3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 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.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

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

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

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

3.2.1 Inhalation Exposure

Adverse effects on the nervous system are 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 was not a developmental toxicant at levels below those inducing maternal toxicity. At doses that impaired maternal body weight gain, developmental effects observed included retarded fetal growth and skeletal development, and altered development of behavior in offspring. At high concentrations modeling solvent abuse, increased malformations and fetal death were also observed.

3.2.1.1 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 Virginia, 39 deaths were attributed to inhalant abuse from 1987 to 1996 (Bowen et al. 1999). The majority of deaths (70%) occurred at ≤22 years of age, and males accounted for 95% of all inhalant abuse deaths; however, only two deaths were attributed to intentional toluene inhalation. In a case-series report from Mexico, 3/20 patients with a history of substance abuse died due to severe acidosis and renal failure following acute toluene intoxication (Camarra-Lemarroy et al. 2015). 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). Toxicokinetic extrapolation from blood toluene concentration in a deceased patient was used to estimate that 1 hour of exposure to 1,800–2,000 ppm toluene may be fatal to humans (Hobara et al. 2000a, 2000b).

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 3-1 and plotted in Figure 3-1.

Table 3-1 Levels of Significant Exposure to Toluene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Mouse (Swiss-Webster)	7 hr				5320 (LC50)	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 (Occup)	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	40 min (WB)	Endocr	200			Kobald et al. 2015	Endpoint examined: Cortisol levels in saliva.
6	Human	2 hr	Resp	1862 M			Meulenbelt et al. 1990	Non-fatal case report.
			Cardio		1862 M (sinus tachycardia)			
			Hemato	1862 M				
			Hepatic		1862 M (liver enlargement)			
			Ocular		1862 M (ocular irritation)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
7	Human	3 hr	Resp	1862 M			Meulenbelt et al. 1990	Non-fatal case report.
			Cardio		1862 M (sinus bradycardia)			
			Hemato	1862 M				
			Hepatic	1862 M				
			Ocular		1862 M (ocular irritation)			
8	Human	4.5 hr	Resp		50 M (irritation of the throat)		Muttray et al. 2005	
9	Human	6.5 hr	Renal	102 M			Nielsen et al. 1985	Endpoint examined: renal excretion rates of albumin and beta-2-microglobulin examined.
10	Human	2 hr	Resp		48 M (mucous membrane irritation)		Orbaek et al. 1998	
11	Human	2 hr	Resp		48 F (mucous membrane irritation)		Osterberg et al. 2003	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
12	Human	6 hr/d	Resp	100			Rahill et al. 1996	No change in lung function.
13	Human	3 hr	Resp	800			von Oettingen et al. 1942	No effects of irritation and no change in respiratory rate, minute volume, leukocyte counts, blood pressure, or pulse in 3 exposed subjects.
			Cardio	800				
			Hemato	800				
14	Rat (Sprague-Dawley)	3 d 6 hr/d	Endocr		500 M (increased serum corticosterone)		Andersson et al. 1980	Endpoints assessed: Serum levels of corticosterone, prolactin, growth hormone, FSH, and LH.
15	Rat (Sprague-Dawley)	5 d 6 hr/d	Endocr	1000 M			Andersson et al. 1980	Endpoints assessed: Serum levels of corticosterone, prolactin, growth hormone, FSH, and LH.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
16	Rat (Sprague-Dawley)	3 d 6 hr/d	Endocr		80 M (increased serum prolactin)		Andersson et al. 1983a	Endpoints assessed: Serum corticosterone, prolactin, growth hormone, FSH, and LH.
17	Rat (Wistar)	7 d 4 hr/d	Endocr		1500 M (18% increase in adrenal weight and increased adrenocortical cell size; increased serum ACTH and corticosterone)		Gotohda et al. 2005	
			Bd Wt		1500 M (8% decreased body weight)			
18	Rat (Wistar)	7 d 4 hr/d	Hepatic		3000 M (increased biochemical markers of liver fibrosis)		Gotohda et al. 2009	
19	Rat (Wistar)	Gd 7-20 6 hr/d	Endocr		1500 F (reduced maternal plasma corticosterone levels)		Hougaard et al. 2003	
20	Rat (Fischer- 344)	6 hr/d 3 or 7 d	Endocr		1000 M (signficantly increased serum corticosterone)		Little et al. 1998	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
21	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%)			
22	Rat	7 d 8 hr/d	Hepatic		795 F (increased liver weight 12%, increased smooth and rough endoplasmic reticulum)		Ungvary et al. 1982	
23	Rat (Wistar)	6 hr	Hepatic		4000 (increased CYP2E1 and decreased CYP 2 C11 in liver)		Wang et al. 1996	
24	Mouse	7 d 8 hr/d	Hepatic		795 F (increased liver weight 11% and cytochrome P-450 30%)		Ungvary et al. 1982	
25	Dog	1 hr	Hemato	200	500 (decreased leukocytes)		Hobara et al. 1984a	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
26	Dog	4 hr	Hemato		700 (decreased leukocytes)		Hobara et al. 1984a	
27	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	
Immuno/ Lymphoret								
28	Rat (Sprague-Dawley)	Gd 7-17 6 hr/d			600 F (decreased thymus weights in dams)		Ono et al. 1995	
29	Mouse (CD-1)	3 hr		1 F	2.5 F (decreased survival following infection with S. zooepidemicus)		Aranyi et al. 1985	
Neurological								
30	Human	6 hr		40 M	100 M (headaches, dizziness, intoxication)		Andersen et al. 1983	
31	Human	6.5 hr (Occup)			100 M (intoxication, dizziness, decreased manual performance and color perception)		Baelum et al. 1985	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
32	Human	8 hr			100 (impaired performance on visual-vigilance task)		Dick et al. 1984	
33	Human	7 hr			75 (dose-related impairment of performance on recognition, pattern memory, and one-hole test results)		Echeverria et al. 1991	
34	Human	20 min		100 M	300 M (increased simple and choice reaction time)		Gamberale and Hultengren 1972	
35	Human	4 hr		80 M			Iregren 1986	Endpoints evaluated: choice reaction time, simple reaction time, color-word vigilance, or memory reproduction.
36	Human	40 min (WB)			200 (altered EEG; impaired performance on visual attention task)		Kobald et al. 2015	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
37	Human	4 hr		40 F			Lammers et al. 2005a	Endpoints evaluated: battery of neurobehavioral tasks, sleep quality questionnaire.
38	Human	4 hr 30 min on, 30 min off		110 M			Lammers et al. 2005a	Endpoints evaluated: battery of neurobehavioral tasks, sleep quality questionnaire.
39	Human	20 min			15[b] (decreased performance on neuropsychological tests in toluene-sensitive subjects)		Little et al. 1999	
40	Human	28-41 min (Occup)		332 M			Muttray et al. 1999	Endpoint evaluated: color vision before and after cleaning a print machine with toluene.
41	Human	4.5 hr		50 M			Muttray et al. 2005	Endpoints evaluated: subjective symptoms, pupillographic sleepiness test.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
42	Human	4 hr		80 M			Olson et al. 1985	Endpoints evaluated: subjective symptoms, simple reaction time, choice reaction time, and memory-reproduction.
43	Human	2 hr			48 M (self-reported fatigue in individuals with toxic encephalopathy)		Orbaek et al. 1998	Endpoints assessed: subjective symptoms in healthy subjects and subjects with toxic encephalopathy.
44	Human	2 hr		48 M			Osterberg et al. 2000	Endpoints assessed: attention and motor speed in healthy subjects and subjects with toxic encephalopathy.
45	Human	2 hr			48 F (self-reported fatigue in individuals with mutliple chemical sensitivity)		Osterberg et al. 2003	Endpoints assessed: subjective symptoms and attention and motor speed in healthy subjects and subjects with MCS.
46	Human	6 hr			100 (decreased perfomance on neuropsychological tests)		Rahill et al. 1996	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
47	Human	3 or 8 hr		100	200 (drowsiness, headache, confusion, weakness)		von Oettingen et al. 1942	
48	Monkey (Cynomolgus)	50 min		1000 F	2000 F (cognitive and motor skills impaired)	3000 F (overt signs of neurotoxicity)	Taylor and Evans 1985	
49	Rat	8 hr			900 M (altered patterns of sleep and wakefulness)		Arito et al. 1988	
50	Rat (Long- Evans)	1-4 hr			1000 M (decreased F2 amplitude in VEPs)		Boyes et al. 2007	
51	Rat (Fischer- 344)	2 hr (W)			110 M (decreased REM sleep)		Ghosh et al. 1989	
52	Rat (Fischer- 344)	2 hr			110 M (changes in sleep pattern)		Ghosh et al. 1990	
53	Rat (DA/HAN)	4 hr or 5 d, 3 hr/d			100 M (nystagmus and altered opticokinetic response)		Hogie et al. 2009	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
54	Rat (Wistar)	30 min			1000 M (impaired learning and memory, decline in conditioned avoidance response)		Huerta-Rivas et al. 2012	
55	Rat (Sprague-Dawley)	10 d 16 hr/d			1000 M (diminished auditory response)		Johnson 1992	
56	Rat (Sprague-Dawley)	2 wk 5 d/wk 16 hr/d			1000 M (diminished auditory response)		Johnson et al. 1988	
57	Rat	20 min			1000 (increased locomotor activity)		Kim et al. 1998	
58	Rat (Wistar)	4 hr			125 M (a temporary decline in conditioned avoidance response)		Kishi et al. 1988	
59	Rat (Fischer- 344)	6 hr/d 3 or 7 d			1000 M (decreased GFAP in thalamus and increased corticosterone)		Little et al. 1998	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
60	Rat (Wistar)	10 d 6 hr/d		1500 M			Lund and Kristiansen 2008	Endpoints examined: brainstem auditory responses, distortion product otoacoustic emissions.
61	Rat (CD)	4 hr		810 M	1660 M (decreased lift reflex, vertical bar placing, and horizontal rod grasping)	3100 M (overt signs of neurotoxicity)	Mullin and Krivanek 1982	
62	Rat (Fischer- 344)	30 min			500 M (changes in flash-evoked and somatosensory -evoked potentials)		Rebert et al. 1989b	
63	Rat	4 hr			1000 M (sleep pattern disturbances- reduced slow wave sleep and increased paradoxical phase)		Takeuchi and Hisanaga 1977	
64	Rat	2-4 hr			480 (decreased performance in rewarded task)		Wood et al. 1983	
65	Mouse CFW (ChasRiver Swiss) albino	30 min		250	500 (increased locomotor activity)		Bowen and Balster, 1998	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
66	Mouse (CFW)	30 min		1000 M	2000 M (decreased anxiety)		Bowen et al. 1996	
67	Mouse (Multiple)	30 min			100 M (increased locomotor activity)		Bowen et al. 2010	Balb/CBYJ, C57BL/6J, and DBA/2J showed effects at identified LOAEL; Swiss Webster mice showed similar effects at higher concentrations.
68	Mouse (C57BL/6N)	5 d 72 min/d		100 M	1000 M (increased locomotor activity)		Bushnell et al. 1985	
69	Mouse (C57BL/6N)	30 min		1000 M	2000 M (increased locomotor activity)		Conti et al. 2012	
70	Mouse (Swiss-Webster)	30 min		500 M	1000 M (increased nociception)		Cruz et al. 2001	
71	Mouse (CBA/CA; C57BL/6J)	7 d 12 hr/d			1000 F (accelerated hearing loss in genetically predisposed mice)		Li et al. 1992	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
72	Mouse (129/Sv-ter)	30 min			1000 M (increased locomotor activity)		Lopez-Rubalcava and Cruz 2000	
73	Mouse (ddy)	8 hr		500 M			Matsuoka et al 1997	Endpoints examined: GFAP, c-jun, and c-fos mRNA levels in cerebrum.
74	Mouse (Swiss-Webster)	30 min		500	1000 (increased nociception)		Paez-Martinez et al. 2003	
75	Mouse (Swiss-Webster)	7 d 30 min/d		1000 M	2000 M (increased locomotor activity)		Tomaszycki et al. 2013	
76	Mouse (CD-1)	6x 1 hr/x		300 M	560 M (increased activity)		Wood and Colotla 1990	
77	Gn Pig (pigmented)	5 d 8 hr/d			250 (transient mid- and high-frequency hearing loss)		McWilliams et al. 2000	
78	Other	10 d 8 or 12 hr/d		2000			Davis et al. 2002	Endpoints examined: auditory brainstem responses in chinchillas.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Reproductive								
79	Human	3 hr			50 (altered LH secretion)		Luderer et al. 1999	
80	Rat (Crl:CD (SD) BR VAF/Plus)	Gd 6-15 6 hr/d		3000 F			API 1991; Roberts et al. 2007	Pregnancy outcomes examined.
81	Rat (Crl:CD BR VAF/Plus)	Gd 6-15 6 hr/d		3500 F		5000 F (increased post-implantation loss; total fetal resorption in 6/9 dams)	API 1992	
82	Rat (Sprague-Dawley)	Gd 8-20 2x/d 15 or 30 min		12000 F			Bowen and Hannigan 2013; Bowen et al. 2005, 2007, 2009a, 2009b	Pregnancy outcomes examined.
83	Rat (Wistar)	Gd 7-20 6 hr/d		1800 F			Dalgaard et al. 2001	Pregnancy outcomes examined.
84	Rat (Wistar)	Gd 7-20 6 hr/d		1500 F			Hougaard et al. 2003; Ladefoged et al. 2004	Pregnancy outcomes examined.
85	Rat (Sprague-Dawley)	Gd 7-17 6 hr/d		2000 F			Ono et al. 1995	Pregnancy outcomes examined.
86	Rat (Sprague-Dawley)	Gd 6-20 6 hr/d		1500 F			Saillenfait et al. 2007	Pregnancy outcomes examined.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
87	Rat (Wistar)	7 d 8 hr/d			3000 F (structural variations in antral follicles in ovary)		Tap et al. 1996	
88	Rat (Wistar)	Gd 9-21 6 hr/d		1200			Thiel and Chahoud 1997	Pregnancy outcomes examined.
89	Mouse (CD-1)	Gd 7-16 7 hr/d		400 F			Courtney et al. 1986	Pregnancy outcomes examined.
90	Mouse (CD-1)	Gd 12-17 3x/d 60 min		2000 F			Jones and Balster, 1997	Pregnancy outcomes examined.
91	Rabbit	Gd 6-18 6 hr/d		500 F			Klimisch et al. 1992	Pregnancy outcomes examined.
92	Rabbit	Gd 7-20 24 hr/d		133 F		266 (4/8 dams aborted)	Ungvary and Tatrai 1985	
Developmental								
93	Rat (CRL:COBS CD (SD) BR)	Gd 6-15 6 hr/d		400			API 1978	Endpts examined: number of implantation sites, live and dead fetuses, and resorptions; fetuses were weighed and examined for external malformations.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
94	Rat (Crl:CD (SD) BR VAF/Plus)	Gd 6-15 6 hr/d		1500	3000	(decreased fetal weight and increased incidence of fetuses with unossified sternebrae)			API 1991; Roberts et al. 2007	
95	Rat (Crl:CD BR VAF/Plus)	Gd 6-15 6 hr/d		2000			3500	(20% decrease in fetal body weight, total absorption at higher exposure levels)	API 1992	
96	Rat (Sprague-Dawley)	Gd 8-20 2x/d 30 min			8000	(decreased postnatal growth)	12000	(increased number of litters with malformed, runted, or dead pups)	Bowen and Hannigan 2013	
97	Rat (Sprague-Dawley)	Gd 8-20 2x/day 15 min			8000	(impaired negative geotaxis in offspring)	12000	(decreased pup body weight, increased number of litters with malformed, runted, or dead pups, impaired negative geotaxis in offspring)	Bowen et al. 2005	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
98	Rat (Sprague-Dawley)	Gd 8-20 2x/d 15 min		8000	12000	(decreased pup birth weight; decreased sensitivity to amphetamine-induced locomotion in males on Pnd 28)			Bowen et al. 2007	Endpoints examined: pup weight on Ppd 1 and 21, open-field activity on Ppd 22, 42, and 63, amphetamine-induced locomotion on Ppd 28.
99	Rat (Sprague-Dawley)	Gd 8-20 2x/day 30 min			8000	(decreased fetal weight and length, decreased placental weight)	12000	(decreased fetal weight and length, decreased placental weight, increased skeletal malformations and soft tissue anomalies)	Bowen et al. 2009a	
100	Rat (Sprague-Dawley)	Gd 8-20 2x/d 15 min			8000	(altered reward-seeking behavior and increased impulsivity in offspring)			Bowen et al. 2009b	Endpoints examined: standard dev't endpoints, waiting-for-reward task and amphetamine-induced locomotion on Ppd 60.
101	Rat (Sprague-Dawley)	Gd 8-20 2 x/d 15 min (WB)		8000	12000	(decreased birth weight; impaired learning and memory in offspring)			Callan et al. 2015	Endpoints examined: Pup weight, external malformations, spatial learning and memory on Pnd 28-44.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
102	Rat (Wistar)	Gd 7-20 6 hr/d			1800 M (decreased neonatal weight, increased apoptosis in cerebral granule layer of cerebellum at Ppd 21)		Dalgaard et al. 2001	Endpoints examined: pup weight, external malformations, paired testes weight and histopathology, brain weight, and brain apoptosis.
103	Rat (Wistar)	Gd 7-20 6 hr/d			1500 (reduced pup birth weight)		Hougaard et al. 2003	No other developmental endpoints were examined.
104	Rat CFY	Gd 1-8 24 hr/d			399 (increased incidence of fetuses with skeletal retardation)	399 F (5/14 dams died)	Hudak and Ungvary 1978	
105	Rat CFY	Gd 9-14 24 hr/d			399 (increased incidence of fetal skeletal anomalies)		Hudak and Ungvary 1978	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
106	Rat (Sprague-Dawley)	Gd 8-20 2x/day 15 min		8000	12000 (decreased pup birth weight)		Jarosz et al. 2008	Endpoints examined: pup weight on Ppd 1 and 30, metabolism in offspring (energy expenditure, respiratory quotient, body fat content).
107	Rat (Wistar)	Gd 7-20 6 hr/d			1500 (reduced postnatal growth, increased apoptosis in the cerebellum of offspring)		Ladefoged et al. 2004	Endpoints examined: pup weight (Ppd 1, 7, 23), external malformations, spatial learning in females (Pnw 5); brain apoptosis (Ppd 6, 22, 24, 27).
108	Rat (Sprague-Dawley)	Gd 7-17 6 hr/d		2000			Ono et al. 1995	Dams sacrificed on Gd 20; standard developmental endpoints assessed.
109	Rat (Sprague-Dawley)	Gd 7-17 6 hr/d		600	2000 (decreased pup weight)		Ono et al. 1995	Endpts examined: pup weight, growth, dev't, survival, reflex dev't (Ppd 6-10); neurobehavior (Pnw 4); hemato, biochem, and organ wt (Ppd 21 and 56).

