. The LD_{50} value for 14-day-old rats was 3.0 g/kg, which is markedly lower than the adult values (Kimura et al. 1971).

Mice were more sensitive than rats to the lethal effects of toluene in 13-week gavage studies. All rats and mice that received 5,000 mg/kg died within the first week. Mortality was also high for groups receiving 2,500 mg/kg with eight out of ten male rats, one out of ten female rats, and with four out of ten male and female mice dying before the end of the study. A dose of 1,250 mg/kg/day was lethal in 10% of female mice but no deaths occurred in male mice or in rats of either sex (NTP 1990). LOAEL values from each reliable study for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

Human data pertaining to the systemic effects of oral exposure to toluene are limited to two case studies (Ameno et al. 1989; Einav et al. 1997). Animal data are also limited, but include cardiovascular, hematological, hepatic, and renal effects in animals exposed orally to toluene at dosage levels up to 2,500 mg/kg/day for 13 weeks, or 590 mg/kg/day for 6 months (Hsieh et al. 1989; NTP 1990; Wolf et al. 1956). Exposure of rats or mice to toluene doses #2,500 mg/kg/day for 13 weeks was not found to produce any musculoskeletal, gastrointestinal or respiratory effects (NTP 1990). All systemic 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 2-2 and plotted in Figure 2-2.

Table 2-2. Levels of Significant Exposure to Toluene - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
	ACUTE EXPOSURE							
	Death							
1	Human	once				625	(death in 30 minutes)	Ameno et al. 1989
2	Rat (Sprague-Dawley)	NS (G)				2610	(LD_{50} 14 day-old rat)	Kimura et al. 1971
3	Rat (Sprague-Dawley)	NS (G)				5568	(LD_{50} young adult rat)	Kimura et al. 1971
4	Rat (Sprague-Dawley)	NS (G)				6438	(LD_{50} adult rat)	Kimura et al. 1971
5	Rat	NS (G)				7300	(LD_{50})	Smyth et al. 1969
6	Rat	once (G)				5580 M	(LD_{50} adult rats)	Withey and Hall 1975
7	Rat (Wistar)	once (G)				7000	(LD_{50} young adult rats)	Wolf et al. 1956

Table 2-2. Levels of Significant Exposure to Toluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
8	Human	once	Resp			625 M (lung congestion and hemorrhage)	Ameno et al. 1989
			Cardio			625 M (necrosis of myocardial fibers)	
			Gastro	625 M			
			Hepatic			625 M (enlarged liver)	
			Renal			625 M (acute tubular necrosis)	
9	Rat (Sprague-Dawley)	Gd 6-19 1x/d (GO)	Bd Wt			520 F (24% decrease in maternal body wt gain)	Gospe et al. 1994
	Neurological						
10	Rat (Long- Evans)	once (GO)			250[b] M (decrease in amplitude in FEP N3 peak)		Dyer et al. 1988
11	Rat (Sprague-Dawley)	once (G)			2610 M (increase in motor activity, lacrimation and salivation)		Mehta et al. 1998
	Reproductive						
12	Mouse (CD-1)	Gd 7-14 1x/d (GO)		2350 F			Smith 1983
	Developmental						
13	Rat	Gd 6-19				650 (reduced brain development of fetus)	Gospe and Zhou 1998

Table 2-2. Levels of Significant Exposure to Toluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
14	Rat (Sprague-Dawley)	Gd 6-19 1x/d (GO)			520 (9.4% reduction in fetal weight)		Gospe et al. 1994
15	Rat (Sprague-Dawley)	Gd 6-19 1x/d (GO)				650 (11.9% decrease in fetal brain weights, 21% decrease in fetal weights, delayed skeletal ossification)	Gospe et al. 1996
16	Mouse (ICR)	Gd 8-12 5 d (G)		1800			Seidenberg et al. 1986
17	Mouse (CD-1)	Gd 7-14 1x/d (GO)		2350			Smith 1983
	INTERMEDIATE EXPOSURE						
	Death						
18	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)				2500 (80% of males and 10% of females died)	NTP 1990
19	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)				1250 F (1/10 died) 2500 M (4/10 died)	NTP 1990

Table 2-2. Levels of Significant Exposure to Toluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
20	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)	Resp	2500			NTP 1990
			Cardio	625 F 1250 M	1250 F (11% increase in relative heart weight) 2500 M (38% increase in relative heart weight)		
			Gastro	2500			
			Hemato	2500			
			Musc/skel	2500			
			Hepatic	312 M 625 F	625 M (8% increase in liver weight) 1250 F (22% increase in liver weight)		
			Renal	312 M 625 F	625 M (6% increase in kidney weight) 1250 F (8% increase in kidney weight)		
			Endocr	2500			
			Bd Wt	1250 M 2500 F	2500 M (body weight 19% lower than controls)		
21	Rat (Wistar)	6 mo 5 d/wk 1x/d (G)	Resp	590 F			Wolf et al. 1956
			Cardio	590 F			
			Hemato	590 F			
			Hepatic	590 F			
			Renal	590 F			

Table 2-2. Levels of Significant Exposure to Toluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL: Less Serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference
22	Mouse (CD-1)	28 d (W)	Hemato	105 M			Hsieh et al. 1989
			Hepatic	22 M	105 M (significant increase in liver weight-19%)		
			Renal	105 M			
			Bd Wt	105 M			
23	Mouse (CD-1)	28 d (W)	Bd Wt	105 M			Hsieh et al. 1990b
24	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)	Resp	2500			NTP 1990
			Cardio	2500		5000 (myocardial degeneration)	
			Gastro	2500			
			Hemato	2500			
			Musc/skel	2500			
			Hepatic	625 M	312 F (7% increase in relative liver weight) 1250 M 10% increase in relative liver weight)		
			Renal	2500			
			Endocr	2500			
			Bd Wt	625 M 2500 F	1250 M (body weight 16% lower than controls)		
	Immunological/Lymphoreticular						
25	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)		2500			NTP 1990

Table 2-2. Levels of Significant Exposure to Toluene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
26	Mouse (CD-1)	28 d (W)		22 M		105 M (diminished immune response)	Hsieh et al. 1989
27	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)		2500			NTP 1990
	Neurological						
28	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)		625		1250 (brain necrosis)	NTP 1990
29	Mouse (CD-1)	28 d (W)			5[c] M (significantly increased levels of norepinephrin & dopamine in brain)		Hsieh et al. 1990b
30	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)		625 M	1250 M (12% increase in relative brain weight)	2500 (ataxia, hypoactivity, prostration)	NTP 1990

Table 2-2. Levels of Significant Exposure to Toluene - Oral (continued)

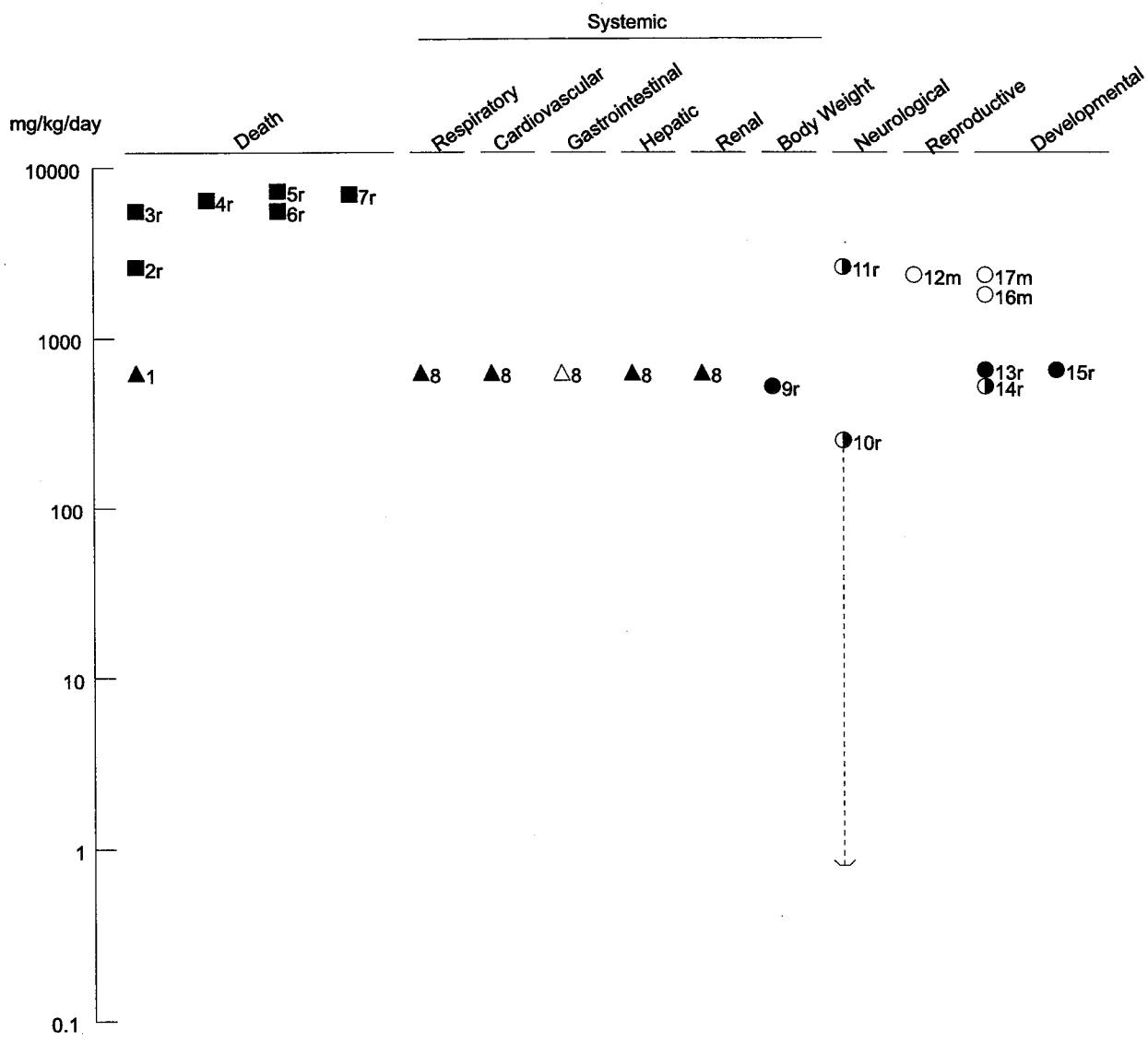
Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)		Serious (mg/kg/day)	
Developmental								
31	Mouse (Hybrid)	Gd 0-21 + ppd 0-55 (W)			4	(impaired rotorod performance, motor coordination)		Kostas and Hotchin 1981

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute oral minimal risk level (MRL); dose divided by an uncertainty factor of 300 (3 for use of a minimally adverse LOAEL, 10 for interspecies differences in response, and 10 for human variability), resulting in an MRL of 0.8 mg/kg/day.
[c]Used to derive an acute oral minimal risk level (MRL); dose divided by an uncertainty factor of 300 (3 for use of a minimally adverse LOAEL, 10 for interspecies differences in response, and 10 for human variability), resulting in an MRL of 0.02 mg/kg/day.

Bd = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; FEP = flash evoked potential; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; LOAEL = lowest-observable-adverse-effect level; M = male; mo = month; NOAEL = no-observable-adverse-effect level; Resp = respiratory; (W) = water; wk = week(s); x = times

Figure 2-2. Levels of Significant Exposure to Toluene - Oral

Acute (≤14 days)



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 for effects other than Cancer

Figure 2-2. Levels of Significant Exposure to Toluene - Oral (Continued)

Intermediate (15-364 days)

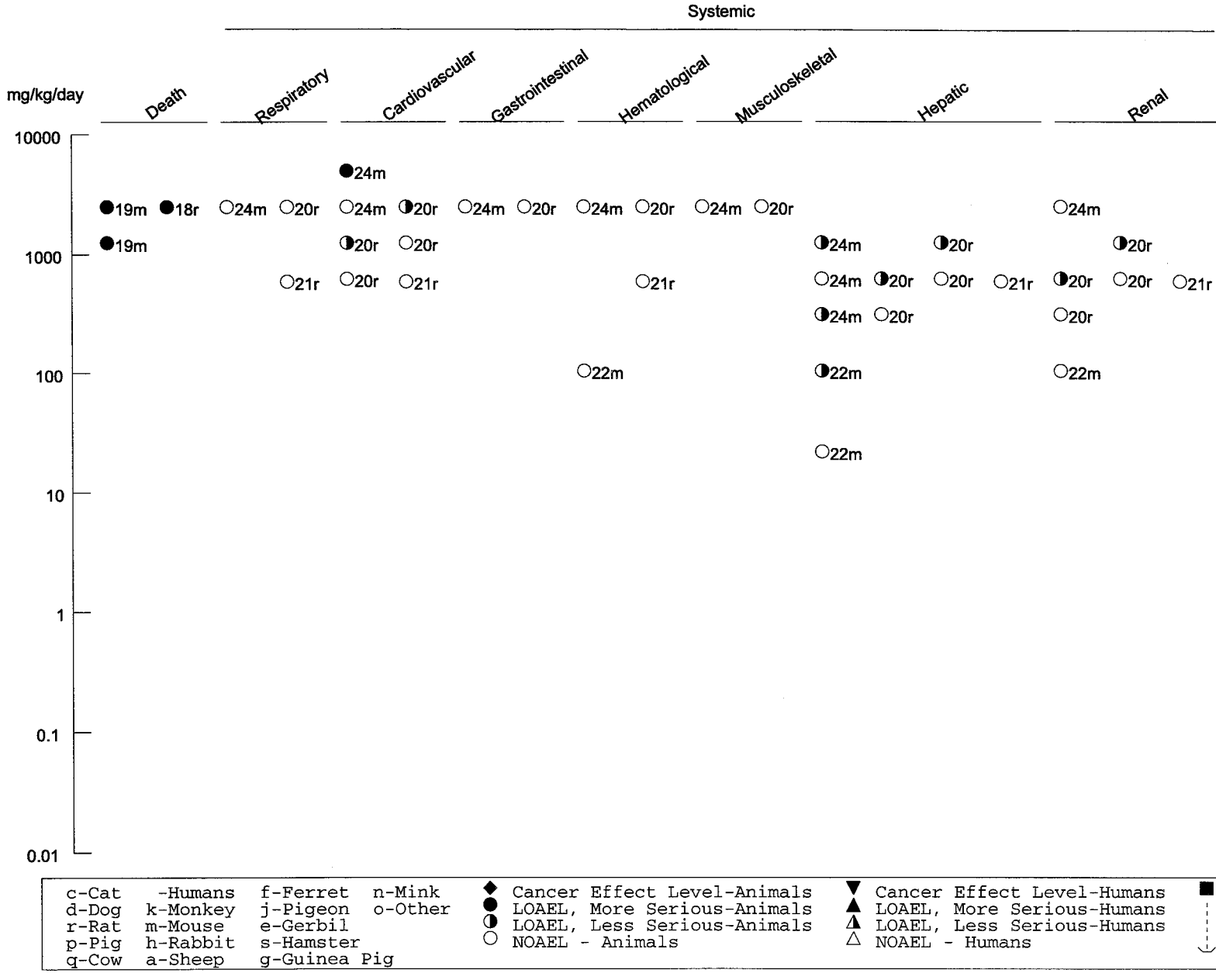
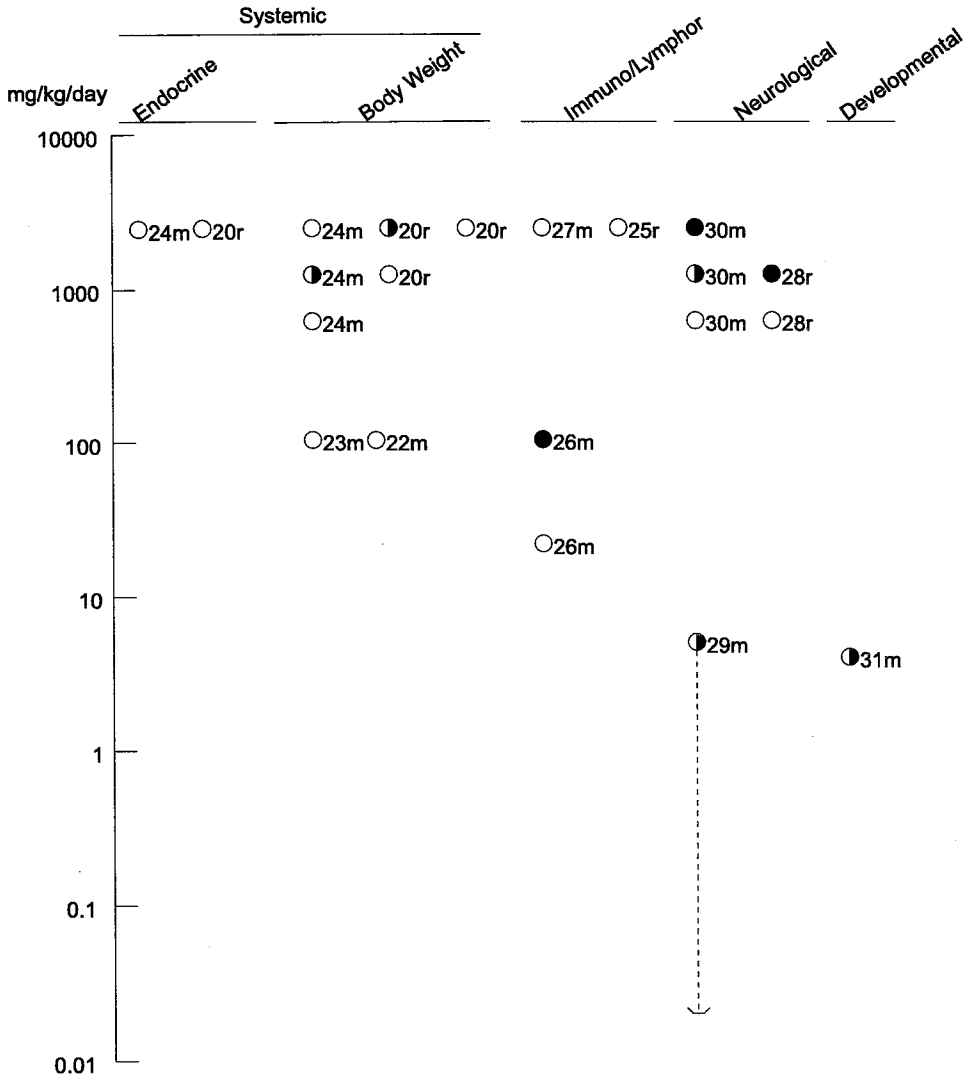


Figure 2-2. Levels of Significant Exposure to Toluene - Oral (Continued)

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 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				

Respiratory Effects. Except for lung congestion and hemorrhage in one case report involving lethality (Ameno et al. 1989), no studies were located regarding respiratory effects in humans after oral exposure to toluene.

No respiratory effects were reported in mice or rats after oral exposure to toluene at dosage levels up to 2,500 mg/kg/day for 13 weeks (NTP 1990) or 650 mg/kg/day for 6 months (Wolf et al. 1956).

Cardiovascular Effects. One case study involving lethality in humans reported necrosis of myocardial fibers after oral exposure to 625 mg/kg toluene (Ameno et al. 1989). Severe sinus bradycardia was reported in a man who accidently ingested 30 mL of an organic solvent containing toluene and other chemicals, the patient was drowsy and complained of dizziness and gastric pain (Einav et al. 1997).

Increased relative heart weights were noted in rats exposed to toluene at 1,250 mg/kg/day for 13 weeks and myocardial degeneration was present in mice exposed to 5,000 mg/kg/day (NTP 1990). All of the mice receiving 5,000 mg/kg/day died during the first weeks of exposure. No effects on the weight or gross morphology of the heart were noted in rats receiving 590 mg/kg/day for 6 months (Wolf et al. 1956).

Gastrointestinal Effects. A case report in humans did not reveal gastrointestinal effects even after oral exposure to a lethal dose of toluene (Ameno et al. 1989).

No gastrointestinal effects were reported in mice or rats after oral exposure to toluene at dosage levels up to 2,500 mg/kg/day for 13 weeks (NTP 1990).

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

There were no changes in total erythrocytes in male mice administered 5–105 mg/kg/day toluene in their drinking water for 28 days, although there was a nonsignificant decrease in the concentrations of leukocytes, lymphocytes, and neutrophils (Hsieh et al. 1989). No effect on erythrocyte counts, leukocyte counts, or hemoglobin concentrations resulted in rats exposed to 590 mg/kg/day for 6 months (Wolf et al. 1956). Neither rats nor mice given doses of 312–2,500 mg/kg/day for 13 weeks displayed any compound-related differences in hematological parameters (NTP 1990).

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

No musculoskeletal effects were reported in mice or rats after oral exposure to toluene at dosage levels up to 2,500 mg/kg/day for 13 weeks (NTP 1990).

Hepatic Effects. The liver of an adult male who died from toluene ingestion (625 mg/kg) was found to be enlarged on autopsy (Ameno et al. 1989).

In mice, there was a significant increase in liver weight after 28 days of ingestion of 105 mg/kg/day toluene in drinking water, but not at doses of 22 mg/kg/day or lower (Hsieh et al. 1989). Relative liver weights increased significantly over control levels in mice administered toluene by gavage for 13 weeks with doses of 312 mg/kg/day and greater in females and 1,250 mg/kg/day and greater in males (NTP 1990). In female rats, the liver weights were increased by exposure to doses of 1,250 mg/kg/day or greater and in male rats by exposure to doses of 625 mg/kg/day or greater. No treatment-related gross or histopathological lesions of the liver were reported (NTP 1990). When rats were exposed for a longer duration, liver weights were not affected and there were no treatment-related lesions in rats that received 590 mg/kg/day toluene by gavage for 6 months (Wolf et al. 1956).

Renal Effects. Data in humans are limited to one case report noting acute tubular necrosis after a lethal exposure to 625 mg/kg (Ameno et al. 1989) and acidosis in another nonlethal case report of thinner consumption (Caravati and Bjerk 1997).

There were no changes in kidney weight for male mice administered doses from 5 to 105 mg/kg/day in drinking water for 28 days (Hsieh et al. 1989), or in female mice given doses of 312–2,500 mg/kg/day by gavage for 13 weeks (NTP 1990). There was a significant decrease in the absolute kidney weight for male mice administered 2,500 mg/kg/day for 13 weeks but no change in the relative kidney weight (NTP 1990). There were significant increases in the relative kidney weights in male rats administered toluene doses of 625 mg/kg/day or greater by gavage for 13 weeks and in females administered doses of 1,250 mg/kg/day (NTP 1990). In addition, lethal exposures of the rats to 5,000 mg/kg/day resulted in hemorrhages of the urinary bladder. No effects on the weight or gross morphology of the kidney were recorded for rats receiving 590 mg/kg/day toluene for months (Wolf et al. 1956).

Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to toluene.

Microscopic examination revealed no effects on the adrenal or thyroid glands in rats and mice administered 312–2,500 mg/kg/day toluene by gavage for 13 weeks (NTP 1990).

Dermal Effects. No studies were located regarding dermal effects in humans or animals after oral exposure to toluene.

Ocular Effects. No studies were located regarding ocular effects in humans or animals after oral exposure to toluene.

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

There were no changes in body weight for male mice administered 5–105 mg/kg/day toluene in their drinking water for 28 days (Hsieh et al. 1989, 1990b). There was also no significant difference in body weights for female rats and female mice given gavage doses of up to 2,500 mg/kg/day for 13 weeks. However, body weights were 16% lower in male mice given 1,250 mg/kg/day and 19% lower in male rats given 2,500 mg/kg/day by gavage for 13 weeks (NTP 1990). Maternal weight gain was 24% lower in rats given 520 mg/kg/day toluene by gavage during gestation days 6–19, compared with control rats (Gospe et al. 1994).

2.2.2.3 Immunological and Lymphoreticular Effects

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

Thymus weights, mixed lymphocyte culture responses, and antibody plaque-forming cell responses were decreased in male mice administered doses of 105 mg/kg/day in their drinking water for 28 days (Hsieh et al. 1989). Mitogen-stimulated lymphocyte proliferation and interleukin-2 immunity were depressed by doses of 22 and 105 mg/kg/day. A dose of 5 mg/kg/day had no effect upon any of these indicators of immune system function. No effects on the histology or weight of the spleen or thymus were reported in rats and mice given gavage doses of up to 2,500 mg/kg/day toluene for 13 weeks (NTP 1990).

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

2.2.2.4 Neurological Effects

Severe depression of central nervous system function was the probable cause of death for a 51-year-old man who ingested approximately 60 mL (625 mg/kg) of toluene (Ameno et al. 1989). No other studies were located regarding neurological effects in humans after oral exposure to toluene.

Male and female rats exposed to single gavage doses of 2,610, 3,915, or 5,220 mg/kg exhibited changes on a variety of neurological tests (Mehta et al. 1998). Significantly greater increases in motor activities were seen at all doses in both male and female rats on day 1; by day 14 after exposure, there were no significant differences, except for vertical motor activity in female rats was significantly reduced at the 2,620 and 3,915 mg/kg/day doses. A dose-dependent increase in abnormal gait was seen on day 1 for male rats at all doses, while female rats exhibited abnormal gait at 3,915 and 5,220 mg/kg. A dose-dependent increase in lacrimation and salivation was seen on day 1 for both males and females at all doses (Mehta et al. 1998).

Single doses of 250–1,000 mg/kg administered by gavage to male rats caused a decrease in the flash evoked potential wave pattern amplitudes (Dyer et al. 1988). This suggests that toluene may have an effect on the visual system at high doses. The LOAEL of 250 mg/kg/day for the effects of the flash evoked potential waveform was used as the basis for the acute-duration oral MRL (0.8 mg/kg/day) (see Section 2.5 and Appendix A).

Brain levels of norepinephrine (NE), dopamine (DA), serotonin (5-HT), and their respective metabolites, vanillylmandelic acid (VMA), homovanillic acid (HVA), and 5-hydroxyindolacetic acid (5-HIAA) were altered in 6 areas of the brain in male CD-1 mice administered toluene (5–105 mg/kg/day) in their drinking water for a 28-day period (Hsieh et al. 1990b). Significant increases of NE, DA, and 5-HT were present in the hypothalamus at all dose levels. The maximum increase occurred with the 22 mg/kg/day dose and there were lesser increases for both the 5 and 105 mg/kg/day doses. Roughly similar fluctuations were seen in the concentrations of VMA and HVA, which are metabolites of DA and NE and 5-HIAA, a serotonin metabolite. In the corpus striatum, the levels of DA and 5-HT were significantly increased at the two highest doses. The level of VMA was also increased significantly at the same doses. In the medulla oblongata, the concentrations of NE, VMA, and 5-HIAA were significantly increased at

the 22 mg/kg/day dose, but not at the other doses, while the levels of 5-HT were significantly increased at the 22 and 105 mg/kg/day doses. NE concentrations were elevated in the midbrain. The 5 mg/kg/day LOAEL from this study was used as the basis for the intermediate-duration oral MRL (0.02 mg/kg/day) (see Section 2.5 and Appendix A).

Exposure to 1,250 and 2,500 mg/kg/day for 13 weeks resulted in increased relative brain weights in male mice (NTP 1990). Necrosis of the brain was present in rats exposed to 1,250 and 2,500 mg/kg/day, but increases in brain weight were only apparent with the 2,500 mg/kg/day dose (NTP 1990). Clinical signs in rats and mice exposed to 2,500 and 5,000 mg/kg/day included ataxia, hypoactivity, prostration, and tremors. No neurological effects were seen in mice or rats at dose levels of 625 mg/kg/day.

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

2.2.2.5 Reproductive Effects

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

There was no effect on the number of mice producing viable litters following oral administration of 2,350 mg/kg on gestational days 7–14 (Smith 1983). Increased relative testicular weights were reported in male mice exposed to 1,250 and 2,500 mg/kg/day by gavage for 13 weeks. However, no effects on the or weight of the prostate, testes, uterus, or ovaries were observed in rats and female mice exposed to 312–2,500 mg/kg/day (NTP 1990).

The highest NOAEL values and all LOAEL values for reproductive effects in mice following acute duration exposure are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

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

Toluene was not a developmental toxicant when administered orally to pregnant mice during the period of organogenesis at doses of 1,800 or 2,350 mg/kg/day (Seidenberg et al. 1986; Smith 1983). Effects on neurological function were observed in mice exposed from 4 to 106 mg/kg/day toluene through their

dams during gestation and lactation and thereafter through their drinking water. Open-field activity was impaired in mice receiving 106 mg/kg/day toluene when measured on postnatal day 35 (Kostas and Hotchin 1981). There was a distinct effect on rotorod performance during the first 2 of 4 consecutive trials in all exposed mice when measured on postnatal days 45–55. However, the effect on rotorod performance was not dose-related.

Following exposure of pregnant rats to gavage doses of 520 or 650 mg/kg/day toluene in corn oil on gestation days 6–19, fetuses showed significantly reduced body weight, delayed skeletal ossification, smaller brain volumes, and decreases in forebrain myelination per cell compared with controls (Gospe and Zhou 1998; Gospe et al. 1994, 1996). The difference in forebrain myelination was the only difference that remained between exposed and control offspring by postnatal day 21 (Gospe and Zhou 1998).

The highest NOAEL values and all LOAEL values for developmental effects in mice following acute and intermediate duration exposures are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

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

2.2.2.8 Cancer

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

There is one oral study on the carcinogenic effects of toluene in animals. Toluene was administered at doses of 500 and 800 mg/kg/day to male and female rats for 104 weeks (Maltoni et al. 1997). A nondose-related increase in total malignant tumors in both males and females at all dose levels, in mammary gland tumors in females at the lower dose, in head cancers in males at the higher dose and females at the lower dose, in lymphomas and leukemias in males at the higher dose and females at both doses, were observed (Maltoni et al. 1997). However, the increased incidences were not dose-related and confidence in the study is low.

2.2.3 Dermal Exposure

There are limited data on the effects of dermal exposure to toluene. There are studies describing occupational exposure of humans to toluene (see Section 2.2.1). Toxicokinetic data (Section 2.3) indicate that humans and animals can absorb toluene across the skin. Studies of dermal exposure to toluene in humans and animals are discussed below.

2.2.3.1 Death

No studies were located regarding lethal effects in humans or animals after dermal exposure to toluene.

2.2.3.2 Systemic Effects

Data are available regarding dermal effects in humans and animals after dermal exposure to toluene. No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, or musculo-skeletal effects in humans or animals after dermal exposure to toluene. In addition, there are data on hepatic, renal, and ocular effects in animals after dermal exposure to toluene. 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 2-3.

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to toluene.

Application of 1 mL toluene to the skin of guinea pigs for 16 hours did not alter liver morphology (Kronevi et al. 1979). Because only one dose of toluene was applied and more sensitive indicators of liver toxicity were not monitored, conclusions cannot be derived regarding the hepatic effects of toluene following dermal exposure.

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to toluene.

Application of toluene to the skin of guinea pigs for 16 hours did not alter renal morphology (Kronevi et al. 1979). The limitations of this study were discussed in the previous section.

Table 2-3. Levels of Significant Exposure to Toluene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
ACUTE EXPOSURE						
Systemic						
Gn pig (albino)	3 d 3x/d	Dermal		10 ul M (skin irritation)		Anderson et al. 1986
Gn pig (albino)	0.25-16 hr	Hepatic	1 mL			Kronevi et al. 1979
		Renal	1 mL			
		Dermal		1 mL (karyopyknosis, karyolysis, perinuclear edema, spongiosis, junctional separation, cellular infiltration)		
Rabbit	once	Ocular		0.1mL (eye irritation)		Hazleton Labs 1962
Rabbit	once	Ocular		0.1 mL (eye irritation)		M B Research Labs 1975b

Gn Pig = guinea pig; LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level

Dermal Effects. In humans, dermal contact with toluene may cause skin damage because it removes skin lipids (EPA 1983a). Workers exposed to mixtures of solvents, of which toluene was generally the major component, reported problems with the skin of their hands (Winchester and Madjar 1986). The specific symptoms associated with the reported skin abnormalities were not reported. Eye irritation in humans occupationally exposed to toluene vapors has also been reported (Meulenbelt et al. 1990). Repeated application of undiluted toluene (amount unstated) to the rabbit ear or shaved skin produced slight to moderate irritation (Wolf et al. 1956). In guinea pigs, continuous contact with toluene resulted in shrinkage and dissolution of the cell nuclei, cellular edema, and cellular infiltration of the dermis (Kronevi et al. 1979). Application of toluene to the skin of guinea pigs, 3 times a day for 3 days, resulted in redness and an increase in epidermal thickness (Anderson et al. 1986). These data suggest that toluene is slightly to moderately irritating to the skin.

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

Slight irritation of the conjunctival membranes, but no corneal injury, was observed in rabbit eyes following direct application of toluene (Hazleton Laboratories 1962; M B Research Labs 1975; Wolf et al. 1956). Moderately severe injury to the eyes of rabbits following direct application of a 40% solution of toluene has also been reported (Carpenter and Smyth 1946). These data suggest that toluene is slightly to moderately irritating to the eyes.

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

2.2.3.3 Immunological and Lymphoreticular Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located for cancer effects in humans after dermal exposure to toluene.

Dermally administered toluene markedly inhibits skin tumorigenesis in the two-stage mouse model utilizing phorbol-12-myristate-13-acetate (PMA) as a promoter (Weiss et al. 1986). The reduction in tumorigenesis was observed in mice initiated with dermal applications of benzo(a)pyrene or 7,12-dimethylbenz(a)anthracene. The pattern of inhibition indicated that the observed effect was not likely to be due to a direct chemical effect on the promoter. The authors speculated that toluene competed for a PMA receptor site, interfered with a biochemical process within the cell membrane, or affected the intracellular cascade between the membrane and the nucleus.

2.3 TOXICOKINETICS

Studies with volunteers and laboratory animals indicate that toluene is readily absorbed from the respiratory and gastrointestinal tracts and, to a lesser extent, through the skin. Animals given toluene orally or by inhalation had high concentrations of toluene in their adipose tissue, brain, and bone marrow, and moderately high concentrations of toluene and its metabolites in liver and kidney. The primary initial steps in toluene metabolism in humans and laboratory animals are side-chain hydroxylation (to form benzyl alcohol) catalyzed predominately by the cytochrome P450 (CYP) isozyme, CYP2E1 (Nakajima and Wang 1994; Nakajima et al. 1991, 1992a, 1992b, 1993, 1997; Tassaneeyakul et al. 1996) followed by oxidation to benzoic acid. Most of the benzoic acid is then conjugated with glycine to form hippuric acid, but a small portion can be conjugated with UDP-glucuronate to form the acyl-glucuronide. Studies with volunteers and human liver microsomes indicate that a very small portion (<1–5%) of absorbed toluene can be converted by CYP1A2, CYP2B2, or CYP2E1 to *ortho*- or *para*-cresol, which are excreted in the urine as sulfate or glucuronate conjugates (Baelum et al. 1993, Nakajima et al. 1997; Tassaneeyakul et al. 1996). In both humans and rats, up to about 75–80% of inhaled toluene that is absorbed can be accounted for as hippuric acid in the urine (Lof et al. 1993; Wang and Nakajima 1992). Much of the remaining toluene is exhaled unchanged. In humans exposed by inhalation, rates of urinary excretion of *ortho*-cresol were about 1,000-fold lower than excretion rates for hippuric acid (Baelum et al. 1993). The excretion of toluene and its metabolites is rapid, with the major portion occurring within 12 hours of exposure.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

In humans exposed to 80 ppm toluene, uptake was rapid as shown by the appearance of toluene (2–5 µmol/L) in the blood within 10–15 minutes of exposure (Hjelm et al. 1988) and by a high correlation between the alveolar and arterial concentrations of toluene both during and after exposure (Carlsson 1982). About 50% of deuterium labeled toluene was absorbed from the lungs in volunteers exposed to 53 ppm for 2 hours during a period of light exercise (Lof et al. 1993). Seven humans exposed to 50 ppm toluene in a closed chamber showed an average retention of 83% of the inspired concentration (Benoit et al. 1985).

Toluene was rapidly absorbed via the lungs of rats; the log concentrations of toluene in the blood and brain were linear functions of the log concentration of toluene in the air (Benignus et al. 1984). In dogs, toluene was found in the arterial and venous blood 2 minutes after the start of exposure (Hobara et al. 1984b).

No information was located regarding possible differences in absorption of inhaled toluene by humans or animals with differences in age.

2.3.1.2 Oral Exposure

Complete gastrointestinal absorption of toluene in human subjects was indicated by monitoring exhaled air for toluene and urine for toluene metabolites (hippuric acid and *ortho*-cresol) following oral administration of toluene as a 2 mg/min infusion for 3 hours through a feeding tube into the stomach (Baelum et al. 1993). Complete absorption of orally administered toluene has also been observed in rats, but oral absorption rates appear to be slower than pulmonary absorption (Pyykko et al. 1977). In this rat study, maximum blood concentrations were observed 1.5–3 hours after administration, whereas maximum blood levels following inhalation were reached in 15–30 minutes.

Ingestion of soil contaminated with toluene can be a concern at hazardous waste sites. Binding to soil does not prevent absorption. The time course for absorption of toluene mixed with sandy soil or clay soil was increased when compared to the time course for pure toluene, but the total amount absorbed was the same based on the area under the blood toluene concentration curve (Turkall et al. 1991).

Studies with brush border membrane vesicles isolated from rat intestines and exposed to toluene indicate that toluene absorption occurs through the lipophilic matrix of the membrane (Alcorn et al. 1991). The removal of proteins from the membrane surface had no effect upon the toluene partition coefficient, but factors affecting the nonesterified membrane fatty acids reduced absorption. In this same *in vitro* study of membrane partitioning, vesicles harvested from the proximal, middle, and distal intestinal segments showed no differences, indicating that concentration and surface area, rather than membrane structure, are the factors determining the amount of toluene absorbed from each portion of the small intestines. Since toluene absorption occurs through the lipid matrix of the membrane, absorption can occur through the mouth and stomach, as well as the small intestines. The amount of toluene absorbed from each organ of the gastrointestinal tract will depend on residence time, absorptive surface area, and partitioning between membrane lipids and lipids in the gastrointestinal tract.

No information was located regarding possible differences in absorption of ingested toluene by humans or animals with differences in age.

2.3.1.3 Dermal Exposure

Toluene is absorbed through human skin slowly (Dutkiewicz and Tyras 1968). The rate of absorption of toluene in human forearm skin was found to range from 14 to 23 mg/cm^2/hour. Based on these estimates, Brown et al. (1984) calculated that bathing in water containing 0.005–0.5 mg toluene/L (15 minutes/day) would result in absorbed dermal dose ranges of 0.0002–0.02 mg/kg/day for a 70-kg adult and 0.0004–0.04 mg/kg/day for a 10.5-kg infant.

Soaking the skin of 2 volunteers with toluene for 5 minutes resulted in a maximum concentration of toluene in blood of 5.4 µmol/L (Aitio et al. 1984). Individual differences were marked, and dramatic changes in blood concentrations were observed over short periods of time. Similar individual differences and highly variable results were reported by Sato and Nakajima (1978) in a study using five volunteers.

Monster et al. (1993) investigated dermal absorption of toluene in 6 rotogravure printing workers. The workers washed their hands with toluene for 5 minutes, and alveolar air samples were collected up to 24 hours after exposure. The concentrations measured the next morning in exhaled air ranged between 0.5 and 10 mg/m^3, clearly demonstrating dermal absorption of toluene.

Toluene in aqueous solution and neat toluene were absorbed through the skin of rats (Morgan et al. 1991). Three solution strengths (0.162, 0.333, and 0.448 mg/L) were tested. Although the blood toluene levels for each strength were near the analytical detection limits, the results of this study indicate that toluene absorption was significant, since only 1% of the body surface was exposed.

Dermal absorption also occurs when animals are exposed to toluene vapors. In nude mice exposure to 300, 1,000, or 3,000 ppm toluene under conditions where there was no respiratory intake of toluene, led to a dose-related and duration-related increase in whole body toluene levels (Tsuruta 1989). The calculated skin absorption coefficient was 1.24 cm/hour. The skin absorption rate for the 300 ppm concentration was 0.0009 mg/cm^2/hour; for the 1,000 ppm concentration, it was 0.0046 mg/cm^2/hour; and for the 3,000 ppm concentration, it was 0.0144 mg/cm^2/hour. Exposure of guinea pigs to an unspecified concentration of toluene for 1 minute, with the skin wiped dry and 1 minute exposures continuing every 30 minutes, for 4 hours, resulted in lower levels of toluene absorption than with continuous exposure for 4 hours (Boman et al. 1995).

No information was located regarding possible differences in absorption of dermally applied toluene by humans or animals with differences in age.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

There is a positive correlation between the levels of toluene in alveolar air and the levels in blood in both humans and animals (Hjelm et al. 1988; Lof et al. 1990; Ovrum et al. 1978). With an exposure in humans of 80 ppm toluene for 4 hours, toluene levels in the blood reached a plateau of 6–7 µmol/L at approximately 2 hours (Hjelm et al. 1988; Lof et al. 1990). In humans, the toluene is distributed between the plasma and red blood cells at approximately a 1:1 ratio according to *in vitro* data; in rats, the ratio is 1:2 based on *in vivo* data (Lam et al. 1990). In the red blood cells, the toluene appears to be associated with the hemoglobin rather than the cell membrane. It is hypothesized that toluene interacts with the hydrophobic core of the heme protein. The interaction of the toluene with the red blood cell increases the amount of toluene that can be accommodated by the aqueous blood medium and facilitates transport of toluene to all areas of the body (including the brain) at a rate that is greater than if toluene was transported only in the plasma.

Autopsies of toluene-exposed humans indicate that absorbed toluene is distributed to lipid-rich and highly vascular tissues such as the brain. For example, toluene levels in the brain and liver of a 16-year-old male who died following an episode of glue sniffing were 297 and 89 µg/mg, respectively (Paterson and Sarvesvaran 1983). Concentrations in the blood were 20.6 µg/mL of toluene and 3.0 µg/mL of acetone. In a man who died following a fall while exposed to toluene during painting, tissue levels of toluene in blood, lung, liver, and brain were 48, 35, 65, and 80 µg/g, respectively (Takeichi et al. 1986).

Within the human brain, toluene has a greater affinity for areas of the brain that contain lipid-rich white matter, such as the brain stem, rather than the areas with larger amounts of gray matter (Ameno et al. 1992). The hippocampus and cerebellum had lower brain: blood toluene ratios than the spinal cord, midbrain, medulla oblongata, and pons. The brain stem controls many involuntary aspects of cardiac, respiratory, and vasomotor function.

Concentrations of toluene in subcutaneous adipose tissue of male subjects exposed during rest or physical exercise to 300 mg/m^3 of toluene were determined (Carlsson and Ljungquist 1982). After exposure at rest for 2 hours, the mean concentration of toluene in adipose tissue was 0.7 mg/kg. The corresponding value after 2 hours of work was 9.9 mg/kg. Linear regression analysis indicated that toluene concentrations in adipose tissue were lower in subjects with large amounts of body fat.

The human data are supported by autoradiography studies using mice. Immediately after inhalation, a high level of radioactivity was found in the body fat, bone marrow, spinal nerves, spinal cord and white matter of the brain of exposed mice (Bergman 1979). Radioactivity was also observed in the blood, kidney, and liver at lower levels. Autoradiography of mice sacrificed immediately after the cessation of exposure revealed a very high concentration of nonvolatile radioactivity in the kidney, particularly the medullary region. Nonvolatile radiation found in the liver and kidney suggests rapid formation and excretion of toluene metabolites.

A one-compartment model was developed for blood and whole-brain toluene levels based on data from rats exposed to 575 ppm toluene for up to 240 minutes (Benignus et al. 1981). Estimated saturation asymptotes were 10.5 ppm for venous blood and 18.0 ppm for brain, respectively. Blood and brain levels achieved 95% of their estimated asymptotes in 53 and 58 minutes, respectively. The distribution half-life for a 30-minute exposure of rats to 2,000 ppm toluene was 0.34 hours, while that for exposure to 10,000 ppm was 0.6 hours (Ameno et al. 1992).

Toluene was rapidly distributed to the tissues in rats after 1, 2, or 3 days of exposure to 100 ppm for 12 hours per day (Zahlsen et al. 1992). Homeostasis was attained in 1 day for the kidney, brain, and liver, whereas toluene concentrations continued to increase in perirenal fat deposits. Once exposures ceased, toluene concentrations declined within 12 hours to near baseline levels for all tissues except in fat. The toluene in rat brains was distributed to the brain stem and midbrain (Ameno et al. 1992), a distribution that parallels that observed in humans and mice (Ameno et al. 1992; Bergman 1979). These regions have a high concentration of white matter.

Toluene distribution to several tissues was followed in dogs exposed through inhalation of 30,000 ppm toluene from a plastic bag for 10 minutes. The toluene level in the arterial blood was 129±54.8 µg/mL while that in the venous blood was 112±48.5 µg/mL. The liver and brain contained roughly equivalent concentrations of toluene (184 and 191 µg/g), while the toluene in the kidneys was 99 µg/g (Ikeda et al. 1990).

Distribution of toluene (assayed by autoradiography and tissue concentrations of radioactivity) in pregnant mice was also characterized by preferential uptake in maternal lipid-rich tissues (brain and fat) immediately after 10-minute inhalation exposures to ^{14}C-labeled toluene at approximately 2,000 ppm (Ghantous and Danielsson 1986). It was thought that toluene, due to its high lipid solubility and low molecular weight, might easily transfer across the placenta, but concentrations of radioactivity in fetal tissues were only about 4% of concentrations in maternal brain and adipose tissue immediately after exposure, and rapidly decreased within 4 hours of cessation of exposure. These results suggest that absorbed toluene is preferentially distributed to maternal adipose tissues in pregnant mice and that distribution to the developing fetus is limited with short-term exposure to the relatively high (compared with occupational exposures) concentration of 2,000 ppm. This concentration, however, is low compared with concentrations experienced by toluene abusers (4,000–12,000 ppm as cited by Gospe et al. 1994). Ghantous and Danielsson (1986) suggested that the lower lipid content in fetal tissue compared with maternal tissue could explain the low uptake of toluene into fetal tissue.

No studies were located that examined *in vivo* distribution of toluene into breast milk in humans or animals. Although breast milk is high in lipid content, it is unknown if there may be preferential uptake of toluene into other maternal lipid-rich tissues. A published estimate of the human milk/blood partition coefficient for toluene, 2.68, was lower than estimates of coefficients for partitioning of toluene between other tissues and human blood, including liver or highly perfused tissues/blood (4.91) and fat/blood (60.01) (Fisher et al. 1997). Transfer from blood to a tissue, however, is also dependent on the rate of

perfusion of the tissue with blood. Fisher et al. (1997) used these partition coefficient values in a physiologically-based pharmacokinetic (PBPK) model designed to predict transfer of volatile chemicals into breast milk, but human or animal pharmacokinetic data for lactational transfer of toluene were not available to validate or modify the model.

Coexposure of rats to xylene increased the concentrations of toluene in the blood and brain in the 19 hours after exposure as compared to exposure to toluene alone (Tardif et al. 1992). This was, apparently, the result of suppressed toluene metabolism because of competition between toluene and xylene for active sites on enzymes responsible for metabolizing both compounds. Pulmonary excretion of toluene was also decreased when exposure to both compounds occurred. As a result, the half-lives for both toluene and xylene were increased.

Blood and brain toluene levels in rats exposed to 2000 or 4000 ppm for 4 hours during daylight were significantly higher at the end of exposure and 40 minutes after the cessation of exposure than in animals exposed in the dark (Harabuchi et al. 1993). This suggests that circadian rhythms may have an influence on toluene absorption, distribution, and excretion.

2.3.2.2 Oral Exposure

In one human who died 30 minutes after ingestion of 625 mg/kg toluene, the liver was found to have the highest concentration of toluene (433.5 µg/g) followed by the pancreas (88.2 µg/g), brain (85.3 µg/g), heart (62.6 µg/g), blood (27.6 µg/g), body fat (12.2 µg/g), and cerebrospinal fluid (11.1 µg/g) (Ameno et al. 1989). The short interval between toluene exposure and death limited the distribution of the toluene to the peripheral body tissues.