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
110	Rat (Sprague-Dawley)	Gd 6-20 6 hr/d		500	1500	(decreased fetal weight)			Saillenfait et al. 2007	
111	Rat (Wistar)	Gd 9-21 6 hr/d		600	1000	(decreased pup weight and delayed vaginal opening)	1200	(significantly increased postnatal/preweaning mortality in offspring)	Thiel and Chahoud 1997	Endpoints examined: pup weight, survival, development, ontogeny of reflexes, neurobehavior, and F1 mating and fertility.
112	Mouse (CD-1)	Gd 7-16 7 hr/d			200	(increased number of litters with fetuses with dilated renal pelvis)			Courtney et al. 1986	
113	Mouse CFLP	Gd 6-13 24 hr/d			133	(decreased fetal body weight)	399	(maternal mortality)	Hudak and Ungvary 1978	
114	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	Endpoints examined: pup weight, growth, reflex ontogeny, neurobehavior.
115	Mouse	Gd 6-15 3-4 hr/d		133	266	(decreased fetal body weight and retardation of fetal skeletal development)			Ungvary and Tatrai 1985	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
116	Rabbit	gd 6-18 6 hr/d		500			Klimisch et al. 1992	
117	Rabbit	Gd 7-20 14 d 24 hr/d		133 F		266 F (4/8 does aborted)	Ungvary and Tatrai 1985	
INTERMEDIATE EXPOSURE								
Death								
118	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d				3000 M (8/10 died)	NTP 1990	
119	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d				3000 M (6/10 died) 625 F (1/10 died)	NTP 1990	
Systemic								
120	Human	2-14 mo 8 hr/d (Occup)	Hepatic	167 F			Seiji et al. 1987	Endpoint evaluated: clinical chemistry.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
121	Rat (CD)	95 d 7 d/wk 6 hr/d	Resp	2000			API 1985; Roberts et al. 2003	Endpts evaluated in 2-generation study: body, liver, kidney, and reproductive organ weight and organ histology in F0/F1 parents and F1/F2 weanlings.
			Cardio	2000				
			Gastro	2000				
			Musc/skel	2000				
			Hepatic	2000				
			Renal	2000				
			Endocr	2000				
			Dermal	2000				
			Ocular	2000				
			Bd Wt	2000				
122	Rat (Fischer- 344)	42 d 5 d/wk 6 hr/d	Bd Wt	1000 M			API 1997	
123	Rat (Long- Evans)	13 wk 5 d/wk 6 hr/d	Bd Wt	1000 M			Beasely et al. 2010	
124	Rat (Long- Evans)	4 wk 5 d/wk 6 hr/d	Bd Wt	1000 M			Beasley et al. 2012	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
125	Rat Long-Evan	4 wk 5 d/wk 6 hr/d (WB)	Ocular	1000 M			Boyes et al. 2015	Ocular endpoint: ophthalmic examination.
			Bd Wt	1000 M				
126	Rat Long-Evan	13 wk 5 d/wk 6 hr/d (WB)	Ocular	1000 M			Boyes et al. 2015	Ocular endpoint: ophthalmic examination.
			Bd Wt	1000 M				
127	Rat (albino)	8 wk 5 d/wk 70 min/d	Resp	12000 M			Bruckner and Peterson 1981b	Endpoints evaluated: body weight, organ weight and histology; upper respiratory tract histology not assessed.
			Cardio	12000 M				
			Hepatic		12000 M (11% decrease in liver weight, elevated liver enzymes in serum)			
			Renal		12000 M (27% decrease in kidney weight)			
			Bd Wt			12000 M (20% reduction in body weight gain)		