When rats were orally exposed to 400 mg/kg toluene, the peak concentration in the blood occurred 1.5 hours after exposure (Ameno et al. 1992). In the brain, the highest brain: blood toluene ratios were found in the pons and caudate-putamen, as opposed to the hippocampus (Ameno et al. 1992). Toluene distribution in the brain was similar with inhalation and oral exposure (Ameno et al. 1992).

2.3.2.3 Dermal Exposure

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

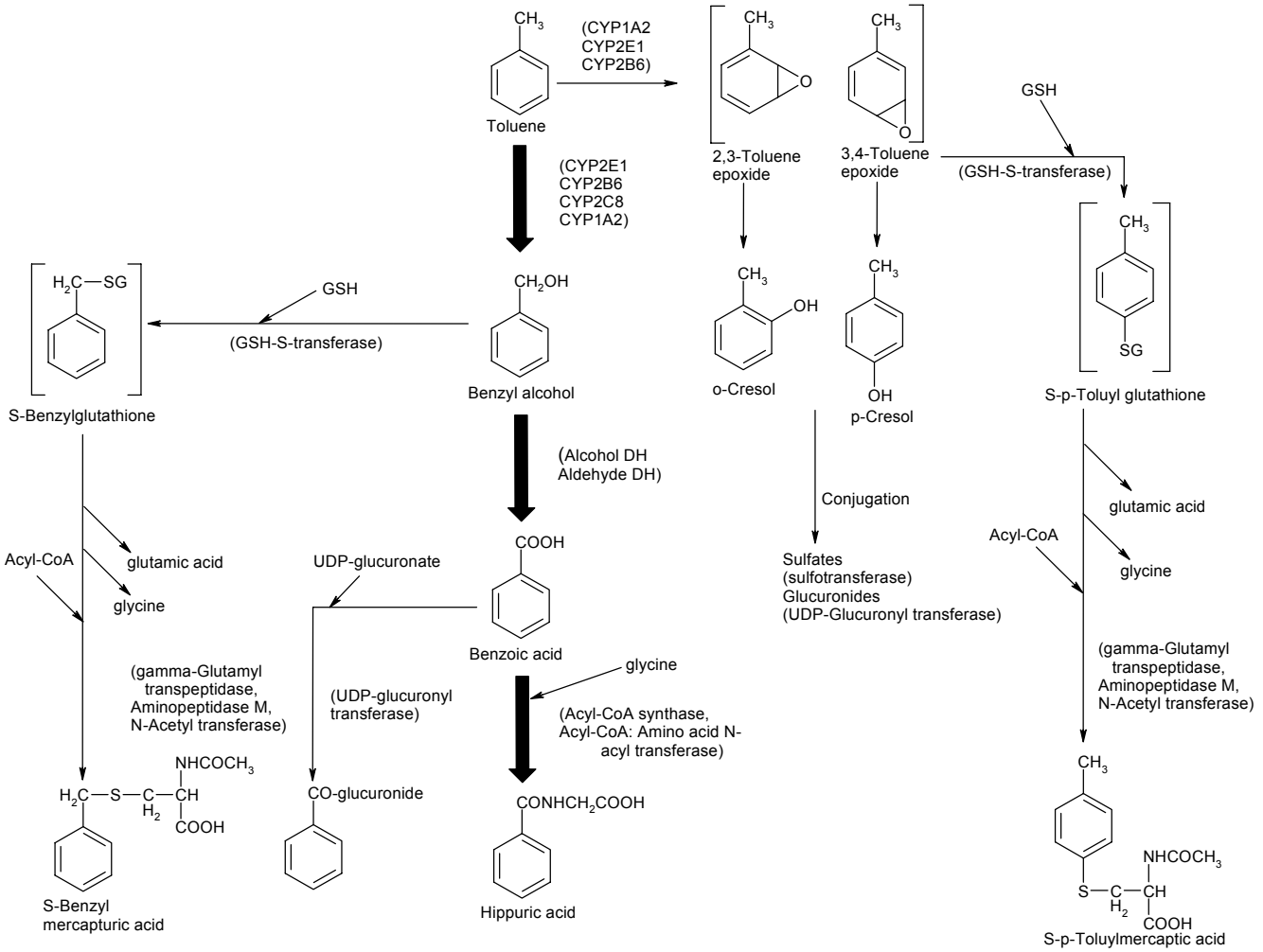
2.3.3 Metabolism

Studies of urinary metabolites in toluene-exposed humans (Andersen et al. 1983; Angerer 1979; Angerer et al. 1998a; Baelum et al. 1987, 1993; Dossing et al. 1983b; Inoue et al. 1986; Jonai and Sato 1988; Kawai et al. 1992a, 1992b, 1993, 1996; Lof et al. 1990, 1993; Maestri et al. 1997; Ng et al. 1990) and rats (Bray et al. 1949; Van Doorn et al. 1980; Wang and Nakajima 1992) have identified hippuric acid (the glycine conjugate of benzoic acid) as the major urinary metabolite of toluene. Minor urinary metabolites (in approximate order of decreasing abundance) include: the glucuronyl conjugate of benzoic acid; sulfate and glucuronide conjugates of *ortho*- and *para*-cresol; S-benzylmercapturic acid; and S-*p*-toluylmercapturic acid. Based on these results and the results from *in vitro* studies, including recent studies with human and rat liver microsomes (Nakajima and Wang 1994; Nakajima et al. 1991,1992a, 1992b, 1993, 1997; Tassaneeyakul et al. 1996), a scheme for toluene metabolism in humans and animals is presented in Figure 2-3.

The initial steps are methyl and ring hydroxylations that are catalyzed by cytochrome P450 (CYP) isozymes. Methyl hydroxylation to form benzyl alcohol was the predominant first step in human (Nakajima et al. 1997; Tassaneeyakul et al. 1996) and rat (Nakajima et al. 1991, 1992a, 1992b, 1993) liver microsomes. Ring hydoxylation to form *ortho*- or *para*-cresols in these studies usually represented less than 5% of total metabolite formation.

Results from *in vitro* studies indicate that CYP2E1 is the most active CYP isozyme in forming benzyl alcohol and CYP1A2 is the most active in forming *ortho*- and *para*-cresols. Using monoclonal antibodies to CYP isozymes as *in vitro* metabolic inhibitors in rat microsome preparations, Nakajima et al. (1991) demonstrated that CYP2E1 (at low toluene concentrations) contributes to the formation of benzyl alcohol and *para*-cresol, CYP1A1/2 contributes to *ortho*- and *para*-cresol formation, and CYP2B1/2 and CYP2C11/6 (at higher toluene concentrations) contribute to the formation of benzyl alcohol and *ortho*- and *para*-cresol. Biphasic enzyme kinetics for the formation of benzyl alcohol from toluene were observed in human liver microsomes, supporting the concept that at least two isozymes with differing affinity for toluene can catalyze benzyl alcohol formation (Tassaneeyakul et al. 1996). The high-affinity component in human liver microsomes was markedly inhibited (about 90% inhibition) by 50 μM diethyldithiocarbamate, an inhibitor of CYP2E1, whereas inhibitors of other CYP isozymes produced generally less than 10% inhibition of the high affinity component (Tassaneeyakul et al. 1996). Other inhibitors tested (and the CYP forms that they are expected to inhibit) were: furafylline (CYP1A2), coumarin (CYP2A6), mephenytoin (CYP2C19), quinidine (CYP2D6), sulfaphenazole (CYP2D6), and

Figure 2-3. Scheme for Toluene Metabolism in Humans and Animals



Proposed enzymes are noted in parentheses.

Sources: Angerer et al. 1998a; IARC 1999; Nakajima and Wang 1994; Nakajima et al. 1997; Tassaneeyakul et al. 1996

CoA = coenzyme A; CYP = cytochrome P-450; DH = dehydrogenase; GSH = glutathione; UPD = uridine 5'-diphosphate

troleandomycin (CYP3A) (Tassaneeyakul et al. 1996). Using microsomes from cells in which cDNAs for eleven different human CYP isozymes were expressed, Nakajima et al. (1997) demonstrated that CYP2E1 was the most active in forming benzyl alcohol, followed in order by CYP2B6, CYP2C8, CYP1A2, and CYP1A1. The activities of CYP2A6, CYP2C9, CYP2D6, CYP3A3, CYP3A4, and CYP3A5 in metabolizing toluene were negligible. CYP1A2 also was active in forming *ortho*- and *para*-cresol (22 and 35% of total metabolites) and CYP2E1 and CYP2B6 catalyzed the formation of *para*-cresol (11–12% of total metabolites) (Nakajima et al. 1997).

Benzyl alcohol is thought to be converted to benzoic acid in two steps by alcohol dehydrogenase and aldehyde dehydrogenase (see Figure 2-3). Conjugation with glycine to form hippuric acid can represent 83–94% of urinary metabolites of toluene in rats (Nakajima and Wang 1994). Hippuric acid formation from benzoic acid (a common component of the diet) is catalyzed by acyl-CoA synthetase and acyl-CoA: amino acid N-acyltransferase. Conjugation of benzoic acid with glucuronic acid to form benzoyl glucuronide is catalyzed by UDP-glucuronyl transferase and can account for 3–9% of urinary metabolites in rats (Nakajima and Wang 1994).

The 2,3- and 3,4-epoxide intermediates, precursors of *ortho*- and *para*-cresol, are thought to be oxidation products of the catalytic actions of CYP1A2, CYP2E1, and CYP2B6 (Nakajima et al. 1997). *Ortho*- and *para*-cresol and their conjugates have been reported to account for 0.5–1.1% and 2.5–14.2%, respectively, of urinary metabolites in rats (Nakajima and Wang 1994). S-benzyl mercapturic acid, a minor urinary metabolite identified in humans, is thought to be formed via conjugation of benzyl alcohol with glutathione (catalyzed by glutathione-S-transferases), followed by the concerted catalytic actions of γ-glutamyltranspeptidase, amino peptidase M, and N-acetyltransferase to release glutamic acid and glycine and add an acetyl group (Angerer et al. 1998a) (see Figure 2-3). The formation of another minor human urinary metabolite, S-*p*-toluylmercapturic acid, is thought to proceed by a similar series of reactions from the proposed intermediate, 3,4-toluene epoxide (Angerer et al. 1998a).

The liver is expected to be the prime site of toluene metabolism, based on the high concentration of CYP isozymes in the liver relative to other tissues. For example, levels of CYP2E1 in human lung microsomes were 10.5% of liver activities (Wheeler et al. 1992). Studies with rats indicate that toluene exposure causes changes in CYP-associated enzyme activities and CYP isozymes themselves in the liver (see Nakajima and Wang 1994 for review). For example, single 6-hour exposures to toluene induced hepatic CYP2E1 levels and associated nitrosodimethylamine demethylase activities (at concentrations $1,000 ppm), induced CYP2B1/2 and CYP3A1/2 levels (at concentrations >2,000 ppm), decreased

CYP2C11/6 levels (at 4,000 ppm), and did not change CYP1A1/2 levels (Wang et al. 1993). Other rat experiments involving longer durations of exposure and potentially higher dose levels have consistently observed induction of hepatic activities of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD), activities associated with CYP1A1/2 (see Nakajima and Wang 1994). Rats given single intraperitoneal injections of 5 mmol toluene/kg showed induction of ethoxycoumarin O-deethylase (ECOD) and EROD activities in liver, but no induction was apparent in lung or kidney tissues (Pyykko et al. 1987). Exposure of rats to 375 ppm toluene, 6 hours/day for up to 5 days or 125 ppm for 6 hours did not significantly change activities of AHH, EROD, or benzyloxyresorufin (BROD) in liver microsomes compared with activities in nonexposed controls, but significantly decreased activities of AHH (by up to about 50%), BROD (by 30–70%), and 2-aminofluorene N-hydroxylase (by up to about 50%) in lung microsomes without altering EROD activities (Furman et al. 1998). The results from these rat studies suggest that toluene at exposure concentrations $1,000 ppm, but not at lower concentrations, induces hepatic CYP enzymes involved in its own metabolism and metabolism of other xenobiotics, and that exposure to 125 or 375 ppm may cause a decrease in pulmonary activities of certain CYP mixed function oxidases. Consistent with the idea of no CYP induction with low-level exposure is the report that workers exposed to 100 ppm toluene did not display increased ability to clear antipyrine (Dossing et al. 1983c).

Levels of CYP isozymes in rat fetal livers are very low, but increase rapidly after birth (Nakajima and Wang 1994). By 10 days after birth, rats of both sexes are capable of responding to toluene exposure by inducing hepatic CYP-associated enzyme activities (Pyykko 1983b). Comparison of rates of metabolism in liver microsomes from male and female rats at 3 weeks (immature) and 18 weeks of age (mature) and in pregnant female rats on gestations days 10 and 21 indicate that age, gender, and pregnancy can influence rates of hepatic toluene metabolism and induction of CYP isozymes (Nakajima et al. 1992b). Rates (on a mg protein basis) of high-affinity toluene metabolism were not statistically significantly different between immature and mature male rats, but rates of low-affinity toluene metabolism were four-fold higher in mature rats compared with immature rats (Nakajima et al. 1992b). In contrast, rates of high-affinity toluene metabolism were lower in mature than in immature female rats, but rates of low-affinity toluene metabolism were not statistically different between immature and mature female rats. Rates of high- and low-affinity toluene metabolism were significantly lower in pregnant rats compared with nonpregnant rats.

Given the lack or low levels of several CYP isozymes in the developing human fetus (Leeder and Kearns 1997), it is expected that the capacity for metabolic detoxification of toluene is low in the developing

fetus. Rat studies indicate that levels of CYP isozymes involved in toluene metabolism, however, are rapidly increased following birth, and suggest that capabilities to carry out Phase I toluene metabolism at low exposure levels during neonatal periods may exceed those at sexual maturity and pregnancy (Nakajima et al. 1992b). CYP2E1, one of the principal CYP isozymes involved in the major toluene pathway (Nakajima et al. 1997; Tassaneeyakul et al. 1996), is reported to be expressed several hours after birth in humans and continues to increase during the first year of life (Vieira et al. 1996). Phase II enzymes involved in toluene metabolism (e.g., N-acetyl transferases, UDP-glucuronyl transferases, and sulfotransferases) also show changes during human neonatal development with adult activities reported to be present by 1–3 years of age (Leeder and Kearns 1997). Although no studies were located directly comparing toluene metabolic capacity in children and adults, the limited available information suggest that children past early neonatal periods may be equally able as adults in metabolically disposing of toluene at low exposure levels expected to be found in the general environment or at sites adjacent to waste sites.

2.3.4 Elimination and Excretion

2.3.4.1 Inhalation Exposure

Studies with humans and laboratory animals indicate that following acute periods of inhalation exposure to toluene, absorbed toluene is excreted predominately in the urine as metabolites (hippuric acid, benzoyl glucuronide, *ortho*- and *para*-cresol and their sulfate and glucuronide conjugates, S-benzyl mercapturic acid, and S-*p*-toluyl mercapturic acid, as discussed in Section 2.3.3) and, to a lesser extent, as non-metabolized toluene in exhaled air (Lof et al. 1993; Ogata 1984; Tardif et al. 1998). For example, following a 2-hour exposure with light physical exercise to deuterium-labeled toluene at a concentration of 200 mg/m^3 (53 ppm), an average 78% of retained label was excreted as urinary hippuric acid within 20 hours by a group of nine volunteers (Lof et al. 1993). A significant portion of absorbed toluene in this and other studies has been estimated to be exhaled as nonmetabolized toluene (7–20% of absorbed toluene) (Carlsson 1982; Leung and Paustenbach 1988; Lof et al. 1993).

Analyses of kinetic data for toluene concentrations in blood, exhaled breath, or adipose tissue following inhalation exposure of humans (Leung and Paustenbach 1988; Lof et al. 1993; Pellizzari et al. 1992; Pierce et al. 1996, 1999) and rats (Rees et al. 1985) indicate that most absorbed toluene is rapidly eliminated from the body and that a smaller portion (that which gets into adipose tissues) is slowly eliminated. Using three-phase exponential mathematical models to describe curves of human blood

concentration as a function of time up to 3–5 hours after 2-hour exposures to 100 or 53 ppm toluene, calculated half-lives (the time to decrease the amount in the phase by one-half) were 1.5 and 3 minutes for the initial phase, 26 and 40 minutes for the second phase, and 3.7 and 12.3 hours for the final phase (Lof et al. 1993; Sato et al. 1974). Elimination half-lives ranged from about 12 to 65 hours (0.5 to 2.7 days) in subcutaneous adipose tissue samples taken from 12 subjects at several times within 8 days of cessation of exposure to about 80 ppm toluene for four consecutive 30-minute periods (Carlsson and Ljungquist 1982). Increasing elimination half-lives were significantly correlated with increasing amounts of body fat (Carlsson and Ljungquist 1982). Using PBPK models, mean terminal half-lives of about 30–38 hours were calculated for changes in blood toluene concentrations between 50 and 100 hours after cessation of 2-hour inhalation exposures of male subjects to 50 ppm $^{1}H_{8}$-toluene and 50 ppm $^{2}H_{8}$-toluene (Pierce et al. 1996, 1999). During this terminal phase of disposition, >95% of toluene is expected to be in adipose tissue and the release of toluene from adipose tissues has been proposed to be the rate-limiting step (Pierce et al. 1999). In studies with rats exposed for 2 hours to 1,000, 1,780, or 3,000 ppm toluene, two-phase exponential models were used to calculate average elimination half-lives of approximately 6 and 90 minutes, but blood toluene concentrations were monitored in this study for no more than 2 hours following exposure (Rees et al. 1985).

Investigators have reported a correlation between occupational exposure to toluene and urinary excretion of hippuric acid, *o*-cresol, and *p*-cresol (De Rosa et al. 1985, 1987; Foo et al. 1991; Kono et al. 1985; Lof et al. 1993; Ogata 1984). However, experts caution that there are individual differences in the amounts of these excreted metabolites, and monitoring of urinary excretion of metabolites can only serve as a qualitative indication of exposure to toluene (Andersen et al. 1983; Baelum et al. 1987; Hasegawa et al. 1983; Kawai et al. 1996; Nise 1992). When volunteers were exposed to 50 ppm deuterium-labeled toluene plus 50 ppm nonlabeled toluene, there was very little variation of labeled hippuric acid excretion between subjects (Lof et al. 1993). After 20 hours, 78% of the absorbed labeled toluene was excreted as labeled hippuric acid. However, unlabeled hippuric acid excretion varied widely between subjects and the total amount of hippuric acid excreted was about 4 times greater than what would have been generated by toluene exposure alone. This indicates that there are a number of hippuric acid precursors present in the environment (e.g., benzoic acid in food) and exposure to these compounds varies, making hippuric acid excretion a poor indicator of toluene exposure.

Studies of workers exposed to low levels of airborne toluene (TWA concentrations below 50 ppm) indicated that correlation coefficients between end-of-shift levels of *ortho*-cresol and hippuric acid in urine and toluene concentrations in breathing zone air, although statistically significant, were not above

0.7, indicating a fair amount of variation not explained by toluene air concentrations (Kawai et al. 1996; Nise 1992). In a study of 17 workers exposed to 8-hour TWA breathing zone toluene concentrations ranging from about 5 to 70 ppm, end-of-shift levels of S-benzylmercapturic acid in urine were correlated with toluene concentrations with a coefficient of 0.74 (Maestri et al. 1997). In addition to the individual differences for urinary metabolites, possible ethnic differences in the excretion of hippuric acid and o-cresol have also been reported in Chinese, Turkish, and Japanese solvent workers (Inoue et al. 1986). Significant differences in *o*- and *p*-cresol/hippuric acid ratios in the urine were also seen in four different strains of rats exposed to toluene (Inoue et al. 1984).

The American Conference of Governmental Industrial Hygienists (ACGIH 1999) recommends using a combination of three biological exposure indices to assess exposure of workers to toluene in the workplace: *ortho*-cresol and hippuric acid levels in urine at the end of a workshift and toluene levels in blood immediately prior to the last shift of a workweek. Angerer et al. (1998a) proposed that S-*p*-toluylmercapturic acid levels in urine may also be useful as a biological indicator of toluene exposure.

2.3.4.2 Oral Exposure

Following oral administration of toluene to eight male subjects by a 2 mg/minute infusion for 3 hours through a feeding tube into the stomach, nonmetabolized toluene was detected in alveolar air samples for up to 4 hours after cessation of exposure and rates of urinary excretion of hippuric acid and *ortho*-cresol were elevated compared with values under nonexposed conditions (Baelum et al. 1993). A 6 mg/minute infusion for 30 minutes did not change the rates of urinary excretion of hippuric acid and *ortho*-cresol, but increased, by four-fold, the area-under-the-curve (AUC) for alveolar toluene concentration compared with the values for the 2-mg/minute exposure protocol. Accompanying the 2-mg/minute exposure protocol with oral doses of ethanol (0.32 g/kg, corresponding to two alcoholic drinks) decreased hippuric acid urinary excretion and dramatically increased the AUC for alveolar toluene concentration (by about 850-fold in one experiment and 56-fold in another). These data indicate that orally administered toluene is eliminated similarly to inhaled toluene, (i.e., by urinary excretion of metabolites and exhalation of nonmetabolized toluene), and that ingestion of ethanol can have a dramatic effect on metabolism and subsequent elimination of toluene. The results are consistent with other studies showing that ethanol inhibits the major toluene metabolic pathway, side-chain oxidation (Dossing et al. 1984; Wallen et al. 1984).

No other studies were located regarding the excretion of toluene in humans or animals after oral exposure.

2.3.4.3 Dermal Exposure

Following a 5-minute episode of hand-washing in toluene while wearing an airstream helmet to limit inhalation exposure, toluene concentrations in exhaled air from human subjects peaked at about 1 ppm at 22 minutes and declined to about 0.03 ppm at 24 hours (Monster et al. 1993). The results from this study indicate that dermally absorbed toluene can be eliminated as the parent compound in exhaled breath, but provide no information concerning the possible urinary excretion of metabolites.

No other studies were located regarding elimination of toluene following dermal exposure.

2.3.5 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 parametrization, (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.

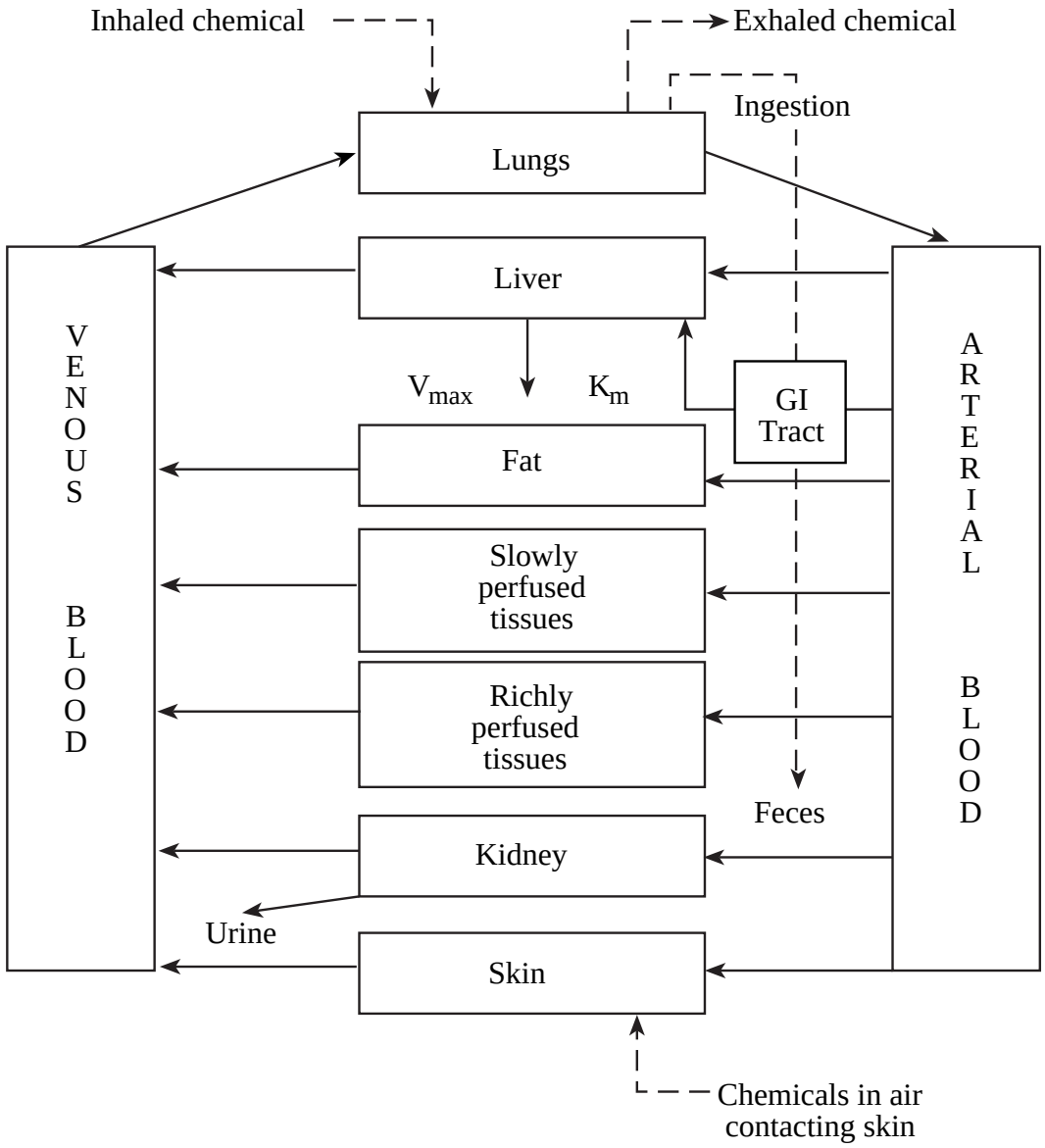
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many 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 2-4 shows a conceptualized representation of a PBPK model.