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
128	Rat (Wistar)	4-8 wk 3 d/wk 1 hr/d (WB)	Hepatic	3000 M			Dick et al. 2014	Endpoints evaluated: organ weight, histopathology.
			Bd Wt	3000 M				
129	Rat (Wistar)	4 wk 3-5 d/wk 1 hr/d (WB)	Bd Wt	3000 M	10000 M (14% decrease in body weight)		Dick et al. 2015	
130	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d	Endocr	320 M			Hillefors-Berglund et al. 1995	No change in serum prolactin levels.
			Bd Wt	320 M				
131	Rat (Wistar)	30 d 24 hr/d	Hepatic	400 M			Ikeda et al. 1986	
			Bd Wt		200 M (decreased body weight gain)			
132	Rat (Wistar)	20 d 4 hr/d	Bd Wt		1500 M (body weight decrease 18%)		Ishigami et al. 2005	
133	Rat (Wistar)	28 d 5 d/wk 2 hr/d (N)	Resp	200 M			Jain et al. 2013	Endpoints examined: lung histopathology, lung and BAL biochemistry.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
134	Rat (Wistar)	12 wk 6 d/wk 8 hr/d	Resp		3000 M (increased pulmonary inflammation, edema, fibrosis, and necrosis)		Kanter 2011a	
135	Rat (Wistar)	12 wk 6 d/wk 8 hr/d	Hepatic		3000 M (enlarged hepatic sinusoids filled with blood and minimal hepatic fibrosis, increased number of apoptotic liver cells)		Kanter 2012	
136	Rat (Sprague-Dawley)	30 d 24 hr/d	Hepatic	320 M			Kyrklund et al. 1987	Endpoint assessed: liver weight.
			Bd Wt		320 M (10% decreased body weight)			
137	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	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
138	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d	Resp	1250	2500	(9-15% increased relative lung weight)			NTP 1990	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	1250	2500	(6-11% increased relative heart weight)				
			Gastro	3000						
			Hemato	3000 M	1250 F	(decreased leukocytes)				
				625 F						
			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	(15% decrease in body weight)	3000 M	(25% decrease in body weight)		
139	Rat (Sprague-Dawley)	21 d 6 hr/d	Ocular	600	2000 F	(lacrimation)			Ono et al. 1996	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
140	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				
141	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d	Resp	300			Poon et al. 1994	Endpoints evaluated: organ weight and histology, upper respiratory tract histology.
			Hemato	300				
			Hepatic	30	300 (significantly increased serum alkaline phosphatase in males & variation hepatocellular size in females)			
			Renal	300				
			Endocr	300 M	30 F (mild reduction in follicle size in thyroid)			
			Bd Wt	300				
142	Rat (Fischer- 344)	23 wk 7 d/wk 8 hr/d 60, 30, or 15 min/hr	Bd Wt		2200 M (decreased body weight gain)		Pryor 1991	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
143	Rat (Fischer- 344)	11 wk 7 d/wk 8 hr/d	Bd Wt		2000 M (decreased body weight gain)		Pryor 1991	
144	Rat (Wistar)	30 d 1 hr/d	Hepatic		1000 M (hepatocyte degeneration, pericentral fibrosis, increased markers of apoptosis)		Tas et al. 2011	
145	Rat (Wistar)	30 d 1 hr/d	Hepatic		1000 M (hepatocyte degeneration, increased number of apoptotic liver cells)		Tas et al. 2013a	
146	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d	Endocr		80 (increase in serum prolactin levels)		von Euler et al. 1994	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
147	Rat (NS)	5 wk 5 d/wk 7 hr/d	Resp		600	(irritation of the lung)	2500	(pulmonary lesions)	von Oettingen et al. 1942	Endpoints evaluated: hematology, organ histology.
			Hemato	600	2500	(transient decrease in leukocytes)				
			Hepatic	5000						
			Renal		600	(renal casts)				
			Endocr	5000						
148	Mouse (BALB/c)	8 wks 6 hr/d	Musc/skel		300 M	(decreased bone mineral density and content in femoral neck)			Atay et al. 2005	
149	Mouse (Swiss-Webster)	40 d 30 min/d	Bd Wt	6000 M					Bowen and McDonald 2009	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
150	Mouse (ICR)	8 wk 5 d/wk 70 min/d	Resp	12000 M			Bruckner and Peterson 1981b	Endpoints evaluated: body weight, organ weight and histology; upper respiratory tract histology not assessed.
			Cardio	12000 M				
			Hepatic		12000 M (decreased liver weight)			
			Renal		12000 M (decreased kidney weight)			
			Bd Wt		12000 M (20% reduction in body weight)			
151	Mouse (ICR)	8 wk 3 hr/d 5 d/wk	Resp	4000 M			Bruckner and Peterson 1981b	Endpoints evaluated: body weight, organ weight and histology; upper respiratory tract histology not assessed.
			Cardio	4000 M				
			Hepatic		4000 M (increased relative liver weight, elevated serum glutamic oxaloacetic transaminase)			
			Renal	4000 M				
			Bd Wt		4000 M (5-10% decrease in body weight gain)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
152	Mouse (C3H/HeN)	12 wk 5 d/wk 6 hr/d	Resp	50 F			Fujimaki et al. 2007	Endpoints examined: histology of nose, trachea, lung.
153	Mouse	20 d 6 hr/d	Hemato		10 M (decreased white blood cells and thrombocytes)		Horiguchi and Inoue 1977	
			Bd Wt	1000 M				
154	Mouse (OF-1)	4 wk 5 d/wk 5 hr/d	Resp		1000 F (inflammation and decreased cell number in olfactory epithelium)		Jacquot et al. 2006	
155	Mouse	30 d 24 hr/d	Hepatic		150 F (increased liver weight [9.6%])		Kjellstrand et al. 1985	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
156	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d	Resp	1250 M	2500 M (5% increase in relative lung weight)		NTP 1990	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
					100 F (12% increase in relative lung weight)			
			Cardio	2500 M 1250 F	2500 F (14% increase in relative heart weight)			
			Gastro	2500				
			Hemato	2500				
			Hepatic	625 M 100 F	1250 M (9% increase in relative liver weight)			
					625 F (6% increase in relative liver weight)			
			Renal	2500 M 625 F	1250 F (7% increase in relative kidney weight)			
			Endocr	2500				
			Bd Wt	1250 M	2500 M (12% decrease in body weight)			
					100 F (13% decrease in body weight)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
157	Rat (CD)	95 d 7 d/wk 6 hr/d		2000			API 1985; Roberts et al. 2003	Endpoints evaluated: thymus and spleen histology in F0/F1 parents and F1/F2 weanlings.
158	Rat (Fischer- 344)	42 d 5 d/wk 6 hr/d		1000 M			API 1997	Endpoint evaluated: thymus weight.
159	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d		3000			NTP 1990	Endpoints evaluated: spleen and thymus weight and histology.
160	Rat (Sprague-Dawley)	90 d 6 hr/d		600	2000 M (decrease in thymus weights)		Ono et al. 1996	
161	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		300			Poon et al. 1994	Endpoints evaluated: spleen and thymus weight and histology.
162	Rat (NS)	5 wk 5 d/wk 7 hr/d		5000			von Oettingen et al. 1942	Endpoints evaluated: spleen and thymus weight and histology.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
163	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d		2500			NTP 1990	Endpoints evaluated: spleen and thymus weight and histology.
Neurological								
164	Rat (CD)	95 d 7 d/wk 6 hr/d		2000			API 1985; Roberts et al. 2003	Endpoints evaluated in 2-generation study: brain weight, brain and spinal cord histology in F0/F1 parents and F1/F2 weanlings.
165	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	
166	Rat	3 wk 5 d/wk 8 hr/d			900 M (prolonged slow-wave sleep and paradoxical sleep latencies)		Arito et al. 1988	
167	Rat (Long- Evans)	4 or 13 wk 5 d/wk 6 hr/d		1000 M			Beasely et al. 2010	Endpoints evaluated: neurobehavioral battery.
168	Rat (Long- Evans)	4 wk 5 d/wk 6 hr/d		1000 M			Beasley et al. 2012	Endpoints evaluated: neurobehavioral battery.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
169	Rat (Sprague-Dawley)	16 wk 104 hr/wk			40 (decreased rearing behavior)		Berenguer et al. 2003	
170	Rat (Sprague-Dawley)	16 wk 104 hr/wk			40 (decreased rearing activity)		Berenguer et al. 2004	
171	Rat Long-Evan	4 wk 5 d/wk 6 hr/d (WB)		1000 M			Boyes et al. 2015	
172	Rat Long-Evan	13 wk 5 d/wk 6 hr/d (WB)		100 M	1000 M (decrease in wave amplitudes of ERG)		Boyes et al. 2015	Endpoints examined: VEP at 2-3 weeks post-exposure, ERG and photoreceptor density at 5-6 weeks and 1 year post-exposure.
173	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	
174	Rat (Sprague-Dawley)	30 d 24 hr/d			320 M (decreased weight of brain and cerebral cortex)		Kyrklund et al. 1987	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
175	Rat (Wistar)	90 d 5 d/wk 6 hr/d		500 M			Lund and Kristiansen 2008	Endpoints examined: brainstem auditory responses, distortion product otoacoustic emissions.
176	Rat (Fischer- 344)	50 d 24 hr/d		600 M			Miyagawa et al. 1995	Endpoint examined: learning and memory (radial arm maze).
177	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d		1250	2500 (ataxia, increased relative brain weight)		NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.
178	Rat (Fischer- 344)	16 wk + 5 wk 7 d/wk 14 hr/d		700 M	1000 M (diminished auditory response)		Pryor et al. 1984b	
179	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		80			Rogers et al. 1999	Endpoints examined: operant training (appetitively-motivated lever-press).
180	Rat (Sprague-Dawley)	13 wk 8 hr/d		1000 M			Tahti et al. 1983	Endpoint examined: motor coordination (tilting plane test, rotarod test).