PBPK models are available that describe the kinetics of toluene after inhalation exposure; two for humans (Fisher et al. 1997; Pierce et al. 1996, 1999) and two for rats (DeJongh and Blaauboer 1996, 1997; Tardif et al. 1993). These models are all modifications of the standard four-compartment PBPK model developed for styrene (Ramsey and Andersen 1984) in which:

(1) absorption into the lung blood is assumed to be dependent on the inhaled concentration of toxicant, the concentration of toxicant in alveolar air, blood flow to the lung, the blood/air partition coefficient, and alveolar ventilation rates,
(2) exchange of toxicant between arterial blood and tissue compartments is flow-limited,

Figure 2-4. 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.

(3) changes in the amount of toxicant in three nonmetabolizing tissue compartments (adipose tissue, slowly perfused tissues, and rapidly perfused tissues) are described by mass transfer differential equations with tissue volume, blood flow through the tissue (i.e., tissue perfusion rate), arterial blood toxicant concentration, and tissue/blood partition coefficients as explanatory variables, and
(4) changes in toxicant amount in the liver (the fourth compartment) are described by similar differential equations that additionally include a Michaelis-Menten term for overall rates of toxicant metabolism.

The five-compartment human model for toluene developed by Pierce et al. (1996) includes an additional equation describing mass balance across the lung that has a Michaelis-Menten metabolic term (Pierce et al. 1996). The model assumes that toluene metabolism in the liver and lung are adequately described by subject-specific maximal rate constants for liver and lung ("Vmax-h and Vmax-p" of $52.1 \times BW^{0.7}$ µmol/hour and $0–0.7 \times 52.1 \times BW^{0.7}$ µmol/hour, respectively) and a common Km (5.97 µmol/L). The Km and Vmax-h values were based on those derived by fitting a Ramsey and Andersen-type four-compartment PBPK model (in which all parameters were constant except Vmax and Km) to toluene uptake data for rats placed in closed chambers at several initial toluene concentrations (Tardif et al. 1993). The human Vmax-h was estimated for each subject by multiplying the rat Vmax-h by the subject's body weight to the 0.7 power; the rat Km was taken as the human value (Pierce et al. 1996). The lung Vmax (Vmax-p) was estimated by model-fitting for each subject, allowing the value to range between 0 and 70% of the liver Vmax, Vmax-h. This procedure was based on observations that levels of CYP2E1 in human lung microsomes were 10.5% of liver activities (Wheeler et al. 1992), and 12 human liver samples showed a seven-fold range of CYP2E1 contents (Thummel et al. 1993).

Another singular feature of the Pierce human model is that subject-specific parameters such as age, height, weight, alveolar ventilation rate, adipose tissue fraction, and blood/air partition coefficient are put into the model (Pierce et al. 1996). Volumes of the tissue compartments in the model were scaled to each subject's body weight. Blood flows to the slowly and rapidly perfused tissues and the liver were taken as fractions of a standard human cardiac output scaled to body weight to the 0.74 power (in units of liter-hour), whereas subject-specific blood flows to the adipose tissue were estimated by model fitting (holding other parameters constant) allowing the fraction of cardiac output that perfuses adipose tissue to range between 0.06 and 0.18. The decision to "model-fit" this parameter within these bounds was based on published observations that adipose blood flows among individuals range widely from about 0.06 to

0.18 of total cardiac output. Tissue/blood partition coefficients used in the model for the slowly and rapidly perfused tissue, the liver, and adipose tissue were 1.54, 4.64, 4.64, and 55.9, respectively.

The initial development and validation of the human model involved comparing model fits with measured data (blood concentrations) for a group of 26 male volunteers who were exposed to 100 ppm toluene (50 ppm $^{1}H_{8}$-toluene and 50 ppm $^{2}H_{8}$-toluene) for a 2-hour period (Pierce et al. 1996). Venous blood concentrations of $^{1}H_{8}$- and $^{2}H_{8}$-toluene were measured at intervals for 120 hours post exposure. Prior to exposure, information on age, body weight, and adipose tissue fraction were obtained. During exposure, individual ventilation rates and blood/air partition coefficients for toluene were measured. Measures of the goodness-of-fit of the model predictions to the data were compared using subject-specific values, average values from the 26 subjects, and average literature values for: body weight, adipose tissue fraction, ventilation rate, blood/air partition coefficient, maximum velocity of pulmonary metabolism, and fraction of cardiac output to adipose tissue. The measured concentrations of toluene in blood showed a ten-fold interindividual range of variation. Subject-specific modeling explained 91% of the data variability, compared with 53% using literature values for model parameters (Pierce et al. 1996).

Pierce et al. (1998) used the human model to estimate toluene concentrations in alveolar breath reflective of exposure to 50 ppm toluene for 8 hours/day (the current ACGIH 8-hour TWA threshold limit value (TLV) for toluene). Calculated values were #10 μmol/m^3 for samples taken just before the final shift of a workweek and #150 μmol/m^3 postexposure. It was proposed that toluene breath sampling would be a rapid, noninvasive biomarker of toluene exposure in workers that is not contaminated by endogenous sources. Pierce et al. (1999) also used the human model as a research tool to ascribe differences in toxicokinetic behavior of $^{1}H_{8}$- and $^{2}H_{8}$-toluene to underlying physiological mechanisms.

Another human PBPK model has been developed for volatile organic compounds that models transfer of toxicant via lactation from a mother to a nursing infant, but *in vivo* pharmacokinetic data for toluene in breast milk were not available to validate this model (Fisher et al. 1997). This model is an adaptation of the Ramsey and Andersen design with the addition of a fifth compartment, a nonmetabolizing milk compartment with a varying volume. The model includes equations describing the rate of change in the amount of toxicant ingested by a nursing infant from the milk compartment, the rate of change in the amount of milk in the mammary tissue lumen, and the rate of change in the amount of toxicant in breast milk. The model used Michaelis-Menten kinetic constants for toluene metabolism in the liver estimated for rats (Vmax 7.5 mg/kg/hour; Km 0.3 mg/L); the rat Vmax was scaled for use in the model by multiplying it by a reference body weight (60 kg) to the 0.74 power. Human milk/blood partition

coefficients for 19 volatile organic chemicals were experimentally determined using samples from volunteers; the coefficient for toluene was 2.68. Other tissue/blood partition coefficients for toluene used in the model included 4.91 for rapidly perfused tissue and for liver, 1.61 for slowly perfused tissue, and 60.01 for adipose tissue.

Fisher et al. (1997) used the model to estimate the amount of toluene an infant would ingest via milk if the mother was occupationally exposed to toluene at the ACGIH (1999) Threshold Limit Value (TLV=50 ppm) throughout a workday. The model predicted that such an infant would have a daily intake of 0.46 mg toluene/day, which is below the U.S. EPA Health Advisory, 2.0 mg/day, for chronic ingestion of 1 L/day of toluene-contaminated water by a 10-kg child.

The four-compartment rat PBPK model for toluene developed by Tardif et al. (1993) restricted metabolism to the liver compartment. As described before, the Km (0.55 mg/L) and Vmax (4.8 mg/hour/kg) values were derived by fitting the model (in which all parameters were held constant except Vmax and Km) to toluene uptake data for rats housed in closed chambers for 5 hours at several initial toluene concentrations (75, 150, or 225 ppm) (Tardif et al. 1993). The model used 18.0 as the blood/air toluene partitioning coefficient, and the following for partitioning between blood and tissue groups: 4.64 for liver and rapidly perfused tissue, 1.54 for slowly perfused tissue, and 56.7 for adipose tissue. Reference rates for alveolar ventilation (15 L/hour/kg) and cardiac output (15 L/hour/kg) were scaled by a factor of body weight to the 0.74 power. Model predictions of venous blood concentrations in rats during and after 5-hour exposures to toluene concentrations of 75, 150, or 225 ppm compared favorably (by visual inspection) with empirical data.

Tardif et al. (1993) linked the rat PBPK model for toluene to a similar PBPK model for xylene via the metabolism term in the liver compartment to test if there was no metabolic interaction between these compounds or if a metabolic interaction existed that could be described by competitive, noncompetitive, or uncompetitive inhibitory interaction. A model with a competitive inhibition metabolic term provided the best visual fit to empirical data for air concentrations of toluene and xylene during 5-hour exposures of rats in a closed chamber to mixtures of toluene and xylene at several initial concentrations.

A five-compartment rat PBPK model developed by DeJongh and Blaauboer (1996) is similar in design to the Tardif rat PBPK model except that it contains an additional compartment, (i.e., the brain, which is assumed to be nonmetabolizing). The model used the same toluene partition coefficients used in the Tardif et al. (1993) rat model; the brain/blood partition coefficient, 2.0, was estimated from a published

value for the human brain/air coefficient and the rat blood/air coefficient. Reference rates for alveolar ventilation (14 L/hour/kg) and cardiac output (14 L/hour/kg) were scaled by a factor of body weight to the 0.74 power. With other parameters in the model held constant, models with different published values of Vmax and Km for toluene metabolism in rat liver from two *in vivo* and six *in vitro* rat studies were compared for their ability to fit empirical data from several studies for toluene blood concentrations or toluene brain concentrations in rats exposed to inhaled toluene. DeJongh and Blaauboer (1996) judged that a Vmax of 4.31 mg/kg/hour and Km of 0.26 mg/L gave the overall best fit to the empirical data, but noted that differences were generally small among predictions from models with the various values of Vmax and Km.

Dejongh et al. (1998) used their rat PBPK model for toluene and similar models for 14 other volatile organic chemicals to examine a hypothesis that the acute lethality of volatile organic chemicals is related to their ability to distribute into the brain. Using these models to calculate the dose in the brain associated with the LC_{50} for the compounds, it was noted that the products of the LC_{50} and their respective exposure durations ranged by about 60-fold, whereas the PBPK-derived brain doses associated with the LC_{50} ranged by about 6-fold. Dejongh et al. (1998) concluded that this observation supports the hypothesis that the acute lethality of volatile organic chemicals, including toluene, is directly related to the extent of their distribution into the brain.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

Absorption. In humans and animals, toluene is rapidly absorbed by inhalation exposure (Benignus et al. 1984; Hjelm et al. 1988; Hobara et al. 1984b; Lof et al. 1993). Animal studies have shown that toluene is absorbed less rapidly by the oral route (Ameno et al. 1992; Pyykko 1983b), while toluene is absorbed slowly through human skin (Dutkiewicz and Tyras 1968). Studies with brush border membrane vesicles isolated from rat intestines and exposed to toluene indicate that toluene absorption occurs through the lipid matrix of the membrane (Alcorn et al. 1991).

Distribution. Toluene has been identified in brain, liver, lung, and blood in humans following toluene exposure (Paterson and Sarvesvaran 1983; Takeichi et al. 1986). Within the human brain, toluene has a greater affinity for areas of the brain that contain lipid-rich white matter, such as the brain stem, rather than the areas with larger amounts of grey matter (Ameno et al. 1992). The human data are supported by

animal studies where distribution of toluene was found to be characterized by uptake in lipid tissues (brain and fat) immediately following inhalation exposure (Ghantous and Danielsson 1986).

Excretion. In both humans and rats, up to about 75–80% of inhaled toluene that is absorbed can be accounted for by urinary excretion of the principal metabolite, hippuric acid (Lof et al. 1993; Ogata 1984; Tardif et al. 1998). Excretion of minor metabolites including S-benzyl mercapturic acid, S-*p*-tolulyl mercapturic acid, and conjugates of *ortho*- and *para*-cresol account for less than 5% of absorbed toluene. Excretion of nonmetabolized toluene in exhaled air can represent from 7 to 20% of absorbed toluene (Carlsson 1982; Leung and Paustenbach 1988; Lof et al. 1993). Although the liver is expected to be the main site of metabolism of toluene, CYP2E1, one of the principal isozymes catalyzing the initial reaction in the principal toluene metabolic pathway, has been detected in human lung microsomes at concentrations about 10-fold less than in liver microsomes (Wheeler et al. 1992). Under conditions in which the main pathway of toluene metabolism is inhibited by co-exposure with ethanol, exhalation of nonmetabolized toluene can become a principal route of excretion (Baelum et al. 1993).

2.4.2 Mechanisms of Toxicity

The mechanism by which acute exposure to toluene brings about neurological effects such as central nervous system depression and narcosis is generally thought to involve, at least in part, reversible interactions between toluene (the parent compound and not its metabolites) and components (lipids or proteins) of nervous system membranes. Support of parent-material involvement comes from the observation that pretreatment of rats with phenobarbital increased the rate of *in vivo* toluene metabolism and shortened the time of recovery from narcosis from single intraperitoneal doses of toluene (Ikeda and Ohtsuji 1971). Other support for this hypothesis includes the transient nature of anesthesia from acute high level exposure to toluene and the rapidity with which toluene-induced changes in brain biochemical variables can be measured. For example, within 0.25–1 hour of intraperitoneal injection of 1-g/kg doses of toluene into rats, brain synaptosomes showed decreased phosphatidylethanolamine content, altered phospholipid methylation activities, altered outer membrane fluidity, and increased Na+-K+-ATPase activities (Lebel and Schatz 1988, 1989, 1990). Decreased Mg++-ATPase activities were measured in brain synaptosomes isolated from rat brains immediately following a 2-hour exposure to 2,000 ppm toluene (Korpela and Tahti 1988). Average whole brain concentrations of several biogenic amines (dopamine, norepinephrine, and 5-hydoxytrytamine) were increased in rats immediately following an 8-hour exposure to 1,000 ppm toluene (Rea et al. 1984). On a molecular scale, the acute anaesthetic actions of toluene and other agents have been postulated to involve intercalation of toluene into the lipid

bilayer of nerve membranes and/or reversible interactions with proteins in the membrane (Franks and Lieb 1985, 1987).

Clinically obvious neurological impairment (e.g., gait and speech abnormalities) and brain atrophy have been observed in several cases of chronic toluene-inhalation abuse. MRI of the brain of solvent abusers (Filley et al. 1990; Rosenberg et al. 1988a, 1988b) suggest preferential atrophy in lipid-rich regions of the brain. Rosenberg et al. (1988a,1988b) found MRI evidence of diffuse central nervous system demyelination in 6 toluene abusers with clinically obvious neurological impairment, whereas Filley et al. (1990) noted that the degree of MRI-detected white matter abnormality in 14 solvent abusers was correlated with neurological impairment. The observed changes in MRI signals may be related to lipid compositional changes in the white matter, since these regions are more lipid-rich than gray matter (Ameno et al. 1992). These observations are consistent with a hypothesis that chronic exposure to high concentrations of toluene brings about structural changes in the brain related to lipid compositional changes. Supporting evidence for this hypothesis includes observations of changed phospholipid composition of rat brain synaptosomes following acute exposure to toluene (Lebel and Schatz 1988, 1989, 1990), decreased phospholipid concentrations in the cerebral cortex of rats following 30 days of continuous exposure to 320 ppm (Kyrklund et al. 1987), and decreased number of neurons in the hippocampus of rats, 4 months after exposure to 1,500 ppm toluene, 6 hours/day, 5 days/week for 6 months (Korbo et al. 1996). It is uncertain if toluene-induced changes in membrane phospholipid content may be caused by increased breakdown of phospholipids or inhibition of synthesis.

Mechanistic understanding is poor of effects that have been associated with intermediate and chronic exposure to toluene in workplace air such as increased symptoms of mild neurological impairment (Boey et al. 1997; Orbaek and Nise 1989; Ukai et al. 1993; Yin et al. 1987), performance deficits on neurobehavioral tests (Foo et al. 1990; Iregren 1982), hearing loss and changes in brainstem auditory-evoked potentials (Abbate et al. 1993; Morata et al. 1997; Vrca et al. 1996), and color vision impairment and changes in brainstem visual-evoked potentials (Muttray et al. 1997, 1999; Vrca et al. 1995, 1997a, 1997b; Zavalic et al. 1998a, 1998b, 1998c), but several mechanistic actions have been postulated.

One mechanistic hypothesis postulates that repeated interaction of toluene with membrane proteins and/or phospholipids in brain cells can change activities of enzymes involved in the synthesis and/or degradation of neurotransmitters and that levels of neurotransmitters at particular sites in the brain may be involved in producing subtle neurological effects. Some evidence for this hypothesis comes from observations of increased concentrations of dopamine, norepinephrine, and 5-hydroxytryptamine in rats exposed to

1,000 ppm for 8 hours (Rea et al. 1984) and in rats exposed to up to 105 mg/kg/day in drinking water for 28 days (Hsieh et al. 1990b), decreased dopamine levels and rates of turnover in several areas of the nucleus caudate in the brain of rats exposed to 80 ppm toluene, 6 hours/day for 3 days (Fuxe et al. 1982), increased levels of dopamine and noradrenaline in several brain regions in rats exposed to 80–3,000 ppm, 6 hours/day for 3 days (Andersson et al. 1983), and decreased activities of aromatic acid decarboxylase, an enzyme involved in synthesis of neurotransmitters, in the brain stem of rats exposed to 250 or 1,000 ppm, 8 hours/day, 5 days/week for 4 weeks (Bjornaes and Naalsund 1988).

Another mechanistic hypothesis postulates that repeated exposure to toluene may cause neurological effects by changing the binding of neurotransmitters to membrane receptors. In support of this hypothesis, persistent changes in brain-tissue dopamine D2 receptor binding and increased serum prolactin levels were found in rats 17 days after exposure to 80 ppm toluene 6 hours/day, 5 days/week for 4 weeks (von Euler et al. 1993, 1994). It was speculated that the increase in serum prolactin level could be related to a possible interaction between toluene and the pituitary dopamine D2 receptor; this receptor normally inhibits the release of prolactin into serum. Correlated with these biochemical changes were a significantly increased locomotor activity (approximately 2-fold) in response to injections of apomorphine (a dopamine) and a significantly increased escape latency (indicating impaired spatial learning and memory) in a water maze task, both observed 14–17 days after the 4-week toluene exposure (von Euler et al. 1994). Whether or not this hypothesis is related to effects observed in occupationally exposed humans is uncertain; Svensson et al. (1992b) did not find changes in serum prolactin levels in toluene-exposed printing workers compared with controls.

Significant decreases (28 or 47%) in rat brain glial fibrillary acidic protein (GFAP) induced by exposure to 1,000 ppm toluene, 6 hours/day for 3 or 7 days have been associated with increased serum levels of corticosterone (Little et al. 1998). The decreases in GFAP were observed in the thalamus and hippocampus, regions of the brain that are reported to be involved in controlling serum glucocorticoid levels and have high concentrations of glucocorticoid receptors, respectively (Little et al. 1998). Little et al. (1998) postulated that decreases in brain GFAP may be a consequence of toluene disruption of the hypothalamic-pituitary-adrenal axis and/or hormonal homeostasis, but noted that the available evidence is inadequate to firmly establish cause and effect. The possible mechanistic connections of these observations to toluene-induced changes in neurobehavior are uncertain.

There is evidence that hearing loss in animals induced by inhalation exposure to toluene is produced by toluene itself and not by its metabolites. Phenobarbital pretreatment, which increases the rate of *in vivo*

metabolism of toluene, prevented hearing loss in rats exposed to 1,500–2,000 ppm toluene, 8 hours/day for 7 days (Pryor et al. 1991). Rats that were given large gavage doses of ethanol (4 g/kg/day) and daily inhalation exposure to toluene concentrations of 1,750 ppm, 6 hours/day, 5 days/week for 4 weeks showed significantly greater hearing loss (as measured by auditory-evoked brainstem potentials) and outer hair cell loss in the ear than those exposed to toluene alone (Campo et al. 1998). Co-exposure to ethanol caused a significant decrease in hippuric acid urinary excretion rates compared with exposure to toluene alone, indicating that these large doses of ethanol inhibited the metabolism of toluene (Campo et al. 1998). Since exposure to ethanol alone in this study did not affect hearing or outer hair cell loss in the ear, ethanol inhibition of toluene metabolism and subsequent potentiation of toluene-induced loss of hearing are consistent with the idea that toluene itself is responsible for these effects. Mechanistic understanding at the molecular and cellular level is poor regarding how toluene exposure leads to a loss of outer hair cells in the ear and the degree to which toluene effects on neural cell membranes may be involved.

The molecular mechanism and pathogenesis of color vision impairment (dyschromatopsia) associated with occupational and intentional abusive exposure to toluene and other organic solvents are not clearly understood, but it has been postulated that toluene interference with dopaminergic mechanisms of retinal cells or toxic demylelinizaton of optic nerve fibers may be involved (Muttray et al. 1997, 1999; Zavalic et al. 1998a, 1998b, 1998c).

The postulated arene oxide intermediates formed in the metabolic pathway from toluene to *ortho*- or *para*-cresol are highly reactive and expected to bind to cell proteins and RNA, thereby potentially leading to cellular dysfunction and degeneration. Studies with human and rat liver microsomes and tissue slices showed that incubation with labeled toluene leads to incorporation of the label into microsomal proteins and RNA in an NADP-requiring reaction (Chapman et al. 1990). It does not appear likely, however, that this mechanism of action is the primary mode of toluene's toxicity, especially at air concentrations below 100 ppm that are of occupational and public health concern, because:

(1) the liver is expected to be the main site of toluene metabolism,
(2) the pathway to the cresol isomers accounts for less than 1–5% of metabolized toluene (see Section 2.3.3),
(3) results from animals studies and studies of toluene-exposed workers do not identify the liver as the most sensitive target organ (see Section 2.2.1.), and

(4) degenerative lesions in nervous tissues have not been detected by light microscopy in rats and mice exposed to concentrations as high as 1,200 ppm 6.5 hours/day, 5 days/week for up to 2 years (CIIT 1980; NTP 1990).

The available evidence, however, is not sufficient to discard the hypothesis that this mode of action (i.e., cellular degeneration caused by reactive metabolic intermediates) may play some role in toluene toxicity, especially with high-level exposures such as those experienced by toluene abusers.

2.4.3 Animal-to-Human Extrapolations

Many laboratory animal species have been used to describe toluene toxicity, but the most commonly used species is the rat. Generally, the toxicokinetic data gathered from rat studies compare favorably with the information available from human studies. In addition, neurological effects observed in rats including changes in locomotor activity, changes in visual- and auditory-evoked brainstem potentials, hearing loss, and changes in brain chemistry appear to be related to critical neurological effects observed in humans after acute or repeated exposure to toluene including self-reported neurological symptoms, impaired performance in neurobehavioral tests, hearing loss, and color vision impairment. Given the availability of data for humans exposed by inhalation, MRLs for inhaled toluene are derived without extrapolating from the available animal toxic-effects data. In contrast, acute and intermediate MRLs for oral exposure to toluene are based on extrapolating neurological effects in rats to humans (see Section 2.5 and Appendix A).