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
181	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d			80 M (decreased cortical area in parietal cortex, impaired motor coordination)		von Euler et al. 2000	No changes in brain weight, overall brain volume, or cortical thickness.
182	Rat (NS)	5 wk 5 d/wk 7 hr/d		600		2500 (overt signs of neurotoxicity)	von Oettingen et al. 1942	
183	Rat (Wistar)	4 wk 5 d/wk 6 hr/d		25 M	100 M (increased escape response in hot-plate test)		Wiaderna and Tomas 2002	
184	Rat (Long- Evans)	3 wk 2x/wk 2 hr/d			178 M (increased nose poking)		Wood and Cox 1995	
185	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d		2500			NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.
186	Mouse (C3H/HeN)	6 wk 5 d/wk 6 hr/d		50 M			Win-Shwe et al. 2010c	Endpoint examined: learning and memory (Morris water maze).

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
187	Mouse (BALB/c)	30 min/d for 3 d, then 1 d/wk for 4 wk		90 M			Win-Shwe et al. 2010d	Endpoint examined: learning and memory (Morris water maze).
188	Gn Pig (pigmented)	4 wk 5 d/wk 8 hr/d			500 (transient mid- and high-frequency hearing loss)		McWilliams et al. 2000	
Reproductive								
189	Rat (CD)	95 d 7 d/wk 6 hr/d		2000			API 1985; Roberts et al. 2003	Endpoints examined: reproductive performance in 2-generation study (F0 and F1 parental animals).
190	Rat (Wistar)	Gd 7 - Ppd 18 6 hr/d		1200 F			Dalgaard et al. 2001	Pregnancy outcomes examined.
191	Rat (Wistar)	Gd 7 - Ppd 18 6 hr/d		1200 F			Hass et al. 1999	Pregnancy outcomes examined.
192	Rat (Wistar)	20 d 4 hr/d		1500 M			Ishigami et al. 2005	Endpoints examined: reproductive organ weights, spermatogenesis.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
193	Rat (Wistar)	12 wk 6 d/wk 8 hr/d				3000 M (impaired spermatogenesis, decreased seminiferous tubule diameter, ultrastructural abnormalites in testes)	Kanter 2011b	
194	Rat (Fischer- 344)	15 wk 5 d/wk 6.5 hr/d		1250 M 3000 F	2500 M (15% increase in testis weight)		NTP 1990	Endpoints evaluated: organ weight and histology; reproductive function not assessed.
195	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 weights of epididymides, but no effect on indices of fertility)	Ono et al. 1996	
196	Rat (Sprague-Dawley)	5 wk 7 d/wk 2 hr/d		4000 M		6000 M (significantly decreased sperm count [66%], motility [78%], and ovum penetration [76%])	Ono et al. 1999	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
197	Mouse (CD-1)	8 wk 5 d/wk 6 hr/d		400 M			API 1981	Endpoints examined: dominant lethal mutations, pre- and postimplantation losses after mating with unexposed females.
198	Mouse (B6C3F1)	14 wk 5 d/wk 6.5 hr/d		2500			NTP 1990	Endpoints evaluated: organ weight and histology; reproductive function not assessed.
Developmental								
199	Rat (CD)	95 d + Gd 1-20 and Ppd 5-21 7 d/wk 6 hr/d		500	2000 (reduced fetal and pup body weights in F1 and F2 generations)		API 1985; Roberts et al. 2003	2-generation reproduction study.
200	Rat (Wistar)	Gd 7 - Ppd 18 6 hr/d			1200 M (decreased neonatal body weight)		Dalgaard et al. 2001	Endpoints examined: pup weight, external malformations, offspring sperm parameters at Ppd 100.
201	Rat (Wistar)	Gd 7 - Ppd 18 6 hr/d				1200 (decreased neonatal body weight, delayed development of reflexes and increased locomotor activity in offspring)	Hass et al. 1999	Pregnancy outcomes examined.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
202	Rat CFY	Gd 1-21 8 hr/d			266 (increased incidence of fetal skeletal retardation)		Hudak and Ungvary 1978	
203	Rat (Wistar)	Ppd 1-28 12 hr/d			100 (decreased growth of hippocampus)		Slomianka et al. 1990	Endpoints examined: body weight, brain histology and hippocampal volume on Ppd 29.
204	Rat (Wistar)	Ppd 1-28 12 hr/d			500 M (reversible decrease in growth of hippocampus)		Slomianka et al. 1992	Endpoint examined: hippocampal volume on Ppd 29 and 120.
CHRONIC EXPOSURE								
Systemic								
205	Human	NS (Occup)	Renal	93 M			Askergren et al. 1981a,b	Endpoints measured: glomerular filtration rate, albumin and beta-2-microglobin excretion.
206	Human	>3 yr	Hemato	600			Banfer 1961	
207	Human	>5 yr (avg = 20 yr) (Occup)	Resp		186 (mucosal irritation)		Deschamps et al. 2001	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
208	Human	20+ yr (Occup)	Cardio	24 M			Gericke et al. 2001	Endpoints examined: blood pressure, clinical chemistry, glomerular filtration rate.
			Hepatic	24 M				
			Renal	24 M				
209	Human	40 mo (avg) (Occup)	Hemato	83 F			Matsushita et al. 1975	Endpoints evaluated: hematology, clinical chemistry.
			Hepatic	83 F				
210	Human	16 +/- 13 years	Renal	44			Stengel et al.1998	No changes in biomarkers of renal damage when 17 subjects with hypertension were excluded.
211	Human	3-39 yr (Occup)	Hepatic		29 M (increased levels of alkaline phosphatase)		Svensson et al. 1992b	
212	Human	>10 yr	Hemato		110 (increased leukocytes)		Tahti et al. 1981	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
213	Human	NS (Occup)	Hemato	24.7			Ukai et al. 1993	Endpoints evaluated: hematology, clinical chemistry.
			Hepatic	24.7				
214	Human	73-96 mo (Occup)	Hemato		46 M (significant decrease in relative number of lymphocytes)		Yin et al. 1987	
					41 F (significant decrease in relative number of lymphocytes)			
215	Rat (Fischer- 344)	106 wk 5 d/wk 6 hr/d	Resp	300			CIIT 1980; Gibson and Hardisty 1983	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	300				
			Hemato		100 (decreased hematocrit)			
			Hepatic	300				
			Renal	300				
			Bd Wt	300				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)	Reference Chemical Form	Comments
216	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	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	1200					
			Gastro	1200					
			Hemato	1200					
			Musc/skel	1200					
			Hepatic	1200					
			Renal		600	(increased severity of nephropathy)			
			Endocr	1200					
			Bd Wt	1200					