As discussed in Section 2.3.5, PBPK models describing the kinetics of toluene after inhalation exposure have been developed for humans (one with four compartments— adipose tissue, liver, and rapidly and slowly perfused tissues, and another with a fifth compartment—breast milk) and rats (one with the basic four compartments and another model with a fifth compartment—the brain). Further development of a human PBPK model that includes partitioning of inhaled and ingested toluene to the brain and a similarly designed rat PBPK model may be useful in improving extrapolation from the oral exposure rat data and in comparing model-based predictions of human effect levels based on neurological effects in inhalationally exposed rats with observed effect levels in humans exposed to airborne toluene.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview.

Adverse effects on the nervous system are the critical effects of concern from acute, intermediate, or chronic exposure to toluene. Acute exposure is associated with reversible neurological symptoms progressing from fatigue, headaches, and decreased manual dexterity to narcosis with increasing exposure levels. Reversible neurological impairment from acute exposure likely involves the direct interaction of toluene with nervous system membranes. Degenerative changes in white matter regions of the brain have been correlated with the severity of persistent neurological impairment in individuals who abused solvents and have repeatedly inhaled toluene at high exposure levels (4,000–12,000 ppm). Results from studies of groups of occupationally exposed workers suggest that chronic exposure to toluene at lower exposure levels (from about 50 to 200 ppm) can produce subtle changes in neurological functions including cognitive and neuromuscular performance, hearing, and color discrimination. Supporting data come from studies of toluene-exposed animals showing changes in behavior, hearing loss, and subtle changes in brain structure, electrophysiology, and levels of neurotransmitters. Case reports of birth defects in children of mothers who abused toluene during pregnancy suggest that exposure to high levels of toluene may be toxic to the developing fetus. However, results from animal studies indicate that toluene is not a teratogenic agent, but can retard fetal growth and skeletal development and adversely influence behavior of offspring at exposure levels that overwhelm maternal mechanisms protecting the developing fetus from exposure. Other adverse health effects, including cancer or effects on reproductive performance, do not appear to be of concern for persons who may experience low exposures to toluene by living or working near hazardous waste sites containing toluene.

Issues relevant to children are explicitly discussed in Section 2.7, Children's Susceptibility and Section 5.6, Exposures of Children.

Minimal Risk Levels for Toluene.

Inhalation MRLs.

- An MRL of 1 ppm (3.8 mg/m^3) has been derived for acute-duration (14 days or less) inhalation exposure to toluene.

This MRL is based on a study by Andersen et al. (1983) in which the effects of toluene on 16 healthy young subjects with no previous regular exposure to organic solvents were investigated (see Appendix A). Groups of 4 subjects were in a chamber for 6 hours a day on 4 consecutive days. The concentration of toluene was 0, 10, 40, or 100 ppm, with the subjects exposed to a different concentration each day. Physiological measurements were performed, including nasal mucociliary flow, and subjective measurements of discomfort. Eight different performance assessment tests were carried out. There was a statistically significant increase (P<0.05%) in the occurrence of headaches, dizziness, and feelings of intoxication during the 100 ppm exposure, but not during exposure to the other concentrations. No statistically significant effects of toluene occurred in the eight performance tests. For 3 of the tests, there was borderline significance (P<0.10%): the subjects felt that the tests were more difficult and strenuous during the 100 ppm exposure. No adverse effects were reported at the 10 and 40 ppm levels. The NOAEL of 40 ppm was adjusted to continuous exposure basis
(40 ppmx5 days/daysx8 hour/24 hour=9.5 ppm) and divided by an uncertainty factor of 10 (to account for human variability) to derive the MRL of 1 ppm.

- No MRL has been derived for intermediate-duration (15–364 days) inhalation exposure to toluene.

No data were considered suitable for use in deriving an intermediate-duration MRL for inhalation exposures. ATSDR believes that the chronic inhalation MRL would also be protective for intermediate-duration exposures.

- An MRL of 0.08 ppm (0.3 mg/m^3) was derived for chronic-duration (365 days or more) inhalation exposure to toluene.

The chronic inhalation MRL is based on a LOAEL of 35 ppm toluene for color vision impairment in a group of toluene-exposed shoemakers studied by Zavalic et al. (1998a) and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 to account for human variability). The study examined color vision abilities in three groups of workers: (1) 46 shoemakers exposed for an average of 16 years to a median toluene concentration of 32 ppm; (2) 37 rotogravure printing workers exposed for an average of 18 years to a median toluene concentration of 132 ppm; and (3) 90 control workers without any known exposure to solvents or neurotoxic agents. Average scores in a color confusion index (based on results of color vision tests and adjusted for age and alcohol intake) were significantly increased in the toluene-exposed shoemakers and printers compared with scores for control workers. The chronic LOAEL of 32 ppm is supported by observations of other subtle neurological effects in other groups of toluene-

exposed workers including altered visual-evoked brainstem potentials in printing press workers exposed to 50 ppm for 30 years (Vrca et al. 1995, 1996, 1997a, 1997b), altered auditory-evoked brainstem potentials in printers exposed to 97 ppm for 12–14 years (Abbate et al. 1993), hearing loss in printers exposed to 0.04–245 ppm toluene (Morata et al. 1997), changes in electro cardiographic R-R intervals in printers exposed to 83 ppm for 1–36 years (Murata et al. 1993), performance deficits in neurobehavioral tests in electronics workers exposed to 88–90 ppm (Boey et al. 1997; Foo et al. 1990), and increased incidence of self-reported neurasthenic symptoms in printers exposed to an average concentration of about 140 ppm over a 29-year period (Orbaek and Nise 1989).

Most of the data on health effects in humans exposed to toluene come from occupational studies or medical reports of solvent abusers. In both situations, concurrent exposure to other chemicals can limit the usefulness of the data for development of guidelines or standards. In addition, there are other confounding variables, especially in the occupational setting, such as alcohol consumption patterns, employment history, diet, use of medications, noise, and fluctuations in atmospheric toluene levels during different portions of the day, all of which complicate evaluation of dose-response patterns. These limitations were considered in selecting the studies for derivation of the MRLs.

ACGIH has recommended a TLV of 50 ppm toluene based on reports of headache and irritation associated with 4–6 hours continuous inhalation of toluene (Andersen et al. 1983; Baelum et al. 1985; Echeverria et al. 1989; Wilson 1943). This value is designed to be protective for healthy adult workers exposed 8 hours/day, 5 days/week for up to 45 years. Adjusting the value for a continuous exposure lasting up to 70 years yields a value of 8 ppm (50 ppm x 5 days/7 days x 8 hours/24 hours x 45 years/70 years=8 ppm). This figure is somewhat higher than the current chronic-duration MRL, but does not include an uncertainty factor to protect susceptible populations. Use of an uncertainty factor of 100 (10 for human variability and 10 for use of a LOAEL) would arrive at a value to 0.08 ppm, which is identical to the current MRL.

Oral MRLs.

- An MRL of 0.8 mg/kg has been derived for acute (14 days or less) oral exposure to toluene.

This MRL was based on a LOAEL of 250 mg/kg from a study of flash-evoked potential (FEP) wave forms in male Long-Evans rats administered doses of 0, 250, 500, or 1,000 mg/kg toluene by gavage (Dyer et al. 1988). Flash-evoked potential tests were administered 45 minutes later as a test of the ability

of the nervous system to process visual information. The amplitude of the N3 peak of the FEP was decreased by toluene exposure at all doses (P<0.0001). This decrease in peak amplitude was not dose-related. Dyer et al. (1988) also carried out a time-course study in which toluene was administered to male Long-Evans rats (16 per group) at doses of 0 and 500 mg/kg by gavage and flash-evoked potential tests were performed 4, 8, 16, and 30 hours later. In the time course study, 500 mg/kg also decreased the amplitude of the flash-evoked potential; at this dose, little change in magnitude of peak N3 depression had occurred 8 hours posttreatment; by 16 hours recovery was complete. An uncertainty factor of 300 was used for this determination (3 for use of a minimally adverse LOAEL, 10 for interspecies extrapolation, and 10 for intraspecies variability).

- An MRL of 0.02 mg/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to toluene.

This MRL was derived from a LOAEL of 5 mg/kg/day based on regional increases in monoamine neurotransmitters in the brains of CD1 mice exposed to toluene through their drinking water for 28 days (Hsieh et al. 1990b). Based on water consumption and average toluene concentrations, the authors calculated toluene doses for the 4 treatment doses of 0, 5, 22, and 105 mg/kg/day over this period. Brain levels of norepinephrine, dopamine, serotonin (5-hydroxytryptamine), and their metabolites vanillylmandelic acid, 3,4-dihydroxy-phenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid were measured in six areas of the brain in the mice. Significant increases (P<0.05) in neurotramsmitter levels were seen in all six regions of the brain of animals gavaged with toluene; in general, the increase was maximal at 22 mg/kg/day. Significantly increased norepinephrine levels were present in the hypothalamus, midbrain, and medulla oblongata. Serotonin levels were significantly increased in the hypothalamus, midbrain, and cerebral cortex. The maximum increase of serotonin (P<0.005), dopamine (P<0.05), and norepinephrine (P<0.05) in the hypothalamus occurred at 22 mg/kg/day. In the corpus striatum, the levels of dopamine and serotonin were significantly increased at the two highest doses. In the medulla oblongata, significant toluene increases of norepinephrine and homovanillic acid were seen only at 22 mg/kg/day. It should be noted that these are minimal effects, and it is unclear how they are related to neurobehavioral changes. An uncertainty factor of 300 was used for this determination (3 for the use of a minimally adverse LOAEL, 10 for interspecies extrapolation, and 10 for intraspecies variability).

No MRL was derived for chronic-duration oral exposures because there were no suitable data for toluene.

Death. Case studies that reported on deaths in humans due to exposure to toluene have generally not provided information on dose and thus, do not provide a basis for quantitative estimates. In one instance, intake of 625 mg/kg resulted in death within 30 minutes (Ameno et al. 1989).

It has been suggested that the mechanism for death due to solvent abuse might be apparent sensitization of the myocardium and consequent sudden and severe arrhythmia resulting from mild anesthesia, and intensified by hypercapnia (excess of carbon dioxide) (Bass 1970). Studies in dogs further suggest that some individuals may be more sensitive to arrhythmic responses to toluene than others (Ikeda et al. 1990). In a single report of human death following oral ingestion of toluene, the cause of death appeared to be profound disruption of central nervous system function (Ameno et al. 1989). Exposure to toluene at a hazardous waste site is not likely to be of lethal magnitude.

Systemic Effects.

Respiratory Effects. The primary effect of toluene on the respiratory tract following inhalation is irritation. Studies with volunteers (Andersen et al. 1983; Carpenter et al. 1944; Baelum et al. 1985) and exposed workers (Parmeggiani and Sassi 1954) have demonstrated that toluene is a mild-to-moderate respiratory irritant. Early animal studies by von Oettingen et al. (1942) reported respiratory irritation and pulmonary lesions in rats exposed to high concentrations of toluene. The findings of von Oettingen et al. (1942) are supported by more recent observations of nasal lesions (including metaplasia of olfactory epithelium and degeneration of respiratory epithelium) in rats exposed to concentrations ranging from 600 to 1,200 ppm, 6.5 hours/day, 5 days/week for 2 years (NTP 1990). Mice exposed by the same exposure protocol to a similar range of concentrations, however, did not display upper or lower respiratory tract lesions (NTP 1990). Acute, intermediate, or chronic inhalation exposure to toluene at a hazardous waste site might result in respiratory tract irritation, especially if release of toluene is into an enclosed space where higher concentrations may develop, but other adverse effects on the lungs or breathing passages are not expected.

Cardiovascular Effects. Inhalation exposure to toluene at concentrations above 1,000 ppm has been associated with alterations of the heart rhythm in both humans and animals (Anderson et al. 1982; Einav et al. 1997; Ikeda et al. 1990; Magos et al. 1990; Meulenbelt et al. 1990; Vidrio et al. 1986), but exposure of rats or mice to concentrations as high as 12,000 ppm (3 hours/day) for intermediate durations or 1,200 ppm (6.5 hours/day) for chronic durations produced no histological changes in heart tissue (Bruckner and Peterson 1981b; CIIT 1980; NTP 1990). There may be intraspecies differences in the

cardiac response to toluene that make some individuals more susceptible than others to potentially fatal arrhythmias; the degree of hypoxia may also be important (Ikeda et al. 1990).

The exposure scenarios associated with cardiac rhythm disturbances were of the short-term, high-level type experienced by substance abusers. Accordingly, cardiovascular responses are not expected to occur following toluene exposure at or near a hazardous waste site, unless some occurrence releases a high concentration of toluene into an enclosed space.

Gastrointestinal Effects. The only gastrointestinal effect reported after exposure to toluene was ulceration of the forestomach of rats exposed to 600 and 1,200 ppm by inhalation for 2 years (NTP 1990). Similar effects were not seen in mice exposed under the same conditions or to rats or mice orally exposed to 2,500 mg/kg/day for 13 weeks (NTP 1990). There is a slight possibility that long-term toluene exposure resulting from the contamination of hazardous waste sites would cause gastrointestinal irritation in the exposed population.

Hematological Effects. Before the mid-1950s, chronic occupational exposure to toluene was associated with hematological effects (Greenburg et al. 1942; Wilson 1943). However, these effects are now attributed to benzene, a common contaminant of toluene at that time (EPA 1985c). In several recent studies, no significant effects of toluene on hematological parameters in workers exposed to toluene or a to mixture of solvents has been observed (Banfer 1961; Capellini and Alessio 1971; Ukai et al. 1993; Yin et al. 1987). In contrast, Tahti et al. (1981) reported a slight positive correlation between exposure and decreased blood leukocyte counts in workers. However, the authors did not report whether the workers were also exposed to other organic solvents. Therefore, effects that were observed cannot be attributed solely to toluene. Decreased leukocyte and white blood cell counts were observed in dogs and rats repeatedly exposed to airborne toluene (Hobara et al. 1984a; Horiguchi and Inoue 1977; von Oettingen et al. 1942), but have not been observed consistently in other studies of rats and mice exposed by inhalation (NTP 1990; Ono et al. 1996; Poon et al. 1994) or by oral administration (Hsieh et al. 1989; NTP 1990; Wolf et al. 1956). In the study by von Oettingen et al. (1942), rats exposed to high concentrations (2,500 or 5,000 ppm) of toluene for 7 hours each day had decreased leukocyte counts following exposure; however, the leukocyte numbers generally returned to normal by the next day. The toxicological significance of a transitory decrease in numbers of leukocytes is not apparent. Since effects on hematological variables have not been observed consistently in studies of occupationally exposed humans or in animals exposed to toluene, they are not expected to occur from acute, intermediate, or exposures at or near hazardous waste sites.

Musculoskeletal Effects. There is one case report of a man who had been sniffing glue containing toluene for 18 years. He complained of severe muscle weakness and was diagnosed with rhabdomyolysis (an acute disease of the skeletal muscles evidenced by myoglobin in the blood and urine) (Hong et al. 1996). However, it is not expected that any such effects would result from toluene exposure at or near a hazardous waste site.

Hepatic Effects. Studies of chronic toluene abusers or occupationally exposed humans have provided little evidence for serious liver damage due to inhaled toluene. Some studies of workers occupationally exposed to average concentrations between about 30 and 350 ppm toluene have reported liver effects such as increased serum levels of enzymes leaked from the liver (Guzelian et al. 1988; Svensson et al. 1992b), but others have recorded no adverse effects (Lundberg and Hakansson 1985; Seijii et al. 1987; Ukai et al. 1993). Results from studies of animals exposed by inhalation for acute (Ungvary et al. 1982; Wang et al. 1996), intermediate (Bruckner and Peterson 1981b; Kjellstrand et al. 1985; Kyrklund et al. 1987; NTP 1990; Poon et al. 1994), or chronic (NTP 1990) durations indicate that daily 6- to 8-hour exposures to concentrations above 300 ppm, but not below, can lead to increased liver weights (Bruckner and Peterson 1981b; NTP 1990; Poon et al. 1994; Ungvary et al. 1982) and induction of hepatic cytochrome P450 levels (Ungvary et al. 1982; Wang et al. 1996). There are a few reports of toluene-induced effects that may be associated with liver damage [e.g., increased serum levels of liver enzymes in rats exposed to 2,000 ppm for 48 hours (Tahti et al. 1983) and in rats exposed to 300 ppm, 6 hours/day for 4 weeks (Poon et al. 1994) and increased endoplasmic reticulum in hepatocytes after exposure of rats, mice, and rabbits to 795 ppm 8 hours/day for 7 days (Ungvary et al. 1982)], but no significant histopathological liver changes or liver weight changes were observed in well-conducted chronic-duration studies in which rats and mice were exposed to concentrations as high as 1,200 ppm, 6.5 hours/day, 5 days/week for 2 years (CIIT 1980; NTP 1990). Results from intermediate-duration oral-exposure studies in rats and mice support the idea that toluene does not cause degenerative liver effects, but, at sufficiently high doses, produces liver weight increases that are likely associated with enzyme induction (Hsieh et al. 1989; NTP 1990; Wolf et al. 1956). It is possible that exposure of the general population to toluene at hazardous waste sites may increase the ability of the liver to metabolize xenobiotics, but other hepatic effects are not expected if exposure levels are at or below those normally experienced in workplaces using toluene.

Renal Effects. The kidney may be a target of toluene toxicity in humans exposed to very high levels of toluene. Renal acidosis has been observed in solvent abusers exposed to toluene, but, in most cases, renal dysfunction is transient and normal function returns when exposure ceases (Gerkin and LoVecchio 1998; Goodwin 1988; Kamijima et al. 1994; Kamijo et al. 1998; Kaneko et al. 1992; Patel and Benjamin

1986; Taverner et al. 1988). These cases, however, are frequently confounded by probable exposure to multiple solvents. Renal effects have not been observed in workers exposed to levels of toluene up to 100–200 ppm for long durations (Askergren et al. 1981a; Nielsen et al. 1985). Animal studies indicate that inhalation of toluene causes kidney damage in rats (e.g., renal tubular casts), but only after intermediate or chronic exposure to concentrations $600 ppm for at least 6 hours/day (CIIT 1980; NTP 1990; von Oettingen et al. 1942). Histological evidence of kidney damage was not found in rats and mice exposed to gavage doses as high as 5,000 mg/kg/day for 14 or 15 weeks (NTP 1990). In general, the available human and animal data suggest that kidney damage is not likely to occur with acute, intermediate, or chronic exposure at toluene levels likely to be experienced by people who may live close to, but not work at, hazardous waste sites containing toluene.

Endocrine Effects. As discussed in more detail in Section 2.6, Endocrine Disruption, current data for toluene-exposed humans or animals provide suggestive, but not conclusive, evidence that toluene may cause some effects that may involve endocrine disruption including reports of increased abortions among female electronics workers (Ng et al. 1992b), changed levels of luteinizing hormone, follicular stimulating hormone, and testosterone in male printers exposed to toluene (Svensson et al. 1992a, 1992b), and increased serum levels of prolactin in rats exposed to 80 ppm toluene 6 hours/day, 5 days/week for 4 weeks (Andersson et al. 1983; Hillefors-Berglund et al. 1995; von Euler et al. 1993, 1994).

Other results from animal studies regarding possible endocrine disruption from toluene include decreased sperm counts and epididymides weight in male rats that were exposed to 2,000 ppm, 6 hours/day for 90 days but showed no exposure-related changes in mating behavior or fertility indices when mated after 60 days of exposure (Ono et al. 1996) and abundant vacuoles and mitochondrial degeneration in antral follicles of the ovaries of female rats exposed to 3,000 ppm, 8 hours/day for 7 days (Tap et al. 1996). Histopathological lesions in male or female reproductive organs, however, were not found in rats and mice exposed to gavage doses up to 2,500 mg/kg/day for 13 weeks or exposed (6–6.5 hours/day, 5 days/week) to concentrations up to 2,500 ppm for 14–15 weeks or 1,200 ppm for 2 years (NTP 1990).

Assessment of reproductive performance was not consistently affected by toluene in several other animal studies. Increased fetal mortality occurred after exposure of pregnant rats to 2,000 ppm, but not 600 ppm, 6 hours/day on gestation days 7–17 or 14 days before through 7 days after mating (Ono et al. 1995, 1996), and increased abortions occurred in pregnant rabbits exposed to 267 ppm, but not 133 ppm, 24 hours/day on gestation days 7–20 (Ungvary and Tatrai 1985). However, the number of pregnant mice producing viable litters was not affected following oral administration of 2,350 mg/kg/day on gestation

days 7–14 (Smith 1983). In addition, reproductive performance variables and offspring survival were not significantly affected in two generations of rats exposed to 2,000 ppm, 6 hours/day for up to 95 days (API 1985) or in rats exposed *in utero* to 1,200 ppm, 6 hours/day on gestation days 9–21 (Thiel and Chahoud 1997).

Dermal Effects. Skin irritation can occur in humans and animals dermally exposed to toluene (EPA 1983a; Winchester and Madjar 1986; Wolf et al. 1956). This appears to be due to the degreasing action of toluene and its removal of protective skin oils. It is uncertain if toluene dissolved in water at or near hazardous waste sites would have an effect on the skin of individuals who come in contact with the contaminated water.

Ocular Effects. Humans have reported eye irritation following exposure to toluene vapors (Andersen et al. 1983; Baelum et al. 1985; Carpenter et al. 1944, 1976; Meulenbelt et al. 1990). This is probably the result of direct contact of toluene vapor with the outer surface of the eye and thus, is not a true systemic effect. Slight to moderately severe irritation of rabbit eyes has been reported following direct application of toluene to the conjunctiva (Carpenter and Smyth 1946; Hazleton Laboratories 1962; Wolf et al. 1956). Reports of color vision deficits in occupationally exposed workers have been postulated to involve toluene interference with dopaminergic mechanisms of retinal cells or toxic demylelinizaton of optic nerve fibers (Muttray et al. 1997, 1999; Zavalic et al. 1998a, 1998b, 1998c).

Body Weight Effects. Weight loss has been reported to occur in rats exposed to toluene for periods of 11–23 weeks (Mattsson et al. 1990; Pryor 1991).

Immunological and Lymphoreticular Effects. Only limited data are available on the immunological effects of toluene. The studies by Lange et al. (1973) and Moszczynski and Lisiewicz (1985) report decreased T lymphocyte counts and decreased serum IgG and IgA levels in occupationally exposed workers, but no signs of diminished immunological function or disturbances in immune skin reactions against such antigens as tuberculin or distreptase. However, because the specific solvent(s) responsible for the effects observed in these studies was not identified, the significance of the findings for humans exposed to toluene are unclear.

Mice exposed to toluene for 4 weeks exhibited an increased susceptibility to infections (Aranyi et al. 1985). Ingestion of doses of 22 mg/kg/day toluene caused adverse effects on lymphocyte proliferation and interleukin-2 immunity in mice exposed through their drinking water. At higher doses

(105 mg/kg/day), additional effects upon the immune system were observed (decreased thymus weight, lymphocyte culture responses, and antibody plaque-forming cell responses) (Hsieh et al. 1989, 1990b). There are no data to suggest that these same responses occur in humans.

Neurological Effects. The nervous system is the critical target of toluene toxicity following acute, intermediate, or chronic inhalation or oral exposure to toluene. Studies with volunteers under controlled acute (6–8 hours) exposure conditions indicate that subtle neurological impairment is detectable in most subjects at concentrations in the 75–150 ppm range (Andersen et al. 1983; Baelum et al. 1985; Echeverria et al. 1991; Guzelian et al. 1988; Iregren 1986; Rahill et al. 1996). Concentrations of 200–800 ppm can produce exhilaration and light-headedness, and, at higher acute exposure concentrations, intellectual, psychomotor, and neuromuscular abilities are obviously impaired followed by development of narcosis (EPA 1985c; von Oettingen et al. 1942).