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
217	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	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	1200				
			Gastro	1200				
			Hemato	1200				
			Musc/skel	1200				
			Hepatic	1200				
			Renal	1200				
			Endocr	1200				
			Bd Wt	1200				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
218	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d	Resp	1200			NTP 1990	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	1200				
			Gastro	1200				
			Hemato	1200				
			Musc/skel	1200				
			Hepatic	1200				
			Renal	1200				
			Endocr	1200				
			Bd Wt	1200				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
219	Mouse (B6C3F1)	15 mo 5 d/wk 6.5 hr/d	Resp	1200 M 600 F	1200 F (minimal hyperplasia of the bronchial epithelium)		NTP 1990	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	1200				
			Gastro	1200				
			Hemato	1200				
			Musc/skel	1200				
			Hepatic	1200				
			Renal	1200				
			Endocr	1200				
			Bd Wt	1200				
Immuno/ Lymphoret								
220	Human	13 yr (avg)		637 M			Pelclova et al. 1990	Endpoint evaluated: serum immunoglobulin levels.
221	Human	16 +/- 13 yr		44			Stengel et al. 1998	Endpoint evaluated: serum IgE levels.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
222	Human	73-96 mo (Occup)			46 M (significantly decreased lymphocytes and increased eosinophils)		Yin et al. 1987	
					41 F (significantly decreased lymphocytes and increased eosinophils)			
223	Rat (Fischer- 344)	2 yr 5 d/wk 6.5 hr/d		1200			NTP 1990	Endpoints evaluated: spleen and thymus weight and histology.
224	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d		1200 F	120 M (increased incidence of pigmentation of the spleen)		NTP 1990	Endpoints evaluated: spleen and thymus weight and histology.
Neurological								
225	Human	12-14 yr (Occup)			97 M (increased wave latencies for BAEPs)		Abbate et al. 1993	
226	Human	4.9 yr (avg)			90.9 (statistically significant performance deficits on neurobehavioral tests)		Boey et al. 1997	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
227	Human	0-40 yr (avg = 14 yr) (Occup)		27			Chouaniere et al. 2002	Endpoints examined: battery of psychomotor function tasks, self-reported neurotoxic symptoms.
228	Human	>5 yr (avg = 20 yr) (Occup)		186			Deschamps et al. 2001	Endpoints examined: subjective symptoms, battery of psychomotor tests.
229	Human	5.7 yr (Occup)			88 F (statistically significant performance deficits on neurobehavioral tests)		Foo et al. 1990	
230	Human	20+ yr (Occup)		24 M			Gericke et al. 2001	Endpoints examined: subjective symptoms, color vision, battery of psychomotor tests.
231	Human	99 mo (avg) (Occup)		20	75 (statistically significant impairments in attention and motor performance)		Kang et al. 2005	
232	Human	40 mo (avg) (Occup)			83 F (abnormal tendon reflex, decreased grasping power and agility of fingers, general weakness)		Matsushita et al. 1975	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
233	Human	1-25 yr (Occup)			122 M (hearing loss)		Morata et al. 1997	
234	Human	1-36 yr (Occup)			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	
235	Human	NS (Occup)		46 F			Nakatsuka et al. 1992	Endpoint evaluated: color vision.
236	Human	unspecified (Occup)		33	75 (impaired visual perception)		Neubert et al. 2001	Endpoints evaluated: subjective symptoms, battery of neurobehavioral tasks.
237	Human	4-43 yr (median = 29 yr) (Occup)			140 M (increased incidence of self-reported neurological symptoms [initial]; statistically significant performance deficits on neurobehavioral tests [follow-up])		Orbaek and Nise 1989; Nordling Nilson et al. 2010	Initial examination was in 1985; follow-up was in 2005.
238	Human	13.4 yr (avg) (Occup)		45[c]			Schaper et al. 2003; 2008	Endpoint examined: audiometry.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
239	Human	7 or 23 yr (avg) (Occup)		43[c]			Schaper et al. 2004	Endpoint evaluated: color vision.
240	Human	13.4 yr (avg) (Occup)		45[c]			Seeber et al. 2004; 2005	Endpoints examined: symbol digit substitution, switching attention, memory span, self-reported symptoms.
241	Human	(Occup)		36	76 (increased self-reported neurological symptoms)		Ukai et al. 1993	
242	Human	21.4 yr (avg) range 4-30 yr (Occup)			50 (increased wave latency and amplitude of visual evoked potentials)		Vrca et al. 1995	
243	Human	4-30 years			50 (decrease in wave amplitudes and increased in wave latencies of BAEP)		Vrca et al. 1996	
244	Human	3-33 years			50 (increased wave latency and decreased amplitude of visual evoked potentials)		Vrca et al. 1997b	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
245	Human	73-96 mo (Occup)			46 M (headaches, dizziness) 41 F (headaches, dizziness)		Yin et al. 1987	
246	Human	17 yr (avg) (Occup)			35 (increased color confusion index)		Zavalic et al. 1998a, c	
247	Human	16.8 +/- 5.94 yr			120 (increased color confusion index)		Zavalic et al. 1998b	
248	Human	15 yr (avg) (Occup)		45 M[c]			Zupanic et al. 2002	Endpoints examined: subjective symptoms, manual dexterity.
249	Rat (Fischer- 344)	2 yr 5 d/wk 6.5 hr/d		1200			NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.
250	Rat (Fischer- 344)	15 mo 5 d/wk 6.5 hr/d		1200			NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.
251	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d		1200			NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
252	Mouse (B6C3F1)	15 mo 5 d/wk 6.5 hr/d		1200			NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.
Reproductive								
253	Human	20+ yr (Occup)		24 M			Gericke et al. 2001	Endpoint examined: serum reproductive hormone levels in male workers.
254	Human	40 mo (avg) (Occup)			83 F (dysmenorrhea)		Matsushita et al. 1975	
255	Human	6 yr (Occup)		88 F			Ng et al. 1992a	Endpoints examined: cycle irregularities, extent of uterine bleeding, and the presence of dysmenorrhea.
256	Human	10 yr (Occup)				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	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
257	Human	25 yr (median) 0.5-37 yr (Occup)			36 M (decreased levels of LH, FSH, and free testosterone)		Svensson et al. 1992a	
258	Human	3-39 yr (Occup)		29 M			Svensson et al. 1992b	Increasing workplace air concentrations were not significantly (p>0.05) associated with plasma concentrations of repro hormones after age adjustment.
259	Rat (Fischer- 344)	106 wk 5 d/wk 6 hr/d		300			CIIT 1980; Gibson and Hardisty 1983	Endpoints evaluated: reproductive organ weight and histology.
260	Rat (Fischer- 344)	2 yr 5 d/wk 6.5 hr/d		1200			NTP 1990	Endpoints evaluated: organ weight and histology; reproductive function was not assessed.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
261	Mouse (B6C3F1)	2 yr 5 d/wk 6.5 hr/d		1200			NTP 1990	Endpoints evaluated: organ weight and histology; reproductive function not assessed.

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

b Used to derive an acute inhalation minimal risk level (MRL); concentration (15 ppm) divided by an uncertainty factor of 9 (3 for use of a minimal LOAEL and 3 for human variability [observed effects were noted in a susceptible/sensitive group of individuals, therefore a full uncertainty factor of 10 for human variability is not necessary]), resulting in an MRL of 2 ppm (7.6 mg/m3).

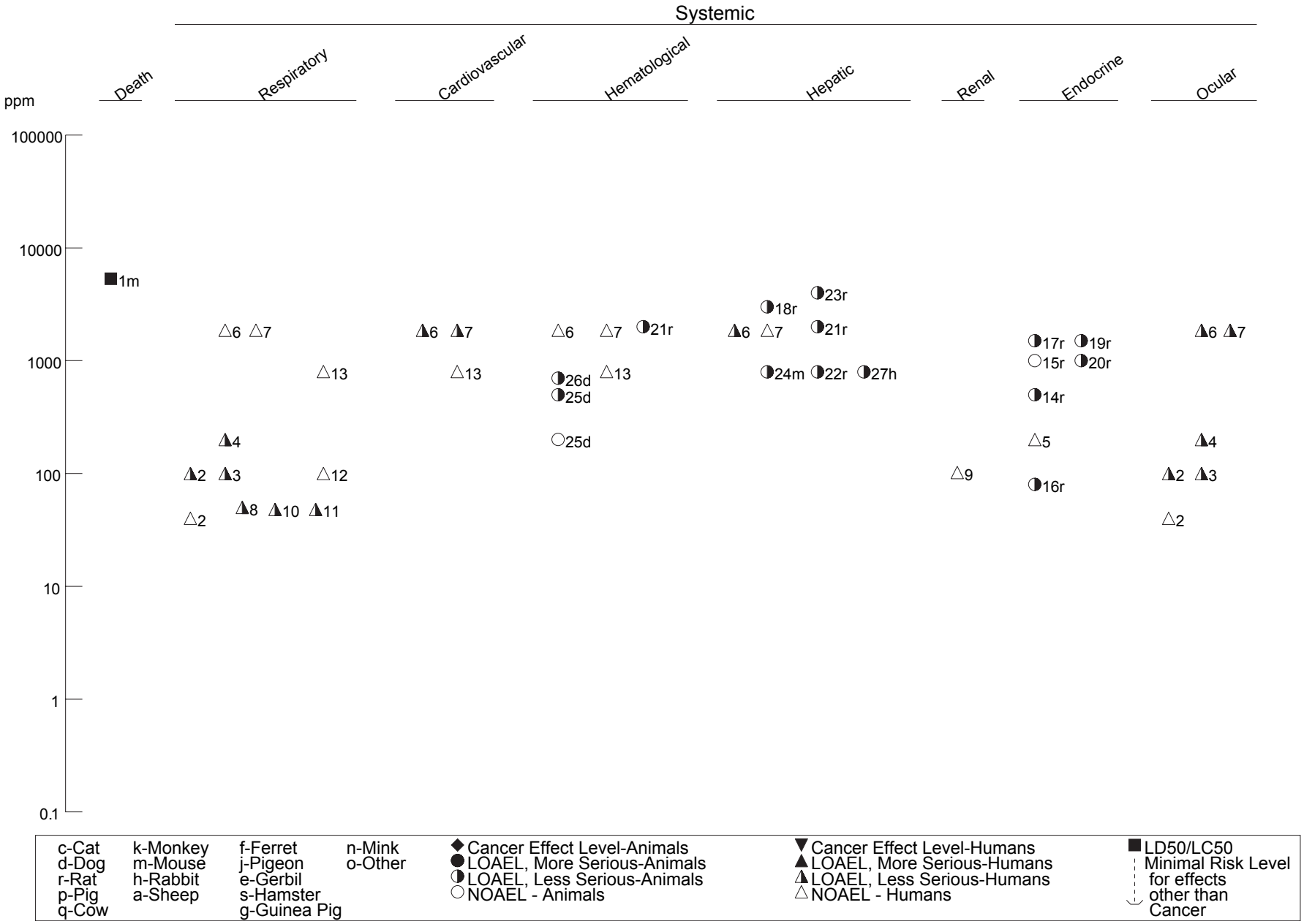
c Used to derive a chronic inhalation minimal risk level (MRL) along with 5 companion studies supporting a NOAEL of 45 ppm for neurological effects; concentration was adjusted to a continuous exposure basis (45 ppm x 5d/7d x 8hr/24hr = 10.7 ppm) and divided by an uncertainty factor of 10 for human variability, resulting in an MRL of 1 ppm (3.8 mg/m3).