Numerous case studies have associated chronic inhalation exposure to toluene at levels inducing narcosis and euphoria (4,000 to 12,000 ppm as estimated by Gospe et al. 1994) with residual or permanent neurological damage as evidenced by abnormal electroencephalograms, structural changes in the brain detected by MRI and SPECT, tremors, paranoid psychosis, recurrent hallucinations, and impaired speech, hearing, and vision (Byrne et al. 1991; Caldemeyer et al. 1996; Devathasan et al. 1984; Filley et al. 1990; Hunnewell and Miller 1998; Ikeda and Tsukagoshi 1990; Kamran and Bakshi 1998; King et al. 1981; Maas et al. 1991; Meulenbelt et al. 1990; Miyagi et al. 1999; Rosenberg et al. 1988a, 1988b; Ryu et al. 1998; Suzuki et al. 1983; Yamanouchi et al. 1995). Studies of workers repeatedly exposed to toluene in workplace air at concentrations ranging from about 30 to 150 ppm have found evidence for increased incidence of self-reported neurological symptoms (Orbaek and Nise 1989; Yin et al. 1987), performance deficits in neurobehavioral tests (Boey et al. 1997; Foo et al. 1990; Orbaek and Nise 1989), hearing loss (Abbate et al. 1993: Morata et al. 1997), changes in visual-evoked brainstem potentials (Vrca et al. 1995, 1997a, 1997b), and color vision impairment (Zavalic et al. 1998a, 1998b, 1998c).

Studies with laboratory animals provide supporting evidence that the nervous system is the critical target of toluene toxicity. Acute (1 to 2 hours) inhalation exposure studies of behavior in rats, mice, and monkeys found evidence for stimulatory effects (e.g., increased locomotor activity, significantly increased response times) at concentrations ranging from 500 to 2,000 ppm, and central nervous system depression (e.g., decreased accuracy in conditioned response tests, decreased locomotor activity, and ataxia) at concentrations greater than 2,000 ppm (Bowen and Balster 1998; Bruckner and Peterson 1981a, 1981b; Bushnell et al. 1985; Hinman 1987; Taylor and Evans 1985). Rats exposed for 4 hours to

concentrations as low as 125 ppm toluene showed performance deficits in several trained neuromuscular responses (Kishi et al. 1988; Mullin and Krivanek 1982; Wood et al. 1983). Acute- and intermediate-duration inhalation exposure studies reported changes in several brain biochemical variables (e.g., dopamine levels, dopamine D2 receptor binding, changes in glial fibrillary acidic protein) in rats at exposure levels as low as 50–80 ppm for 6–8 hours/day (API 1997; Hillefors-Bergllund et al. 1995; Ikeda et al. 1986; Little et al. 1998; von Euler et al. 1989b, 1993, 1994). Neurological effects observed in animals after acute- or intermediate-duration oral exposure include changed flash-evoked potentials in rats given single gavage doses of toluene as low as 250 mg/kg (Dyer et al. 1988), changes in levels of several neurotransmitters (e.g., norepinephrine, dopamine, serotonin) in several brain regions of mice exposed to 5–105 mg/kg/day in drinking water for 28 days (Hsieh et al. 1990b), and clinical signs of central nervous system dysfunction including ataxia, prostration, and tremors in rats and mice exposed to gavage doses $2,500 mg/kg/day for 13 weeks (NTP 1990). Other toluene-induced neurological effects reported in studies of animals with intermediate to chronic inhalation exposure include hearing loss in rats exposed to concentrations as low as 700–1000 ppm, 6–14 hours/day for 2–9 weeks (Campo et al. 1997, 1998; Johnson et al. 1988; Pryor and Rebert 1992; Pryor et al. 1984a, 1984b, 1991;), abnormal flash-evoked brain potential responses in rats exposed to 8,000 ppm for 15–35 minutes, 4–9 times/days for 13 weeks (Mattson et al. 1990), decreased weight of the total brain and the cerebral cortex, associated with decreased phospholipid content, in rats continuously exposed to 320 ppm for 30 days (Kyrklund et al. 1987), and decreased number of neurons in the hippocampus of rats, 4 months after exposure to 1,500 ppm toluene, 6 hours/day, 5 days/week for 6 months (Korbo et al. 1996).

Reproductive Effects. There is some evidence that women occupationally exposed to toluene, or wives of men similarly exposed, have an increased risk of spontaneous abortions (Lindbohm et al. 1992; Ng et al. 1992b; Taskinen et al. 1989). However, interpretation of these results is limited due to small sample size evaluated, an inability to define accurate exposure levels, failure to account for all possible confounding variables, and the difficulty in validating self-reported data. The occurrence of testicular atrophy (Suzuki et al. 1983) in one case of chronic solvent abuse cannot be specifically attributed to toluene exposure. Occupational exposure to increasing concentrations of toluene (8–<111 ppm) has been associated with decreased plasma levels of the luteinizing hormone, follicle stimulating hormone and testosterone levels in males (Svensson et al. 1992a, 1992b).

Results from the moderate number of animal studies examining reproductive end points following toluene exposure were discussed in detail in the Endocrine Effects portion of this section. These studies found some evidence for minor toluene-induced changes in male and female reproductive organs [e.g.,

decreased sperm count in male rats (Ono et al. 1995, 1996) and ultra structural changes in antral follicles in ovary of female rats (Tap et al. 1996)], but no histological evidence of structural damage to the reproductive organs in rats and mice exposed orally for intermediate durations or by inhalation for intermediate or chronic durations (NTP 1990). No evidence for impaired reproductive performance was found in several assays (Ono et al. 1995, 1996; Smith 1983; Thiel and Chahoud 1997), including a 2-generation study of rats exposed to up to 2,000 ppm, 6 hours/day (API 1985), except that exposure during pregnancy produced increased fetal mortality in rats exposed to 2,000 ppm, but not 600 ppm, 6 hours/day, on gestation days 7–17 or for 14 days before through 7 days after mating (Ono et al. 1995, 1996), and increased abortions in pregnant rabbits exposed to 267 ppm, but not 133 ppm, 24 hours/day on gestation days 7–20 (Ungvary and Tatrai 1985).

In general, the available results from studies of humans and animals suggest that toluene is not a potent reproductive toxicant, but may cause some reproductive problems, especially with repeated inhalation exposure during pregnancy to concentrations above 200 ppm.

Developmental Effects. There are a number of published reports of birth defects, similar to those associated with fetal alcohol syndrome, that have been described in children born to women who intentionally inhaled large quantities of toluene or other organic solvents during pregnancy (Arnold et al. 1994; Erramouspe et al. 1996; Goodwin 1988; Hersch 1988; Hersch et al. 1985; Lindemann 1991; Pearson et al. 1994). Defects described include microcephaly, central nervous system dysfunction, growth deficiency, cranofacial and limb abnormalities, and reversible renal tubular acidosis. Studies of women exposed during pregnancy to much lower concentrations of toluene in the workplace are restricted to a retrospective study of 14 women in Finland occupationally exposed to mixed solvents that suggested that solvent exposure may increase risk for central nervous system anomalies and neural tube closure defects (Holmberg 1979).

The reports of birth defects in solvent abusers suggest that high-level exposure to toluene during pregnancy can be toxic to the developing fetus. The available human data, however, do not establish causality between low-level or occupational exposure to toluene and birth defects, because of the small sample size and the mixed solvent exposure experienced by the subjects in the Holmberg (1979) study, the lack of other studies of possible birth defects in children of occupationally exposed women, and the likelihood that the high exposure levels experienced by pregnant solvent abusers (4,000–12,000 ppm) overwhelm maternal protection of the developing fetus from absorbed toluene. Experiments with pregnant mice demonstrated that 10-minute exposures to 2,000 ppm resulted in low uptake of toluene into

fetal tissue and suggest that, at lower exposure levels, absorbed toluene is preferentially distributed to maternal adipose tissue before distribution to the developing fetus (Ghantous and Danielsson 1986).

Studies of animals exposed during pregnancy found evidence for toluene-induced fetal resorptions and abortions in rabbits (Ungvary and Tatrai 1985) and skeletal retardation and anomalies in rats (Hudak and Ungvary 1978) at 24-hour/day exposure levels associated with maternal toxicity and mortalities (399 and 266 ppm for rabbits and rats, respectively). Other developmentally toxic effects in animals associated with less than continuous inhalation exposure to toluene (3–8 hours/day) during pregnancy include retarded skeletal development in rat fetuses at 266 ppm, 8 hours/day (Hudak and Ungvary 1978), decreased body weight in rat fetuses at 2,000 ppm, 6 hours/day (Ono et al. 1995), decreased body weight and increased incidence of unossified sternebrae in rat fetuses at $1500 ppm, 6 hours/day (Huntingdon Research Centre 1992a, 1992b), decreased fetal body weight and delayed vaginal opening in rat offspring at 1,000 ppm, 6 hours/day and increased preweaning offspring mortality at 1,200 ppm (Thiel and Chahoud 1997), decreased body weight and retarded skeletal development in mouse fetuses at 266 ppm, 3–4 hours/day (Ungvary and Tatrai 1985), and increased mouse litters with fetuses with enlarged renal pelves at 200 ppm, 6 hours/day (Courtney et al. 1986). Several inhalation studies have identified no-effect levels for toluene effects on standard developmental end points (e.g., implantations, resorptions, fetal body weight, and fetal visceral and skeletal malformations and variations) including 750 ppm, 6 hours/day on gestation days 6–15 for rats (Huntingdon Research Centre 1992b), 600 ppm, 6 hours/day on gestation day 7–17 for rats (Ono et al. 1995), 600 ppm, 6 hours/day on gestation days 9–21 for rats (Thiel and Chahoud 1997), 133 ppm, 3–4 hours/day on gestation days 6–15 for mice (Ungvary and Tatrai 1985), 133 ppm, 24 hours/day on gestation days 7–20 for rabbits (Ungvary and Tatrai 1985), and 500 ppm, 6 hours/day on gestation days 6–18 for rabbits (Klimisch et al. 1992).

In animal studies of oral exposure during gestation, developmentally toxic effects were not observed in pregnant mice exposed to oral doses of 1,800 or 2,350 mg/kg/day (Seidenberg et al. 1986; Smith 1983), but exposure of pregnant rats to gavage doses of 650 mg/kg /day toluene in corn oil on gestation days 6–19 produced offspring with decreased body weights, delayed ossification, smaller brain volumes, and decreased forebrain myelination per cell compared with controls (Gospe and Zhou 1998; Gospe et al. 1996).

Results from studies of neurobehavioral end points in rats following *in utero* exposure to toluene suggest that maternal exposure to airborne toluene concentrations above 1,200 ppm, 6 hours/day during late embryonic and fetal development can impair behavioral development of rat offspring (Jones and Balster

1997; Ono et al. 1995; Thiel and Chahoud 1997) and that drinking water exposure during gestation and lactation at doses of 106 mg/kg/day changes postweaning open-field locomotor activity in rat offspring (Kostas and Hotchin 1981).

The available animal studies suggest that toluene is not a potent teratogenic agent with *in utero* exposure, but can retard fetal growth and skeletal development and adversely influence development of behavior of offspring at exposure levels above those that form the basis of the inhalation and oral MRLs for toluene.

Genotoxic Effects. Results of *in vivo* studies of exposed humans (see Table 2-4) and *in vitro* microbial assays and other *in vitro* systems generally indicate that toluene is nonmutagenic and nongenotoxic (see Table 2-5). Richer et al. (1993) reported no significant effects on sister chromatid exchanges, cell cycle delay, and cell mortality in lymphocytes following exposure of 5 men to 50 ppm toluene over 3 consecutive days. However, an increase in the incidence of chromatid breaks, micronuclei, and sister chromatid exchanges in lymphocytes of workers exposed to toluene along with other chemicals has been reported (Bauchinger et al. 1982; Nise et al. 1991; Pelclova et al. 1990; Schmid et al. 1985). These studies of workers are confounded by concurrent exposure to other organic chemicals, small cohort size, and a lack of historical exposure monitoring data.

Cancer. Human and animal studies generally do not support a concern for the carcinogenicity of toluene. The only available human epidemiological studies were negative but inconclusive due to limitations in design. The validated animal inhalation bioassays were negative (CIIT 1980; NTP 1990); however, one available oral study showed a nondose-related increase in a variety of tumors (Maltoni et al. 1997). Thus, the data do not support a firm conclusion regarding the carcinogenicity of toluene.

2.6 ENDOCRINE DISRUPTION

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, or otherwise interfere with the normal function of the endocrine system. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. Some scientists believe that chemicals with the ability to disrupt the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. Others believe that endocrine disrupting chemicals do not pose a significant health risk, particularly in light 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

Table 2-4. Genotoxicity of Toluene *In Vivo*

Species (tests system)	End point	Results	Reference
Non-Mammalian cells:			
Grasshopper	Mitotic arrest	+	Liang et al. 1983
Mammalian cells:			
Rats	Chromosomal aberrations in bone marrow cells	+	Dobrokhotov and Enikeev 1977
Mice	Dominant lethal mutations in sperm cells	–	API 1981
Mice	DNA damage in blood, bone marrow and liver	–	Plappert et al. 1994
Human[a]	Chromatid breaks, gaps, and exchanges	+	Bauchinger et al. 1982
Human[a]	Chromosome changes	–	Forni et al. 1971
Human[a]	Sister chromatid exchange	–	Haglund et al. 1980
Human[a]	Chromosome changes	–	Maki-Paakkenen et al. 1980
Human[b]	DNA damage		Pitarque et al. 1999
Human[a]	Micronuclei and chromosome breaks	+	Nise et al. 1991
Human[a]	Aberrant cells and chromosome breaks	+	Pelclova et al. 1990
Human[a]	Sister chromatid exchange, cell cycle delay, cell mortality	–	Richer et al. 1993
Human[a]	Chromosome aberrations	+	Schmid et al. 1985
Human[a]	Sister chromatid exchange	–	Schmid et al. 1985

[a]Detected in peripheral lymphocytes
[b]Detected in leukocytes

+ = positive result; – = negative result

Table 2-5. Genotoxicity of Toluene *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Bos et al. 1981
S. typhimurium (TA98, TA100, UTH8413, 8414)	Gene mutation	–	–	Connor et al. 1985
S. typhimurium (TA1535, PSK1002)	Gene mutation	No data	–	Nakamura et al. 1987
S. typhimurium	Gene mutation	No data	–	Nestmann et al. 1980
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	NTP 1990
Escherichia coli (P3478)	Gene mutation	No data	–	Fluck et al. 1976
Mammalian cells:				
Human lymphocytes	Sister chromatid exchange and chromosomal aberrations	No data	–	Gerner-Smidt and Friedrich 1978
Human lymphocytes	Sister chromatid exchange and chromosomal aberrations	No data	–	NTP 1990

– = negative result

compounds are derived from plants and are similar in structure and action as endogenous estrogen. While there is some controversy over the public health significance of endocrine disrupting chemicals, it is agreed that the potential exists for these compounds to affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (EPA 1997g). As a result, endocrine disruptors may play a role in the disruption of sexual function, immune suppression, and neurobehavioral function. Endocrine disruption is also thought to be involved in the induction of breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Current data provide suggestive, but not conclusive, evidence that toluene may cause some endocrine effects. Most case studies of chronic abusers of toluene and other solvents have not reported effects on endocrine organs, but there are reports of effects that may be associated with endocrine disruption in groups of toluene-exposed workers including changed plasma levels of luteinizing hormone, follicular stimulating hormone, and testosterone in male printers exposed to toluene (Svensson et al. 1992a, 1992b), delayed time to pregnancy among wives of men exposed to mixed organic solvents including toluene (Sallmen et al. 1998), increased incidence of spontaneous abortions in female toluene-exposed electronics workers (Ng et al. 1992b), and incidences of spontaneous abortion above population norms in other small groups of toluene-exposed female workers or wives of male workers (Lindbohm et al. 1992; Taskinen et al. 1989). However, small numbers and lack of adjustment for possible confounding factors in some of these studies precludes drawing definite conclusions.

In animal studies, female rats exposed to 30 or 300 ppm, 6 hours/day, 5 days/week for 4 weeks showed a mild reduction in follicle size of the thyroid in one study (Poon et al. 1994), but results from several other studies in rats and mice found no histological evidence of toluene-induced changes in endocrine organs including the thyroid, adrenal glands, or pancreas following intermediate or chronic, oral, or inhalation exposure (API 1985, NTP 1990, Von Oettingen et al. 1942).

Exposure to toluene may damage the reproductive organs in animals, but whether this affects reproductive performance is unclear. Decreased sperm counts and decreased weights of the epididymides have been reported in male rats exposed to 2,000 ppm, 6 hours/day for 90 days (Ono et al. 1996). Exposure of female rats to 3,000 ppm, 8 hours/day for 7 days produced abundant vacuoles, lytic areas, and mitochondrial degeneration in the antral follicles of the ovaries (Tap et al. 1996). Increased relative testicular weights were reported in male mice exposed to 1,250 and 2,500 mg/kg/day by gavage for 13 weeks. However, no effects on the prostate, testes, uterus, or ovaries were observed in rats and female

mice gavaged with 312–2,500 mg/kg/day (NTP 1990), and no treatment-related histopathological lesions were found in the testes or ovaries of rats and mice exposed (for 6–6.5 hours/day) to concentrations up to 2,500 ppm toluene for 6.5 hours/day for 14–15 weeks or up to 1,200 ppm for 6–6.5 hours/day for 2 years (CIIT 1980; NTP 1990).

Assessment of reproductive performance was not consistently affected by toluene in several other animal studies. Increased fetal mortality was reported for rats exposed to 2,000 ppm for 6 hours/day from days 7–17 of gestation or from 14 days before mating until day 7 of gestation (Ono et al. 1995, 1996). Increased abortion was seen in rabbits continuously exposed to 267 ppm on days 7–20 of gestation, but not in mice exposed to 267 ppm for 3–4 hours/day on gestational days 6–15 (Ungvary and Tatrai 1985). However, the number of pregnant mice producing viable litters was not affected following oral administration of 2,350 mg/kg/day on gestation days 7–14 (Smith 1983). In addition, reproductive performance variables and offspring survival were not significantly affected in two generations of rats exposed to 2,000 ppm 6 hours/day for up to 95 days (API 1985) or in rats that had been exposed *in utero* to 1,200 ppm 6 hours/day on gestation days 9–21 (Thiel and Chahoud 1997).

There is evidence that toluene exposure can perturb the hypothalamic-pituitary axis in rats leading to persistent increases in serum levels of prolactin, but a study of toluene-exposed workers found no evidence for changed prolactin levels compared with control subjects (Svensson et al. 1992a, 1992b). Acute-to-intermediate duration exposure to 80 ppm toluene (6 hours/day for 4 weeks) increased serum levels of prolactin in rats 17 days after cessation of exposure, but not 29–40 days after exposure (Andersson et al. 1983; Hillefors-Berglund et al. 1995; von Euler et al. 1993, 1994). Von Euler et al. (1993, 1994) speculated that the increase in serum prolactin level could be related to a possible interaction between toluene and the pituitary dopamine D2 receptor which inhibits the release of prolactin into serum.

2.7 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 5.6 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 while 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).

Data from controlled-exposure studies of volunteers, studies of occupationally exposed humans, case reports of toluene abuse, and studies of animals after inhalation or oral exposure indicate that the nervous system is the critical target of toluene toxicity (see Sections 2.2, 2.3.4, and 2.5 for more details). The effects of toluene have not been thoroughly studied in children, but the limited available data suggest that the nervous system is also the most likely target of toluene toxicity in children. There are numerous reports of adolescents who repeatedly inhaled high levels (4,000–12,000 ppm) of toluene and developed persistent central nervous system dysfunction (e.g., Byrne et al. 1991; Devasthasan et al. 1984; King et al. 1981). Neurological effects associated with toluene exposure in juvenile animals include changed levels of brain neurotransmitters in rats, 7 weeks after 10-day exposure as newborns to 80 ppm, 6 hours/day (von Euler et al. 1989b); high frequency hearing loss in rats after exposure to 1,200 ppm for 14 hours/day for 5 or 9 weeks starting at weaning (Pryor and Rebert 1992; Pryor et al. 1984a); and decreased latency of escape from an electric shock in young rats (50 days old) exposed to 30,000–40,000 ppm toluene for 15 minutes/day for 30 days (Castilla-Serna et al. 1991).

Available information regarding age-related differences in toluene metabolism suggests that developing fetuses and children at very early stages of development may be more susceptible to toluene toxicity than adults, and that children past early neonatal periods may have the same capability as adults to dispose of toluene at low exposure levels . The capacity for metabolic detoxification of toluene is expected to be low in the developing human fetus because several CYP isozymes are either absent or expressed at very low levels (Leeder and Kearns 1997). However, rat studies indicate that levels of CYP isozymes involved in toluene metabolism are rapidly increased following birth and suggest that capabilities to carry out Phase I toluene metabolism at low exposure levels during neonatal periods may exceed those at sexual maturity and pregnancy (Nakajima et al. 1992b). CYP2E1, one of the principal CYP isozymes involved in the major toluene metabolic pathway (Nakajima et al. 1997; Tassaneeyakul et al. 1996), is expressed several hours after birth in humans and continues to increase during the first year of life (Vieira et al. 1996). Phase II enzymes involved in toluene metabolism (e.g., N-acetyl transferases, UDP-glucuronyl transferases, and sulfotransferases) also show changes during human neonatal development with adult activities present by 1–3 years of age (Leeder and Kearns 1997). There are other physiological differences between adults and children (e.g., children have higher brain mass per unit of body weight, higher cerebral blood flow per unit of brain weight, and higher breathing rates per unit of body weight:

see Snodgrass [1992]), but their contributions to possible age-related differences in susceptibility to toluene toxicity are currently uncertain.

Results from animal studies indicating that younger animals may be more susceptible to toluene toxicity than adults are restricted to markedly lower LD_{50} values for 14-day-old rats compared with adult rat values (Kimura et al. 1971) and more severe high frequency hearing loss in young rats exposed to toluene compared with adult rats (Pryor et al. 1984a). The human brain grows rapidly for the first 2 years life and continues more slowly until full brain cell numbers, complete myelination of subcortical white matter, and complete elaboration of dendrites and axons are attained at adulthood (Snodgrass 1992). It is unknown if the relatively long period of development of the human brain may make juvenile humans more susceptible to toluene toxicity than juvenile non primate animals.

Case reports of birth defects in solvent abusers suggest that high-level exposure to toluene during pregnancy can be toxic to the developing fetus (Arnold et al. 1994; Erramouspe et al. 1996; Goodwin 1988; Hersch 1988; Hersch et al. 1985; Lindemann 1991; Pearson et al. 1994). It is likely that the high exposure levels experienced by pregnant solvent abusers (4,000 to 12,000 ppm) overwhelm maternal mechanisms that protect the developing fetus from absorbed toluene at lower exposure levels. Experiments with pregnant mice demonstrated that 10-minute exposures to 2,000 ppm resulted in low uptake of toluene into fetal tissue and suggest that, at lower exposure levels, absorbed toluene is preferentially distributed to maternal adipose tissue before distribution to the developing fetus (Ghantous and Danielsson 1986).

Studies of pregnant rats, mice, and rabbits found no effects on standard developmental end points such as number of resorptions, fetal body weights, and incidences of fetuses or litters with malformations, after exposure during gestation to inhaled concentrations as high as 500–750 ppm, 6 hours/day (Huntingdon Research Centre 1992b; Klimisch et al. 1992; Ono et al. 1995; Thiel and Chahoud 1997; Ungvary and Tatrai 1985) and 133 ppm, 24 hours/day (rabbits only) (Ungvary and Tatrai 1985) or to oral doses as high as 1,800 or 2,350 mg/kg/day (Seidenberg et al. 1986; Smith 1983). Observed effects on fetal development in the available animal studies, at higher exposure levels that were not fatal to the fetuses or the mothers, were generally restricted to fetal body weight decrease, retardation of skeletal development, or minor skeletal or visceral anomalies without increased incidences of malformations (Courtney et al. 1986; Hudak and Ungvary 1978; Huntingdon Research Centre 1992a, 1992b; Ono et al. 1995; Thiel and Chahoud 1997; Ungvary and Tatrai 1985). Neurological effects observed in animal offspring following gestational exposure to toluene include smaller brain volumes and decreased forebrain myelination per

cell in offspring of pregnant rats exposed to 650 mg/kg/day during gestation (Gospe and Zhou 1998; Gospe et al. 1996) and behavioral changes in offspring of pregnant rats exposed by to airborne concentrations above 1,200 ppm, 6 hours/day (Jones and Balster 1997; Ono et al. 1995; Thiel and Chahoud 1997) or to drinking water doses of 106 mg/kg/day (Kostas and Hotchin 1981).

In general, available information suggests that toluene is not a potent teratogenic agent with *in utero* exposure, but can retard fetal growth and skeletal development and adversely influence development of behavior of offspring at exposure levels above those that form the basis of the inhalation and oral MRLs for toluene.

Transfer of toluene to nursing infants from breast milk of currently exposed mothers is expected to be a possibility because of the lipophilicity of toluene and the relatively high lipid content of breast milk. Elimination kinetics data for nonpregnant or nonlactating humans and rats following toluene exposure, however, indicate that most absorbed toluene is rapidly eliminated from the body and that a much smaller portion (that which gets into adipose tissues) is slowly eliminated (Leung and Paustenbach 1988; Lof et al. 1993; Pierce et al. 1996, 1999; Pellizzari et al. 1992; Rees et al. 1985; see Section 2.3.4). Thus, mobilization during pregnancy or lactation of stored toluene from pre conception exposure does not appear to be a major concern.

Fisher et al. (1997) developed a human PBPK model that predicts transfer of toxicant via lactation from a mother to a nursing infant and used the model to estimate the amount of toluene an infant would ingest via milk if the mother was occupationally exposed to toluene at the ACGIH (1999) Threshold Limit Value (TLV=50 ppm) throughout a workday. The model predicted that such an infant would have a daily oral intake of 0.46 mg toluene/day. This value is below the U.S. EPA Health Advisory, 2.0 mg/day, for chronic ingestion of 1 L/day of toluene-contaminated water by a 10-kg child and a daily oral intake for a 10-kg child (8 mg/day) associated with the acute oral MRL for toluene (0.8 mg/kg/day). However, this value is above the daily oral intake for a 10-kg child (0.2 mg/day) associated with the intermediate oral MRL for toluene (0.02 mg/kg/day), suggesting there may be some concern for neurological effects in suckling infants exposed for more than 14 days to breast milk from mothers exposed during lactation to concentrations of 50 ppm in workplace air. It should be noted, however, that no human (or animal) studies were located regarding *in vivo* distribution of toluene into breast milk or elimination kinetics from breast milk, and the Fisher et al. (1997) PBPK model has not been validated with *in vivo* data.

2.8 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 toluene are discussed in Section 2.8.1.

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 toluene are discussed in Section 2.8.2.

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 2.10 "Populations That Are Unusually Susceptible".

2.8.1 Biomarkers Used to Identify or Quantify Exposure to Toluene

The biological exposure indices recommended by ACGIH (1999) to assess exposure of workers to toluene in the workplace are *ortho*-cresol and hippuric acid levels in urine at the end of a workshift and toluene levels in blood immediately prior to the last shift of a workweek. However, there are no markers of toluene exposure that persist in the body for an extended period of time after exposure has ceased.

The most accurate biomarker of toluene exposure is the presence of toluene in serum or blood, but measurements of toluene or its metabolites in urine are often preferred as urine sampling is less invasive. Measurements of toluene in serum, blood, and urine taken at the end of shift were significantly correlated with measurements of toluene concentrations from personal air monitors (Kawai et al. 1992a, 1992b, 1996). Measurement of toluene in blood was more sensitive than measurement of toluene in urine for detecting toluene at low concentrations (Kawai et al. 1992a). It is not necessary to draw large quantities of blood for analysis since toluene concentrations from capillary blood samples were also highly correlated (r=0.94) with toluene concentrations in exhaled air (Foo et al. 1991).

Although measurement of urinary excretion of toluene metabolites (hippuric acid, mercapturic acids, *ortho*-cresol and *para*-cresol) is a less invasive method than blood sampling for determining toluene exposure, the presence of these compounds in the urine is not definitive proof of toluene exposure since they are also produced by metabolism from the normal diet (Baelum 1990; Hjelm et al. 1988; Lof et al. 1993; Maestri et al. 1997). It has also been reported that the presence of toluene in urine is a more sensitive biomarker for toluene exposure than the presence of hippuric acid or ortho-cresol (Kawai et al. 1996). In addition, the background levels of these metabolites may be affected by individual variability (Lof et al. 1993), ethnic differences (Inoue et al. 1986), or other factors such as alcohol consumption and smoking (Kawamoto et al. 1996; Maestri et al. 1997). Despite these limitations, a number of authors have shown a correlation between the levels of these metabolites in urine and toluene exposure (Angerer and Kramer 1997; Angerer et al. 1998a; Kawai et al. 1992a, 1992b, 1996; Nise 1992; Truchon et al. 1996) and they have been widely used as biomarkers of toluene exposure.

2.8.2 Biomarkers Used to Characterize Effects Caused by Toluene

There are no specific biomarkers used to characterize the effects from toluene exposure. Changes in the brain, which are detected through magnetic resonance imaging (MRI) or brainstem auditory evoked response (BAER) techniques in combination with an exposure history, can be used to evaluate the degree of central nervous system damage experienced by a known toluene abuser. This approach does not appear to offer potential as a method of measuring the effects of short- or long-term minimal exposures as are likely to occur with environmental releases. A detailed discussion of the effects of toluene exposure is included in Section 2.2.

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).

2.9 INTERACTIONS WITH OTHER CHEMICALS

Alteration of toluene metabolism may influence toluene's toxic effects because toluene metabolism predominately represents a detoxification process (see Section 2.4.2). Hypothetically, compounds that stimulate or inhibit metabolism of toluene may respectively decrease or increase toluene toxicity, although the possible exhalation of unmetabolized toluene represents an alternate dispositional pathway that may be utilized under conditions inhibiting mainstream toluene metabolism. Several metabolic interactions between toluene and other chemicals have been studied. The results present evidence that alteration of toluene metabolism may influence toluene toxicity and that toluene can influence the toxicity of other chemicals.

Phenobarbital pretreatment, which increases the rate of *in vivo* metabolism of toluene by inducing CYP isozymes, prevented hearing loss in rats exposed to 1,500–2,000 ppm toluene, 8 hours/day for 7 days (Pryor et al. 1991). Conversely, rats that were given large gavage doses of ethanol (4 g/kg/day) and daily inhalation exposure to toluene concentrations of 1,750 ppm, 6 hours/day, 5 days/week for 4 weeks showed significantly greater changes in auditory-evoked brainstem potentials and outer hair cell loss in the ear than those exposed to toluene alone (Campo et al. 1998). Co-exposure to ethanol caused a significant decrease in hippuric acid urinary excretion rates compared with exposure to toluene alone, indicating that these large doses of ethanol inhibited the metabolism of toluene (Campo et al. 1998).

Consistent with the idea that co-exposure to ethanol inhibits toluene metabolism are observations that ingestion of ethanol prolongs the presence of toluene in blood in humans (Imbriani and Ghittori 1997; Wallen et al. 1984) and rats (Romer et al. 1986). These results indicate that toluene-induced hearing loss is caused by toluene itself and not its metabolites, and that workers exposed to toluene who regularly drink alcohol may be at greater risk of developing toluene-related neurological problems than non drinkers.

Concurrent chronic ethanol ingestion and acute toluene inhalation in rats was associated with a modest elevation in plasma aspartate aminotransferase and increases in relative liver weight and liver triglycerides (Howell et al. 1986). Toluene also antagonized the hypertriglyceridemia associated with chronic ethanol ingestion. This study suggests that combined ethanol and chronic occupational toluene exposure may have the potential to augment alcohol-induced fatty liver.

Benzene, xylene, and toluene are metabolized through cytochrome P-450 oxidation. Benzene is converted to phenol, hydroquinone, catechol, and phenyl mercapturic acid; xylene is converted to methyl hippuric acids, and toluene forms hippuric acid, o-cresol, and p-cresol. The excretion of metabolites was investigated in four groups of workers who were exposed in the workplace to benzene and toluene, to a mixture of both solvents, or to no solvents (Inoue et al. 1988). Analysis of the data on excretion of urinary metabolites indicated that simultaneous exposure to both benzene and toluene inhibited the microsomal metabolism of both compounds through the cytochrome P-450 system. Toluene had more of an inhibitory effect on benzene metabolism than benzene had on toluene metabolism. This observation was confirmed in rodent studies using 6-hour inhalation exposures to benzene, toluene, or a mixture of both compounds, with pharmacokinetic modeling of the exposure data (Purcell et al. 1990). Combinations of either 200 ppm toluene with 1,000 ppm benzene or 1,000 ppm toluene with 200 ppm benzene were tested. The fit of the actual closed chamber concentrations for the individual chemicals with the model results, suggests that the interaction of benzene and toluene are noncompetitive. The data from studies of the benzene-toluene interaction may indicate that workers exposed to mixtures of both solvents have a lower risk of benzene-induced leukopenia than workers exposed to benzene alone (Purcell et al. 1990).

Toluene and xylene are also often found together in mixtures such as paint thinners. Human exposure to low levels of both solvents (50 ppm xylene, 40 ppm toluene) did not modify the conversion of either substance to its urinary metabolites (Kawai et al. 1992b; Tardif et al. 1991). However, at higher concentrations (80 or 150 ppm xylene, 95 or 150 ppm toluene), the blood and exhaled air concentrations

of both solvents were increased compared to the controls exposed to either solvent alone, indicating that metabolism of both solvents was decreased by the coexposure paradigm (Tardif et al. 1991, 1992). Similarly, coexposure of toluene, methyl ethyl ketone and isopropyl alcohol at low concentrations in rats had no effect on the urinary excretion of hippuric acid, while high concentrations resulted in decreased levels of hippuric acid (Uaki et al. 1995). Tardif et al. (1993) reported that a linked PBPK model for toluene and xylene with a competitive inhibition metabolic term provided the best visual fit (compared with non- or competitive inhibition metabolic terms) to empirical data for air concentrations of toluene and xylene during 5-hour exposures of rats in a closed chamber to mixtures of toluene and xylene at several initial concentrations.

Toluene and *n*-hexane, which are used together in some glues and paints, are neurotoxic chemicals that act by different modes at different sites. Toluene effects on the central nervous system are thought to be facilitated by toluene itself, whereas *n*-hexane affects the peripheral nervous system through the production of a toxic metabolite, 2,5-hexanedione (Ali and Tardif 1999). The initial metabolism of both compounds has been demonstrated to principally involve CYP isozymes including CYP2E1 and CYP2B6 (Ali and Tardif 1999). Under *in vitro* conditions with rat liver microsomes, a noncompetitive inhibition of each other's metabolism was demonstrated (Perbellini et al. 1982). In studies comparing urinary excretion of metabolites in rats exposed to mixtures of toluene and *n*-hexane or to each solvent alone, co-exposure inhibited the urinary excretion of 2,5-hexanedione to a larger extent than the urinary excretion of toluene metabolites, hippuric acid, and *ortho*-cresol (Ali and Tardif 1999; Iwata et al. 1983; Perbellini et al. 1982). The results from these studies suggest that toluene is a more effective inhibitor of *n*-hexane metabolism than is *n*-hexane of toluene metabolism. Co-exposure of rats to 1,000 ppm toluene and 1,000 ppm *n*-hexane (12 hours/day for 16 weeks) decreased toxic effects of *n*-hexane on the peripheral nervous system compared with exposure to 1,000 ppm *n*-hexane alone (Takeuchi et al. 1981). Another rat study found confirming results in that co-exposure to 1,200 ppm toluene and 4,000 ppm *n*-hexane (14 hours/day for 9 weeks) decreased *n*-hexane-induced effects on the peripheral nervous system compared with *n*-hexane alone, and had only slight effects on toluene-induced hearing loss and motor dysfunction compared with toluene alone (Pryor and Rebert 1992). Human and rat PBPK models have been developed to model the combined exposure and disposition of inhaled toluene and *n*-hexane (Ali and Tardif 1999; Yu et al. 1998). Model simulations predicted that co-exposure to *n*-hexane and toluene at constant concentrations corresponding to their occupational exposure limits (50 ppm) would lead to only a slight effect on the kinetics of their respective metabolism and disposition, but that the interaction could change with fluctuations in worker activity loads and workplace air concentrations (Ali and Tardif 1999; Yu et al. 1998).

An individual's drug therapy can have an influence on toluene toxicity. Haloperidol (an antipsychotic) functions by blocking dopamine receptors in the brain. The combination of haloperidol with toluene exacerbates dopamine depletion in several areas of the brain, thus changing the pharmacodynamics of the haloperidol. Individuals who take haloperidol should be counseled by their physician if environmental or occupational exposure to toluene is possible (von Euler et al. 1988b).

Studies in humans and rats indicate that the common analgesics, acetaminophen and aspirin, may inhibit toluene metabolism and influence toluene toxicity. CYP2E1 is involved in the initial step of the principal metabolic pathway for toluene and acetaminophen, and represents a potential site for a competitive metabolic interaction. Aspirin and one of the principal downstream metabolites of toluene, benzoyl coenzyme A, are conjugated with glycine. When glycine pools are depleted by competition for glycine by aspirin metabolism, toluene metabolism may be inhibited. In volunteers exposed for 4 hours to 300 mg/m^3 toluene (80 ppm) with or without doses (1,000 mg/70 kg=14.3 mg/kg) of acetaminophen (paracetamol) or acetyl salicylic acid (aspirin), co-exposures with these analgesics increased the concentration of toluene in the blood compared with exposure to toluene alone (Lof et al. 1990). Acetaminophen co-exposure also significantly increased the area under the blood concentration versus time curve and the apparent blood clearance of toluene, consistent with an inhibition of toluene metabolism. Co-exposure of rats for 10 days to higher oral doses of aspirin (acetyl salicylic acid: 100 mg/kg, twice daily) and inhalation exposure to toluene (1,000 ppm, 14 hours/day) caused a more severe loss of hearing (assessed 2–5 days or 4 months after cessation of exposure) compared with exposure to toluene alone (Johnson 1992). Treatment with aspirin alone at these doses did not cause hearing loss in the rats. These results are consistent with the hypothesis that high doses of aspirin may potentiate toluene effects on hearing by inhibiting toluene metabolism.

The benzoic acid metabolite of toluene is conjugated with glycine to produce hippuric acid. Toluene potentiation of developmentally toxic effects in rats from high doses of aspirin has been attributed to metabolic competition for glycine pools (Ungvary et al. 1983). Pregnant rats that were given 250 mg/kg acetyl salicylic acid on gestation day 12 and exposed to toluene at concentrations of 1,000, 2,000, or 3,600 mg/m^3 (265, 531, or 956 ppm) on gestation days 10–13 showed maternal effects (decreased food consumption and body weight gain and increased relative liver weight) and fetal effects (retardation of skeletal development and increased incidence of fetal malformations) that were more severe than those observed in rats exposed to 250 mg/kg acetyl salicylic acid alone. The effects were comparable in severity to those observed in rats exposed to 500 mg/kg salicylic acid alone. In this study, no maternal or fetal effects were observed in a group of rats exposed to 956 ppm toluene on gestation days

10–13 without coexposure to acetyl salicylic acid. The maternal and fetal effects of co-exposure to acetyl salicylic acid and toluene were diminished to the severity of the 250-mg/kg acetyl salicylic acid alone level when the administration of the acetyl salicylic acid dose was preceded by two hours with a gavage dose of 5,000 mg/kg glycine.

2.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to toluene than will most persons exposed to the same level of toluene 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 toluene, or compromised function of organs affected by toluene. Populations who are at greater risk due to their unusually high exposure to toluene are discussed in Section 5.7, Populations With Potentially High Exposures.

The main target organ of toluene is the central nervous system, and it is generally thought to be due at least in part, to reversible interactions between toluene (the parent compound, not its metabolite) and the lipid or protein components of nervous system membranes (mechanisms of toxicity are discussed in detail in Section 2.4.2). The main pathway of toluene metabolism leads to the production of hippuric acid, which is excreted in the urine. The predominant first step in human and rat metabolism of toluene is catalyzed primarily by the CYP 2E1 isozyme. Later steps in this pathway involve the enzymes alcohol dehydrogenase, aldehyde dehydrogenase, acyl-coenzyme A synthase, and acyl-coenzyme A:amino acid N-acyl transferase (metabolism is discussed in detail in Section 2.2.3).

Environmental or genetic factors that decrease the capacity for metabolic detoxification of toluene are likely to increase susceptibility. This is supported by experiments in which inhibiting or enhancing toluene metabolism respectively enhanced of inhibited toluene-induced hearing loss in rats (Campo et al. 1998; Pryor et al. 1991). Chronic consumers of alcohol, and users of any medication that interfered with toluene metabolism, would be likely to have an increased risk for this reason. Differences in the relative efficiency of enzymes found in ethnic populations may also lead to differences in toluene susceptibility. For instance, ethnic variations in the occurrence of CYP isozymes, alcohol dehydrogenase, and aldehyde dehydrogenase are known to exist (Kawamoto et al. 1995, 1996; Kim et al. 1997).

Nutritional status may also affect susceptibility to toluene. Liver metabolism of toluene in rats fasted for 1 day was significantly increased compared with rats that had been fed (Nakajima and Sato 1979).

However, long-term malnutrition may increase susceptibility to the developmental effects of toluene. Skeletal development in the fetuses of rats that were malnourished throughout pregnancy and injected with 1.2 g/kg/day toluene was retarded to a significantly greater extent than in the fetuses of well-nourished dams injected with toluene (da Silva et al. 1990).

Individuals with pre-existing medical conditions may also be more susceptible to the effects of toluene. Individuals with pre-existing defects in heart rhythm may have a greater risk than healthy individuals for experiencing tachycardia or cardiac fibrillation following exposure to high levels of toluene. The presence of toluene in the air reduces the concentration of oxygen and can lead to hypoxia when exposure concentrations are high. Thus, individuals with asthma or other respiratory difficulties may be at increased risk with exposure to high atmospheric concentrations of toluene. Genetic predisposition for hearing loss may increase the risk for toluene-induced ototoxicity (Johnson 1992; Li et al. 1992).

2.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to toluene. 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 toluene. 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 toluene:

Aaron, CK and Howland, MA (eds.). 1994. *Goldfrank's Toxicologic Emergencies*. Appleton and Lange, Norwalk, CT.

Dreisbak, RH (ed.). 1987. *Handbook of Poisoning*. Appleton and Lange, Norwalk, CT.

Ellenhorn, MJ and Barceloux, DG, (eds.). 1988. *Medical Toxicology: Diagnosis and Treatment of Human Poisoning*. Elsevier Publishing, New York, NY.

Haddad, LM and Winchester, JF (eds.). 1990. *Clinical Management of Poisoning and Drug Overdose*. 2nd edition, WB Saunders, Philadelphia, PA.

2.11.1 Reducing Peak Absorption Following Exposure

The absorption of toluene is rapid and virtually complete following inhalation and oral exposures. Toluene appeared in the blood of 10 human subjects within 10–15 minutes of exposure to 78 ppm toluene

in the air, signifying rapid absorption through the lungs. When exposure occurs by the oral route, uptake into the blood is expected to be slightly slower due to the time needed for transit to the small intestines. Since toluene is absorbed across the lipid matrix of the cell membrane (Alcorn et al. 1991) some absorption can occur from the mouth and stomach. However, most of the toluene will be absorbed through the intestines due to large exposed surface area of the villi and microvilli. Other factors that will influence uptake from the gastrointestinal tract are lipid content of the gastrointestinal contents and the magnitude of the toluene exposure. Absorption of inhaled toluene is increased by exercise and so a reduction of physical activity during exposure is likely to reduce absorption (Rahill et al. 1996). However, there is really no effective way to reduce peak absorption following inhalation exposure. Emesis is contraindicated in cases of toluene ingestion due to the risk of aspiration. The use of activated charcoal and lavage may help to reduce oral exposure and rapid rinsing of the skin with water or washing with soap and water will reduce the opportunity for dermal absorption. If the eyes are affected, proper rinsing procedures should be followed.

2.11.2 Reducing Body Burden

The total body burden of toluene is reduced by measures that increase the rate of metabolism and excretion. Oxygen therapy and positive-pressure ventilation have been used as emergency treatments following episodes of toluene abuse (Graham 1990). This procedure promotes the loss of unmetabolized toluene from the lungs. Increased oxygen availability also has a positive effect on the rate of oxidative metabolism in the liver, lungs, intestines, and other tissues.

Increased fluid consumption, which increases the rate of urine production and excretion, will help to decrease the toluene body burden since toluene metabolites are water soluble and excreted in the urine. In cases where kidney function has been impaired, renal dialysis has been used to remove toluene metabolites from the body (Graham 1990).

2.11.3 Interfering with the Mechanism of Action for Toxic Effects

In cases where toluene has caused cardiac arrhythmias, antiarrhythmic medications have been used to control the heart beat (Graham 1990). No other medical practices for ameliorating the toxic effects of toluene were identified in the available literature. When toluene exposures are unavoidable, as in the workplace, avoidance of alcohol or medications that may inhibit metabolic disposition of toluene is another measure that can be taken to reduce health risks from exposure.

2.12 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 toluene 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 toluene.

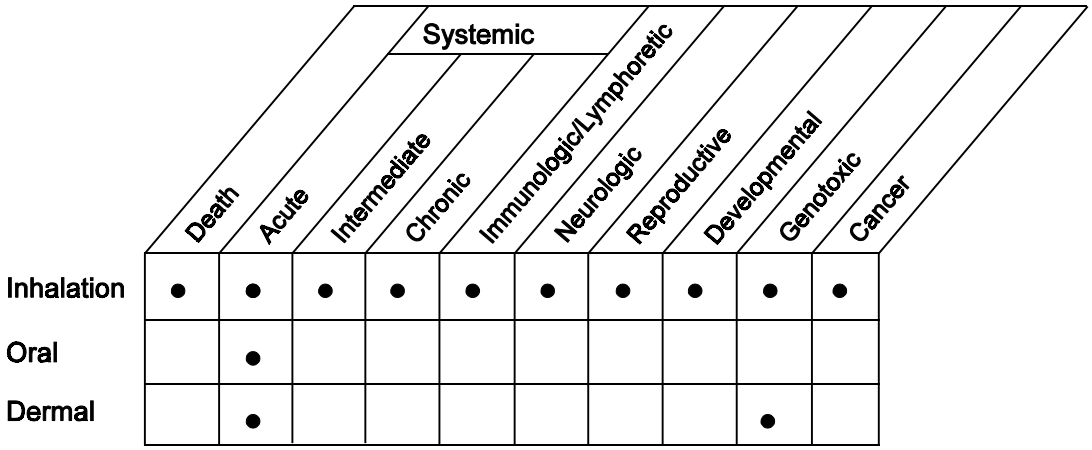
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean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

2.12.1 Existing Information on Health Effects of Toluene

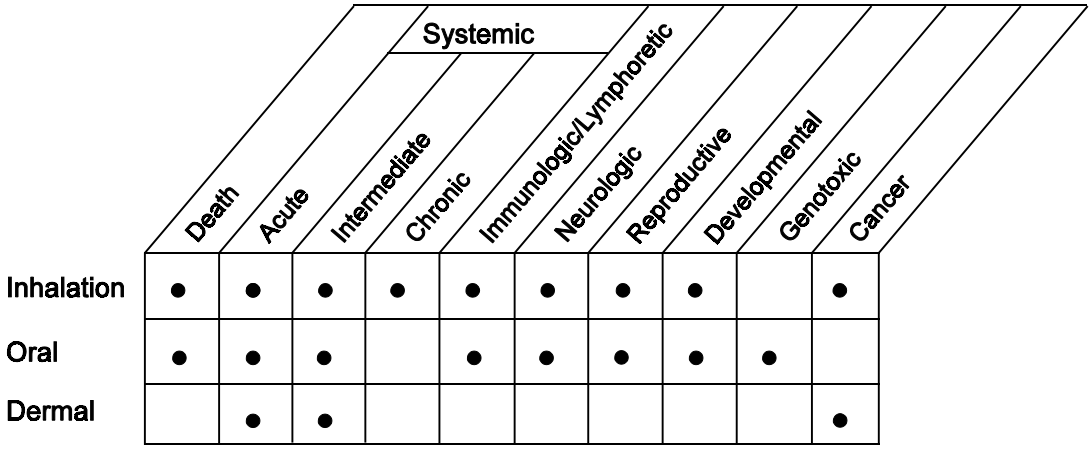
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to toluene are summarized in Figure 2-5. The purpose of this figure is to illustrate the existing information concerning the health effects of toluene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 2-5, there is a considerable body of data on the health effects of toluene in humans following acute, intermediate, and chronic inhalation exposures. It appears that clinical effects of high concentrations on the major target organ, the central nervous system, have been well characterized. However, many of the available reports lack quantitative information on exposure levels and there is still much that must be learned about the ultra structural molecular level of toxicity. There are some oral, but essentially no dermal, data available; however, these are not primary routes by which humans are exposed

Figure 2-5. Existing Information on Health Effects of Toluene



Human



Animal

● Existing Studies

to toluene. Figure 2-5 also shows that considerable animal toxicity data for inhalation exposure are available. However, there are limited oral and dermal data from animal studies.