ACTH = adrenocorticotropic hormone; ALT = alanine amino transferase; AST = aspartate aminotransferase; BAEP = brainstem auditory evoked potential; BAL = bronchioalveolar lavage; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; ERG = electroretinogram; F = Female; FSH = follicle stimulating hormone; Gastro = gastrointestinal; Gd = gestational day; GFAP = glial fibrillary acidic protein; Gn Pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LC50 = lethal concentration, 50% kill; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); MCS = multiple chemical sensitivity; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Occup = occupational; Pnd = post-natal day; Pnw = post-natal week; Ppd = post-parturition day; REM = rapid eye movement; Resp = respiratory; VEP = visual evoked potential; wk = week(s); x = time(s); yr = year(s)

3. HEALTH EFFECTS

Figure 3-1 Levels of Significant Exposure to Toluene - Inhalation

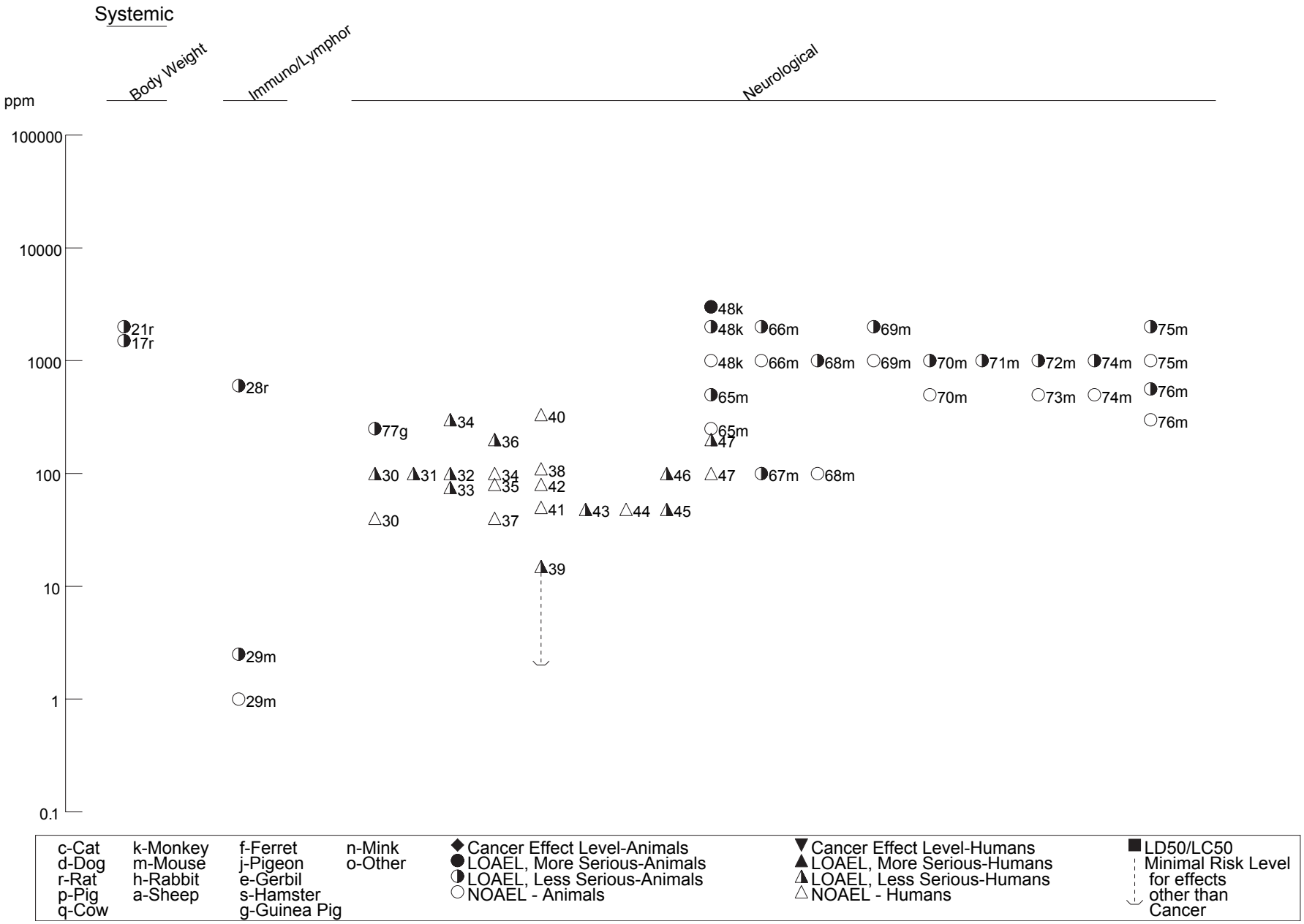
Acute (≤14 days)



3. HEALTH EFFECTS

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

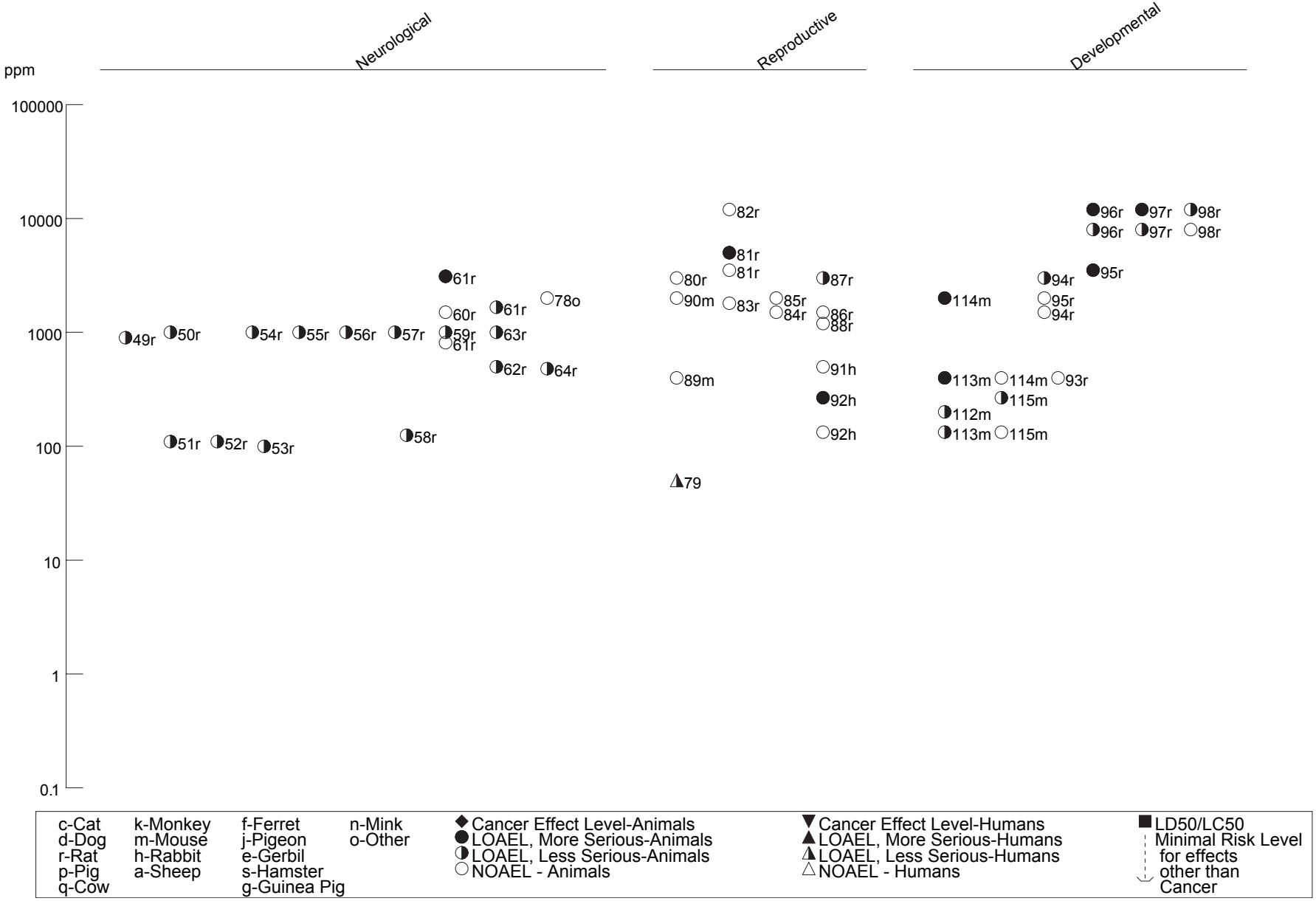
Acute (≤14 days)



3. HEALTH EFFECTS

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

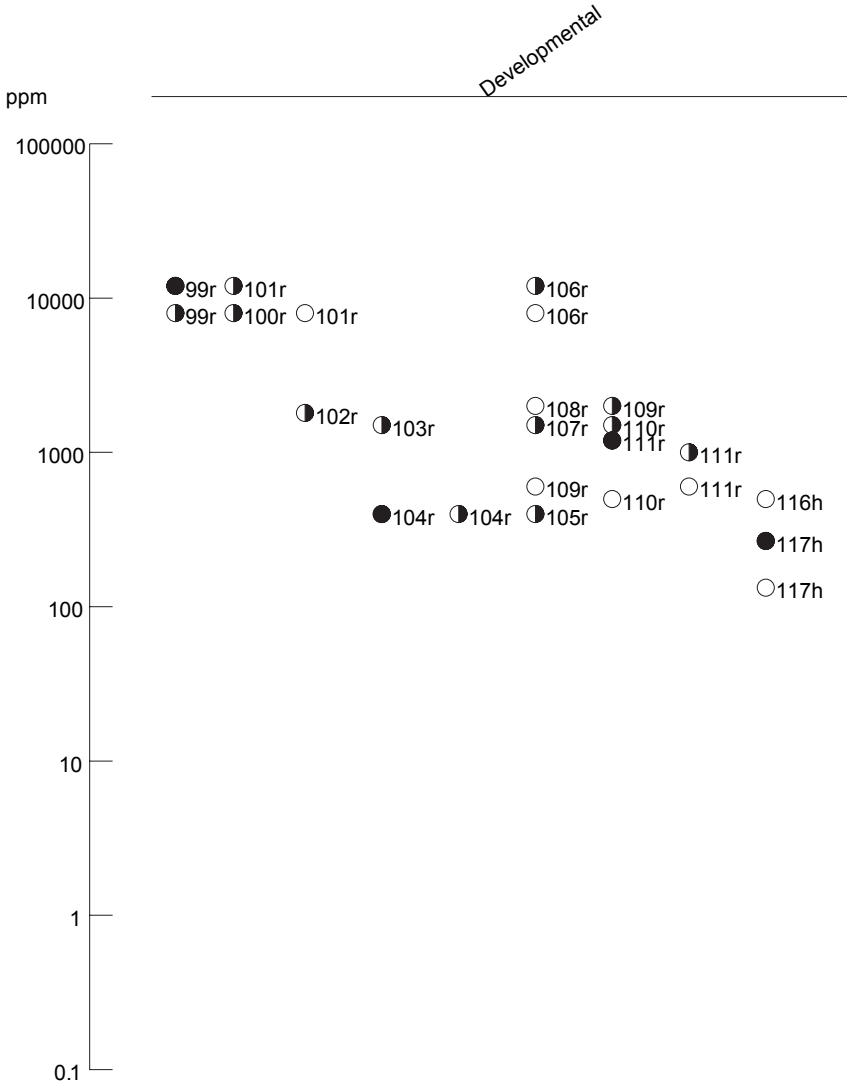
Acute (≤14 days)