2.12.2 Identification of Data Needs

Acute-Duration Exposure. Several studies are available regarding the effects of single exposures to toluene, both in humans and animals (Andersen et al. 1983; Baelum et al. 1985; Echeverria et al. 1991; von Oettingen et al. 1942). These studies clearly identify the nervous system as the critical toxicity target of toluene, and describe dose-response relationships between neurological end points and exposure levels. Supporting data are provided by studies of animals after inhalation (Bowen and Balster 1998; Bruckner and Peterson 1981a, 1981b; Bushnell et al. 1985; Hinman 1987; Kishi et al. 1988; Mullin and Krivanek 1982; Taylor and Evans 1985; Wood et al. 1983) or oral (Dyer et al. 1988) exposure. Further studies of orally-exposed animals involving a range of exposure levels (including low levels) and employing sensitive, behavioral, ultra structural, and biochemical measurements may be useful. Data for the dermal exposure route are limited; however, this is not a primary route of human exposure. Sufficient data for the oral and inhalation routes were available to derive an acute inhalation MRL based on a lack of neurological effects in volunteers exposed to 40 ppm toluene for 6 hours (Anderson et al. 1983) and an acute oral MRL based on changes in flash-evoked brain potentials observed in mice exposed to 250 mg/kg toluene (Dyer et al. 1988).

Intermediate-Duration Exposure. Several studies are available on repeated-dose exposure of humans and animals to toluene after inhalation exposure (Bjornaes and Naalsund 1988; Kyrklund et al. 1987; Mattsson et al. 1990; Pryor 1991; von Oettingen et al. 1942). These studies have elucidated the effects of repeated exposure of toluene on the primary target organ, the central nervous system. No-effect levels for intermediate, low-level inhalation exposure in air have not been thoroughly investigated. Determination of these values would be valuable in evaluating the human health risk. Studies on repeated intermediate-duration exposure of humans and animals to toluene by the oral route are adequate (Hsieh et al. 1989, 1990b; Kostas and Hotchin 1981; NTP 1990), and sufficient data were available to derive an intermediate-duration oral MRL based on toluene-induced changes in brain levels of biogenic monoamines in mice (Hsieh et al. 1990b). Studies following dermal exposure are lacking, however, this is not a primary route by which humans are exposed to toluene.

Chronic-Duration Exposure and Cancer. Numerous case reports have associated chronic inhalation exposure to toluene at levels inducing narcosis and euphoria (4,000 to 12,000 ppm as estimated by Gospe et al. 1994) with persistent neurological damage (Byrne et al. 1991; Caldemeyer et al. 1996; Devathasan et al. 1984; Filley et al. 1990; Hunnewell and Miller 1998; Ikeda and Tsukagoshi 1990; Kamran and Bakshi 1998; King et al. 1981; Maas et al. 1991; Meulenbelt et al. 1990; Miyagi et al. 1999; Rosenberg et al. 1988a, 1988b; Ryu et al. 1998; Suzuki et al. 1983; Yamanouchi et al. 1995). Studies of workers repeatedly exposed to toluene in workplace air at concentrations ranging from about 30 to 150 ppm have found evidence for increased incidence of self-reported neurological symptoms (Orbaek and Nise 1989; Yin et al. 1987), performance deficits in neurobehavioral tests (Boey et al. 1997; Foo et al. 1990; Orbaek and Nise 1989), hearing loss (Abbate et al. 1993; Morata et al. 1997), changes in visual-evoked brainstem potentials (Vrca et al. 1995, 1997a, 1997b), and color vision impairment (Zavalic et al. 1998a, 1998b, 1998c). Two animal studies have investigated the effects of toluene following chronic inhalation exposure (CIIT 1980; NTP 1990). Multiple end points of toxicity were investigated, including carcinogenicity, and the data indicate that toluene is not a carcinogen. Sufficient data for the inhalation route were available to derive a chronic MRL based on color vision impairment in toluene exposed workers (Zavalic et al. 1998a). Additional prospective studies of hearing, color vision ability, and performance in neurobehavioral tests of groups of occupationally exposed workers may decrease uncertainty in the chronic inhalation MRL for toluene. The chronic effects of toluene have not been investigated following oral or dermal exposures, and the carcinogenic potential has not been studied following dermal exposure; however, these are not considered major routes of toluene exposure.

Genotoxicity. To evaluate the potential of toluene to cause chromosomal damage, well designed *in vivo* studies using test material of known purity may be valuable. These tests would aid in determining whether toluene itself has clastogenic potential or whether the positive results that have been reported are due to impurities in the test material (animal studies) or multiple solvent exposures (human studies) (API 1981; Bauchinger et al. 1982; Nise et al. 1991; Pelclova et al. 1990; Schmid et al. 1985). Because it is believed that toluene toxicity may be mediated, at least in part, through a highly reactive and short-lived arene oxide intermediate, which interacts with cellular proteins and RNA (Chapman et al. 1990), further studies of this interaction would provide useful information.

Reproductive Toxicity. In general, available results from studies of toluene-exposed workers and animals suggest that toluene is not a potent reproductive toxicant, but may cause some reproductive problems, especially with repeated inhalation exposure during pregnancy to concentrations above 200 ppm. There are a few reports that women occupationally exposed to toluene, or wives of men

similarly exposed, have an increased risk of spontaneous abortions (Lindbohm et al. 1992; Ng et al. 1992b; Taskinen et al. 1989), but a causal relationship is not established by these studies due to small sample sizes evaluated, inability to define accurate exposure levels, failure to account for potentially important confounding variables, and difficulty in validating self-reported data. In addition, one study reported that toluene-exposed male workers showed decreasing plasma levels of the luteinizing hormone, follicle stimulating hormone and testosterone levels with increasing concentrations of toluene (8–<111 ppm) (Svensson et al. 1992a, 1992b). Animal studies found some evidence for minor toluene-induced changes in male and female reproductive organs (Ono et al. 1995, 1996; Tap et al. 1996), but no histological evidence of structural damage to the reproductive organs in rats and mice exposed orally for intermediate durations or by inhalation for intermediate or chronic durations (NTP 1990). No evidence for impaired reproductive performance was found in several assays (API 1985; Smith 1983; Ono et al. 1995, 1996; Thiel and Chahoud 1997) (including a 2-generation study of rats exposed to up to 2,000 ppm, 6 hours/day [API 1985]), except that exposure to concentrations above 200 ppm during pregnancy produced increased fetal mortality in pregnant rats (2,000 ppm, 6 hours/day) (Ono et al. 1995, 1996) and increased abortions in pregnant rabbits (267 ppm, 24 hours/day) (Ungvary and Tatrai 1985). Additional studies of reproductive end points in groups of occupationally exposed workers may be useful in discerning the possible reproductive hazards of toluene in the workplace, if large enough groups of workers are examined, exposure levels can be accurately monitored, and confounding variables are accounted for or minimalized. Another 2-generation reproductive study in another animal species (e.g., rabbits) may also help to decrease uncertainty in defining no-effect levels for reproductive effects from toluene.

Developmental Toxicity. Published reports of birth defects described in children born to women who abused toluene or other organic solvents during pregnancy suggest that high-level exposure to toluene during pregnancy can be toxic to the developing fetus (Arnold et al. 1994; Erramouspe et al. 1996; Goodwin 1988; Hersch 1988; Hersch et al. 1985; Lindemann 1991; Pearson et al. 1994). Studies of developmentally toxic effects in children of women exposed during pregnancy to much lower concentrations are restricted to a small study of 14 Finnish women exposed to mixed solvents suggesting that solvent exposure may increase risk for central nervous system anomalies and neural tube closure defects (Holmberg 1979). The available human data do not establish causality between low-level or occupational exposure to toluene and birth defects, because of the small sample size and the mixed solvent exposure experienced by the subjects in the Holmberg (1979) study and the lack of other studies of possible birth defects in children of women exposed to toluene in the workplace. Additional studies of

developmental end points in offspring of mothers exposed to toluene in the workplace may help to clarify the potential for human health risk.

Results from several inhalation exposure studies of animals indicate that exposure to levels of toluene that begin to produce maternal toxicity can cause fetal effects, including reduced fetal survival and retardation of growth and skeletal development (Courtney et al. 1986; Hudak and Ungvary 1978; Huntingdon Research Centre 1992a, 1992b; Ono et al. 1995; Thiel and Chahoud 1997; Ungvary and Tatrai 1985). No-effect levels in animals for toluene effects on standard developmental end points range from about 133 ppm for a 24 hour/day exposure protocol (Ungvary and Tatrai 1985) to 133–750 ppm with 3–6 hours/day protocols (Huntingdon Research Centre 1992b; Klimisch et al. 1992; Thiel and Chahoud 1997; Ungvary and Tatrai 1985). In animal studies of oral exposure during gestation, no developmental effects were observed in pregnant mice exposed to oral doses of 1,800 or 2,350 mg/kg/day (Seidenberg et al. 1986; Smith 1983), but exposure of pregnant rats to gavage doses of 650 mg/kg /day produced offspring with decreased body weights, delayed ossification, smaller brain volumes, and decreased forebrain myelination per cell compared with controls (Gospe and Zhou 1998; Gospe et al. 1996).

Results from studies of neurobehavioral end points in rats following *in utero* exposure to toluene suggest that maternal exposure to airborne concentrations above 1,200 ppm, 6 hours/day gestation can impair behavioral development of rat offspring (Jones and Balster 1997; Ono et al. 1995; Thiel and Chahoud 1997) and that drinking water exposure during gestation and lactation at doses of 106 mg/kg/day changes postweaning open-field locomotor activity in rat offspring (Kostas and Hotchin 1981).

Additional studies of sensitive neurological end points, including neurobehavioral end points, in offspring of toluene-exposed pregnant animals may better determine no-effect levels for toluene effects on neurodevelopment. Inhalation exposure studies are likely to be of more relevance to human exposures of concern than oral exposure studies. Developmental effects have not been investigated following dermal exposure; however, this is not a primary route of human exposure.

Immunotoxicity. The only inhalation data available on possible immunological effects of toluene are from studies of exposed workers (Lange et al. 1973; Moszczynsky and Lisiewicz 1984; Yin et al. 1987). In all cases, the workers were exposed to several solvents (toluene, benzene, and xylene), thus making it difficult to associate the effects on the immune system specifically with toluene. Animal data using the oral route of exposure provide some evidence of immunotoxicity from toluene exposure (Hsieh et al. 1989). Accordingly, oral and inhalation studies in animals designed to clarify the effect of toluene on the

immune system, particularly on lymphocyte production and function, antibodies, and interferons, may help determine if toluene was involved in the effects on immunity observed in occupationally exposed workers. Additional studies of the impact of toluene on disease resistance, building on the work of Aranyi et al. (1985), may also be valuable.

Neurotoxicity. Effects on the human nervous system from inhalation exposure to toluene are well documented (Andersen et al. 1983; Baelum et al. 1985; Byrne et al. 1991; Devathasan et al. 1984; Echeverria et al. 1991; Filley et al. 1990; Foo et al. 1990; Hanninen et al. 1976; Ikeda and Tsukagoshi 1990; Orbaek and Nise 1989; Rosenberg et al. 1988a, 1988b; Vrca et al. 1995, 1996, 1997a, 1997b; Zavalic et al. 1998a, 1998b, 1998c) and are the basis for the inhalation exposure MRLs. The central nervous system effects of toluene in animals have also been studied in detail via the inhalation route of exposure (Arito et al. 1988; Bruckner and Peterson 1981a; Bushnell et al. 1985; Hinman 1987; Ikeda et al. 1986; Mattsson et al. 1990; Pryor 1991; Pryor et al. 1991; Rebert et al. 1989a; Taylor and Evans 1985; Wood and Colotla 1990). Available data clearly indicate that the central nervous system is a target, but the molecular mechanisms of toxicity have yet to be elucidated with certainty. Dose-response relationships for central nervous system effects in humans and animals (rats and mice) have been established, but more information concerning the reversibility of effects (especially when exposure is chronic) may be useful. The effects of toluene on neurobehavioral function were used to derive an MRL of 4 ppm for acute inhalation exposure (based on a study by Andersen et al. 1983) and a chronic-duration MRL of 1 ppm (based on a study by Zavalic et al. 1998a). Additional studies concerning the progression of subtle, toluene-induced nervous system defects, such as diminished auditory responses, changes in flash evoked visual responses, and impaired color vision discrimination, may decrease uncertainties in the MRLs.

The neurological effects of toluene via the oral route have not been extensively investigated, but the available data support the inhalation data in identifying the nervous system as the critical target of toluene toxicity. An acute MRL of 0.8 mg/kg/day was developed based on a change in flash-evoked potential waveforms in rats exposed to a single dose of toluene (Dyer et al. 1988). The intermediate-duration MRL (0.02 mg/kg/day) was based on a regional increase in the levels of selected neurotransmitters in the brains of exposed mice (Hsieh et al. 1990b). Additional studies may help define the functional manifestation of regional alterations in levels of neurotransmitters in the brain and of changes in FEP waveforms. No data on dermal exposure are available, but this is not the primary route of human exposure.

Epidemiological and Human Dosimetry Studies. Additional studies of neurological and reproductive end points in groups of toluene-exposed workers may decrease uncertainty in the chronic MRL and may help determine if toluene represents a reproductive health hazard in humans at low exposure levels. These studies will be most useful if groups of workers can be identified whose exposure to other chemicals in the workplace is minimal, if adjustments for lifestyle confounding factors can be made, and if personal air monitoring data are available. Earlier reports of increased risk of spontaneous abortions (Lindbohm et al. 1992; Ng et al. 1992b; Taskinen et al. 1989) and altered plasma levels of male sexual hormones (Svensson et al. 1992a, 1992b) in groups of toluene-exposed workers await confirmation from further research.

Biomarkers of Exposure and Effect.

Exposure. Toluene and its metabolites are easily detected in the blood and urine (DeRosa et al. 1985; Hjelm et al. 1988; Kono et al. 1985; Lof et al. 1990; Ogata et al. 1970). However, many toluene metabolites are also produced by other naturally occurring or xenobiotic materials and, thus, are not specific for toluene. The presence of toluene in exhaled air and blood is the most reliable biomarker of exposure (Foo et al. 1991; Kawai et al. 1992a). The ACGIH (1999) recommends using a combination of three biological exposure indices to assess exposure of workers to toluene in the workplace: *ortho*-cresol and hippuric acid levels in urine at the end of a workshift and toluene levels in blood immediately prior to the last shift of a workweek.

Angerer et al. (1998a) proposed that S-*p*-toluylmercapturic acid levels in urine may also be useful as a biological indicator of toluene exposure. Maestri et al. (1997) reported that end-of-shift levels of S-benzylmercapturic acid in urine of workers were correlated with toluene concentrations with a coefficient of 0.74. Additional studies may help determine whether these are reliable biomarkers of exposure that can improve the accuracy of monitoring workers' exposure to toluene.

Effect. There are no suitable biomarkers of effect except for changes in the brain found in chronic solvent abusers with obvious neurological dysfunction (Filley et al. 1990; Rosenberg et al. 1988a). Additional information on the mechanism of neurotoxicity may suggest a useful biomarker of either exposure or effect. However, at this time, there is little to suggest that such biomarkers are present for anything other than the abuse paradigm.

Absorption, Distribution, Metabolism, and Excretion. The absorption, distribution, metabolism, and excretion of toluene in humans and animals following inhalation exposure are well characterized (Ameno et al. 1992; Andersen et al. 1983; Angerer 1979; Angerer et al. 1998a; Baelum et al. 1987, 1993; Benignus et al. 1981; Benoit et al. 1985; Bergman 1979; Bray et al. 1949; Carlsson 1982; Carlsson and Ljungquist 1982; Chand and Clausen 1982; Dossing et al. 1983c; Furman et al. 1998; Ghantous and Danielsson 1986; Hjelm et al. 1988; Ikeda et al. 1990; Kawai et al. 1992a, 1992b, 1993, 1996; Lof et al. 1990a, 1990b, 1993; Maestri et al. 1997; Nakajima and Wang 1994; Nakajima et al. 1991, 1992a, 1992b, 1993, 1997; Ng et al. 1990; Ogata 1984; Pellizzari et al. 1992; Pierce et al. 1996, 1999; Paterson and Sarvesvaran 1983; Takeichi et al. 1986; Tassaneeyakul et al. 1996; Van Doorn et al. 1980; Wang and Nakajima 1992; Zahlsen et al. 1992).

Sufficient pharmacokinetic data have been generated to support the development of PBPK models that describe the kinetics of toluene after inhalation exposure; two for humans (Fisher et al. 1997; Pierce et al. 1996, 1999) and two for rats (DeJongh and Blaauboer 1996, 1997; Tardif et al. 1993). Further development of a human PBPK model that includes partitioning of inhaled and ingested toluene to the brain and a similarly designed rat PBPK model may be useful in improving extrapolation from the oral exposure rat data and in comparing model-based predictions of human effect levels based on neurological effects in inhalationally-exposed rats with observed effect levels in humans exposed to airborne toluene. Additional studies of the appearance and elimination kinetics of toluene in breast milk may help to validate the human PBPK model developed by Fisher et al. (1997) to estimate transfer of toluene to a nursing infant. It is unlikely that such studies would be done with volunteers, but studies of nursing animals may provide pertinent information if a similar rat PBPK was developed.

Limited data are available on the quantitative absorption and excretion of toluene by the oral and dermal routes. Studies of humans and animals indicate that dermal absorption of toluene is slow (Aitio et al. 1984; Dutkiewicz and Tyras 1968), but can be significant (Aitio et al. 1984; Monster et al. 1993; Morgan et al. 1991; Sato and Nakajima 1978). Additional studies of dermal uptake of toluene from solution may help to further quantify exposure by this pathway.

Comparative Toxicokinetics. Available data suggest that there are species, age, gender, and strain differences in the metabolism of toluene (Chapman et al. 1990; Inoue et al. 1984, 1986; Nakajima et al. 1992b). Further evaluation of these differences, and comparison of metabolic patterns in humans with those of animals, may help determine the most appropriate species and strain of animal to use in

evaluating the risk of human exposure to toluene. Additional evaluation of human variability in disposition of toluene is also warranted.

Methods for Reducing Toxic Effects. Oxygen therapy and positive pressure ventilation have been used to reduce the toluene body burden (Graham 1990). Washing of toluene from exposed body surfaces is beneficial. Other than these general guidelines, there is very little information available on methods of mitigating the toxic effects of toluene. Additional data on the outcome of emergency response procedures would be beneficial. Studies of the benefit of diet, ethanol absence, and controlled exposure to prescription or nonprescription drugs on blood levels of toluene and its metabolites could provide information that would be helpful in understanding the impact of these factors on the risks from occupational exposure.

Children's Susceptibility. The effects of toluene have not been thoroughly studied in children or immature animals, but the effects observed in juvenile toluene abusers (Byrne et al. 1991; Devasthasan et al. 1984; King et al. 1981) and immature animals exposed to toluene (Castilla-Serna et al. 1991; Pryor and Rebert 1992; Pryor et al. 1984a; von Euler et al. 1989b) are consistent with effects observed in adults. Information regarding age-related differences in toluene metabolism suggests that developing fetuses and children at very early stages of development may be more susceptible to toluene toxicity than adults due to lower capabilities to metabolically detoxify toluene, but, by 1–3 years of age, adult capabilities may be attained (Leeder and Kearns 1997; Nakajima et al. 1992b, 1997; Tassaneeyakul et al. 1996; Vieira et al. 1996). An oral lethality study in rats (Kimura et al. 1971) and a study of toluene-induced hearing loss in young rats (Pryor et al. 1984a) provide the only health effect data suggesting that immature animals may be more susceptible than adult animals. Additional research on the development of metabolic capabilities in newborn and very young children, coupled with animal studies examining relevant neurological end points in toluene-exposed animals of varying ages, may lead to better understanding of the susceptibility of children to toluene toxicity.

Studies with pregnant mice suggest that distribution of inhaled toluene to fetal tissue is limited due to maternal metabolic detoxification and preferential distribution of nonmetabolized toluene to maternal adipose tissue (Ghantous and Danielsson 1986). 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.

Transfer of toluene to infants from breast milk of nursing mothers who are concurrently exposed to toluene in the workplace is expected to be a possibility and a concern (see Section 2.7). As discussed in the Absorption, Distribution, Metabolism, and Excretion subsection above, additional studies of the kinetics of elimination of toluene from nursing animals may provide pertinent information to better predict the degree to which toluene may be transferred in breast milk from a toluene-exposed working mother to her nursing infant. Monitoring studies of toluene in breast milk in groups of toluene-exposed lactating women may also provide some pertinent information.

2.12.3 Ongoing Studies

There are several ongoing research efforts that will provide data related to the toxic actions of toluene (FEDRIP 1998). These projects are summarized in Table 2-6. Some of this research will supply information identified in the preceding section on research needs. Three of the investigators are studying the effects of toluene in humans, focusing on different end points. Dr. M. Utell of the University of Rochester is studying the effects of toluene on neurological and respiratory end points in humans, while Dr. E. Faustman of the University of Washington is examining the effects of toluene on the endocrine system. Dr. D.M. Christiani of Harvard University is assessing the reproductive outcomes of occupational exposure to aromatic solvents, including toluene, in the oil refinery system in China.

Two investigators are using animal models to study the effects of toluene. Dr. R. Balster of Virginia Commonwealth University is investigating the abuse of inhalants, including toluene, using behavioral test procedures in mice. D.N. Kurtzman of Texas Tech University is investigating the relationship between ATPase activity and tubular function in rat and rabbit kidneys.

Table 2-6. Ongoing Research for Toluene

Investigator	Affiliation	Research description	Sponsor
Backes, W	Louisiana State University Medical Center, New Orleans, LA	Toxicological significance of alkylbenzene metabolism	National Institute of Environmental Health Sciences
Balster, RL	Virginia Commonwealth University, Richmond, VA	Behavioral pharmacology of abused solvents	National Institute on Drug Abuse
Branch, S	North Carolina State University, Raleigh, NC	DNA methylation effects on mammalian development	National Institute of Environmental Health Sciences
Christiani, DM	Harvard University	Reproductive outcomes of occupational exposure to aromatic solvents in the oil refinery system in China	
Cunningham, ML	National Institute of Environmental Health Sciences, Research Triangle Park, NC	Metabolism and genotoxicity of genotoxic noncarcinogens	National Institute of Environmental Health Sciences
Faustman, EM	University of Washington, Seattle, WA	Human reproductive endocrine effects of solvent exposure	National Institute for Occupational Safety and Health
Fechter, LD	University of Oklahoma Health Science Center, OK	Mechanisms of organic solvent ototoxicity	National Institute of Environmental Health Sciences
Greenberg, A	University of North Carolina, Charlotte, NC	2,3 Eposyoxepins as benzene ring opening metabolities	National Institute of Environmental Health Sciences
Kalman, DA	University of Washington, Seattle, WA	Human dosimetry for assessment of exposure to volatile compounds	National Institute of Environmental Health Sciences
Karol, MH	University of Pittsburgh, Pittsburgh, PA	Chemically induced chronic allergic lung disease	National Institute of Environmental Health Sciences
Kurtzman, DN	Texas Tech University	Relationship between ATPase activity and tubular function in rat and rabbit kidneys	
Lee, L	University of Kentucky, Lexington, KY	Pulmonary afferents in regulation of airway functions	National Heart, Lung, and Blood Institute
Stern, S	University of Rochester, Rochester, NY	Inhalant abuse during gestation	National Institute on Drug Abuse

Table 2-6. Ongoing Research for Toluene *(continued)*

Investigator	Affiliation	Research description	Sponsor
Turtletaub, KW	University of California Berkeley, Berkeley, CA	Protein and DNA adducts following low dose exposure by accelerator, MS	National Institute of Environmental Health Sciences
Utell, M	University of Rochester, Rochester, NY	Effects of toluene on performance in healthy humans	National Center for Research Resources