3. HEALTH EFFECTS

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

Acute (≤14 days)



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

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

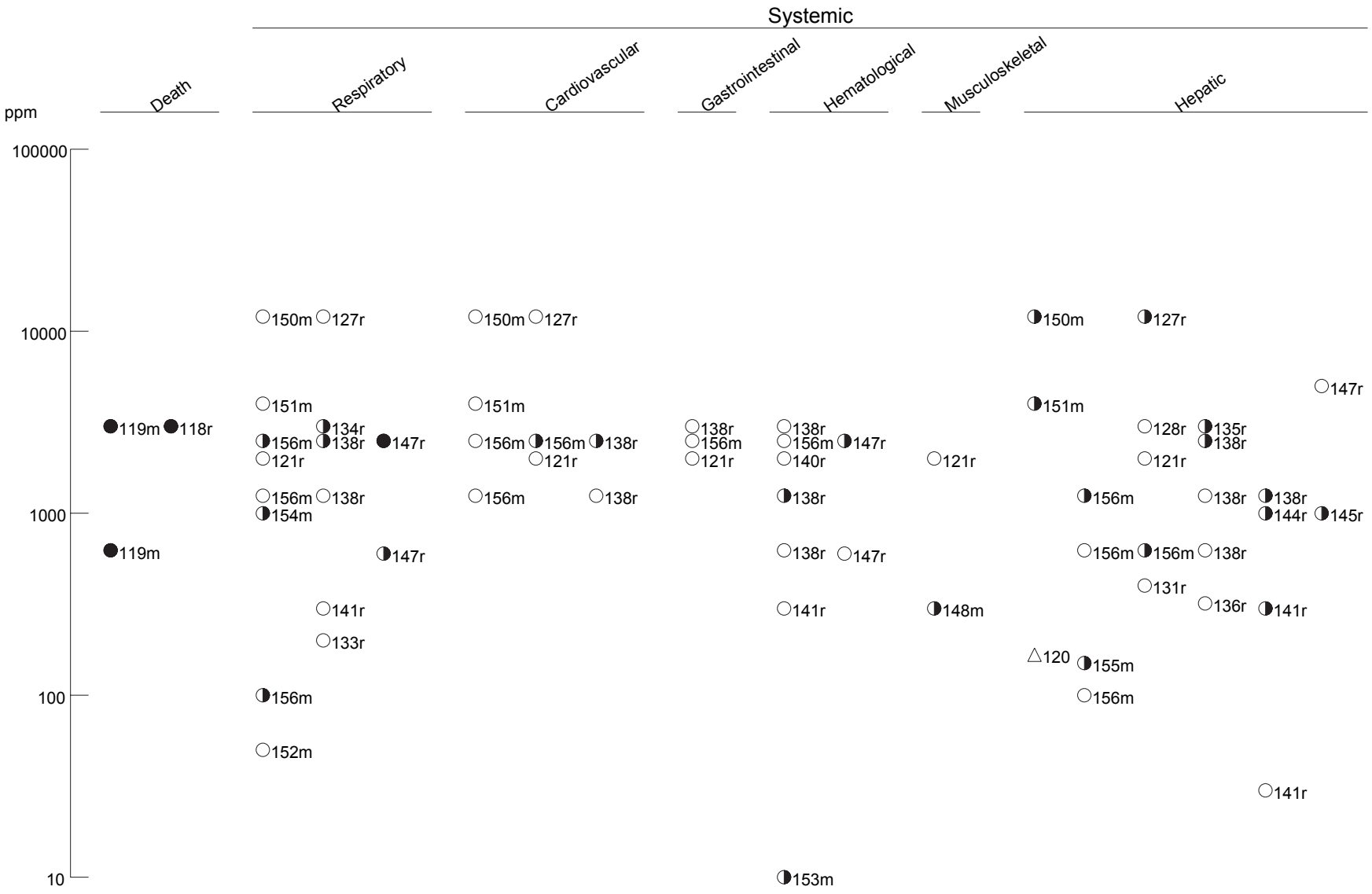
▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

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

3. HEALTH EFFECTS

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

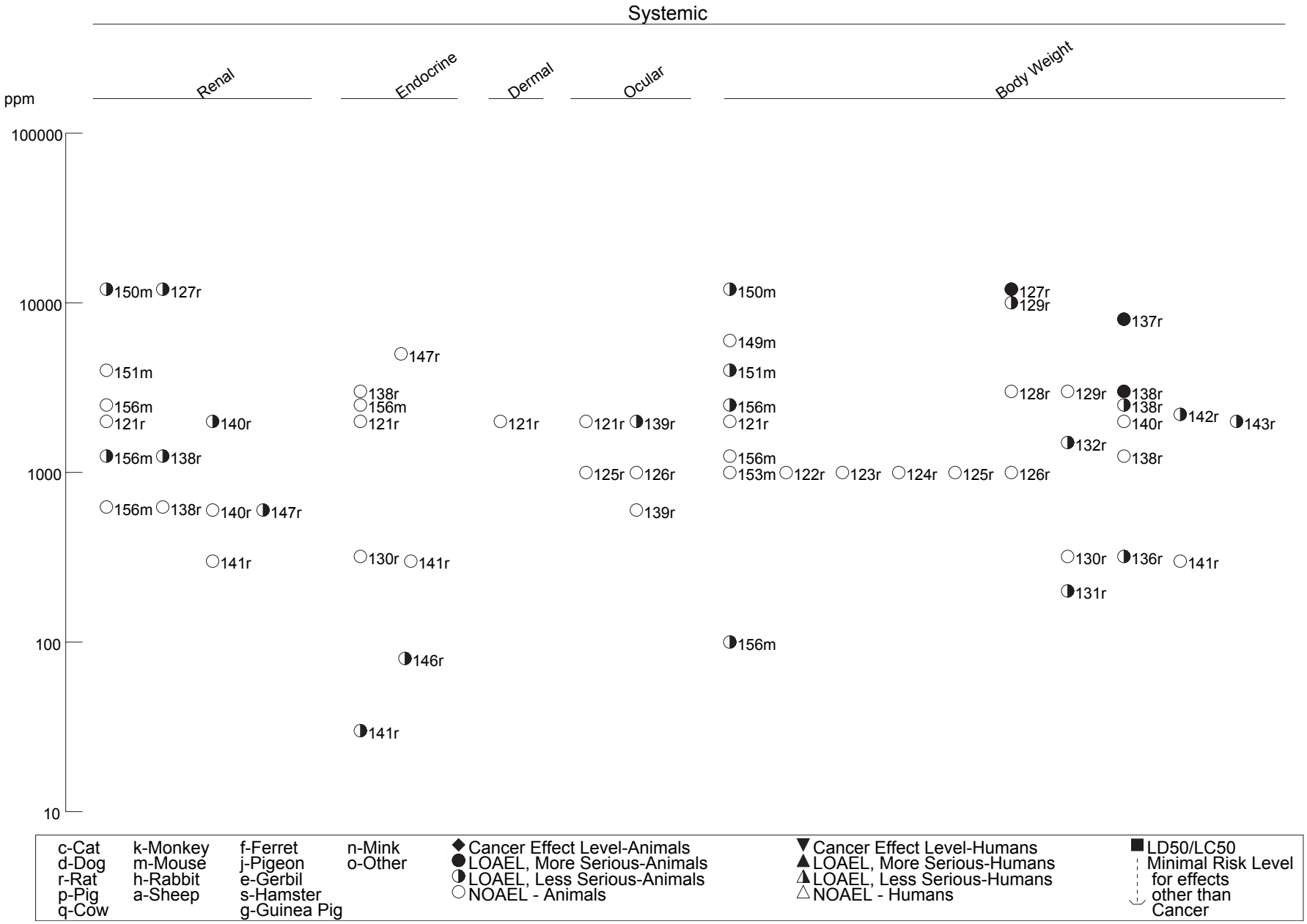
Intermediate (15-364 days)



3. HEALTH EFFECTS

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

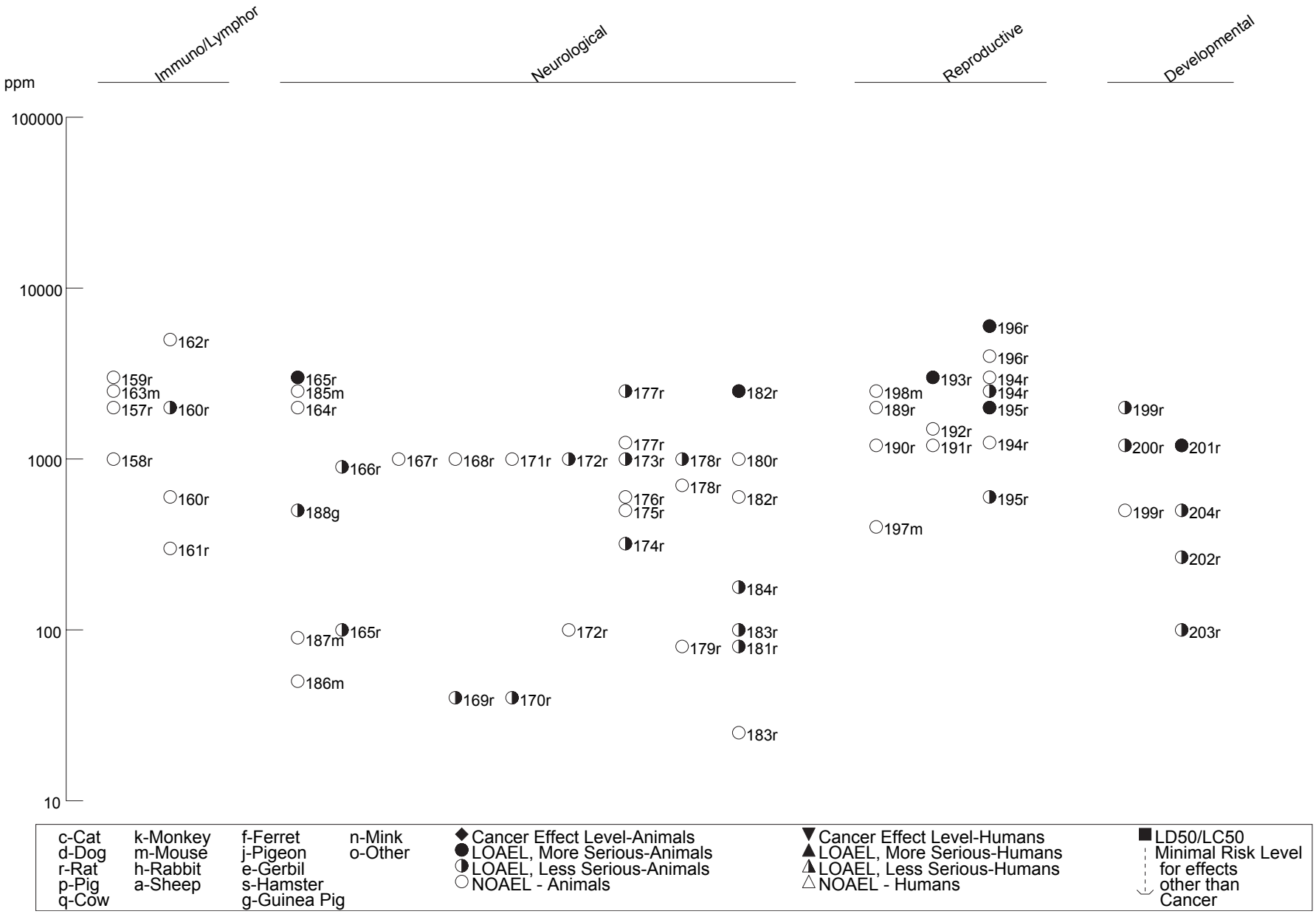
Intermediate (15-364 days)



3. HEALTH EFFECTS

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

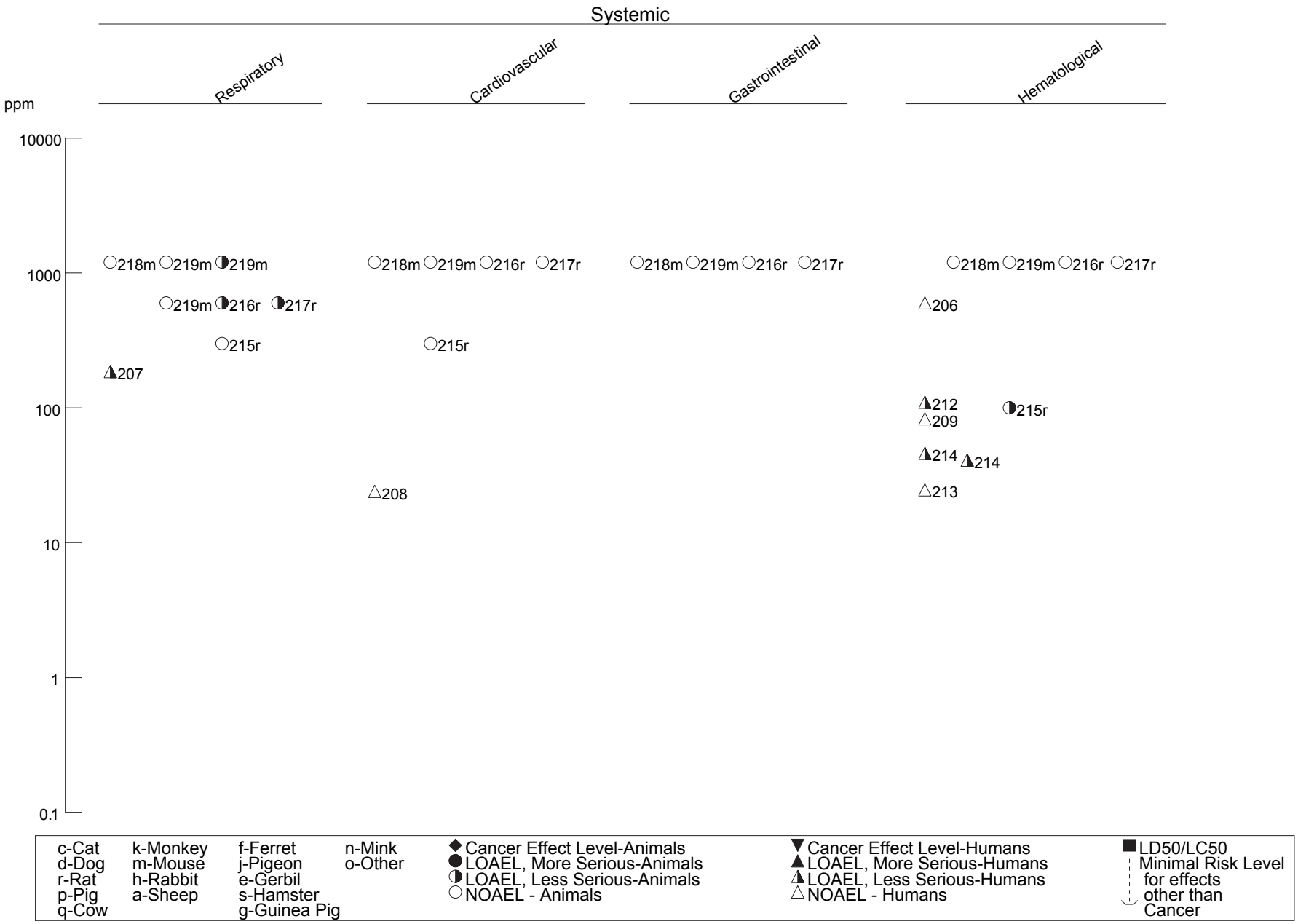
Intermediate (15-364 days)



3. HEALTH EFFECTS

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

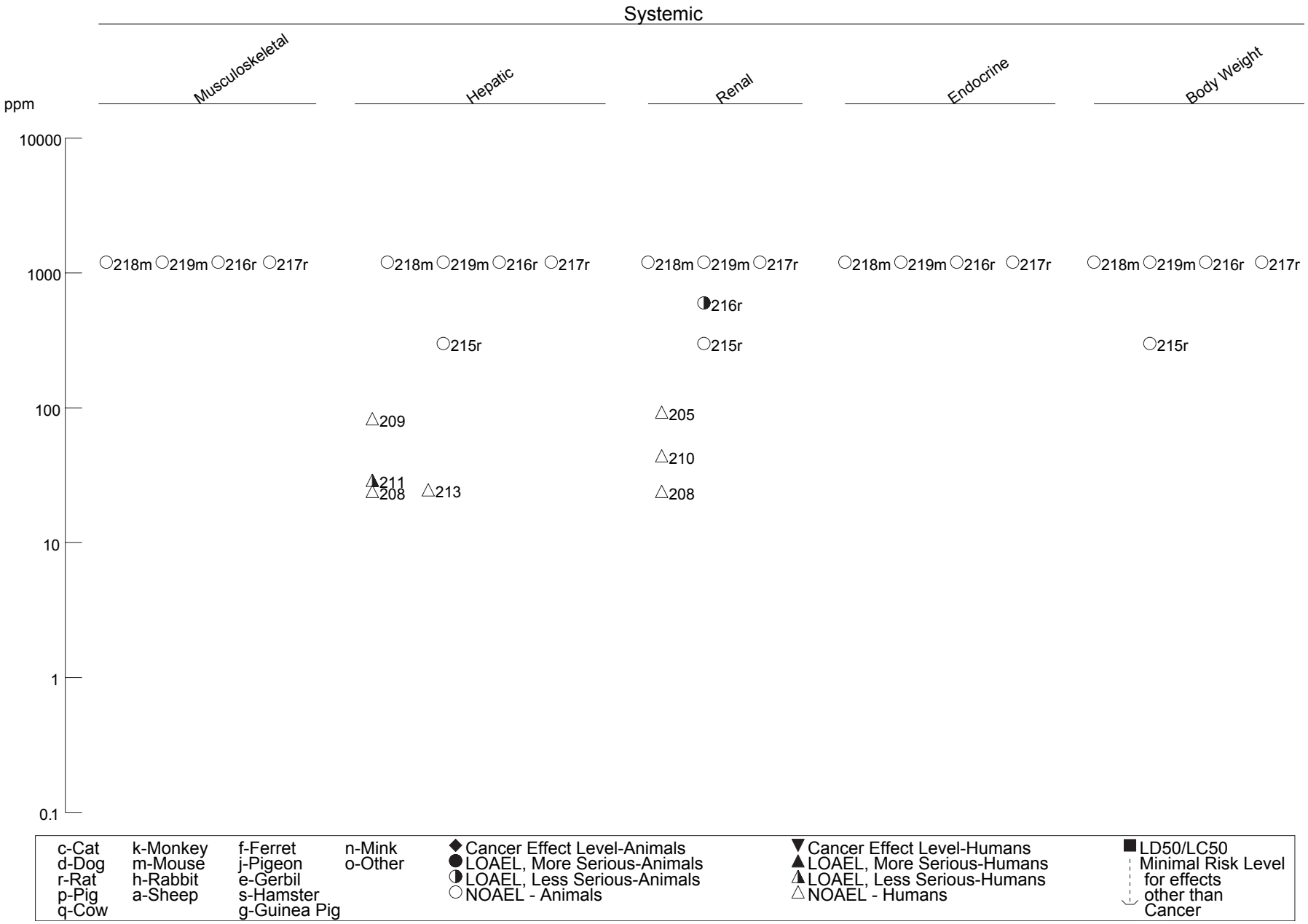
Chronic (≥365 days)



3. HEALTH EFFECTS

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

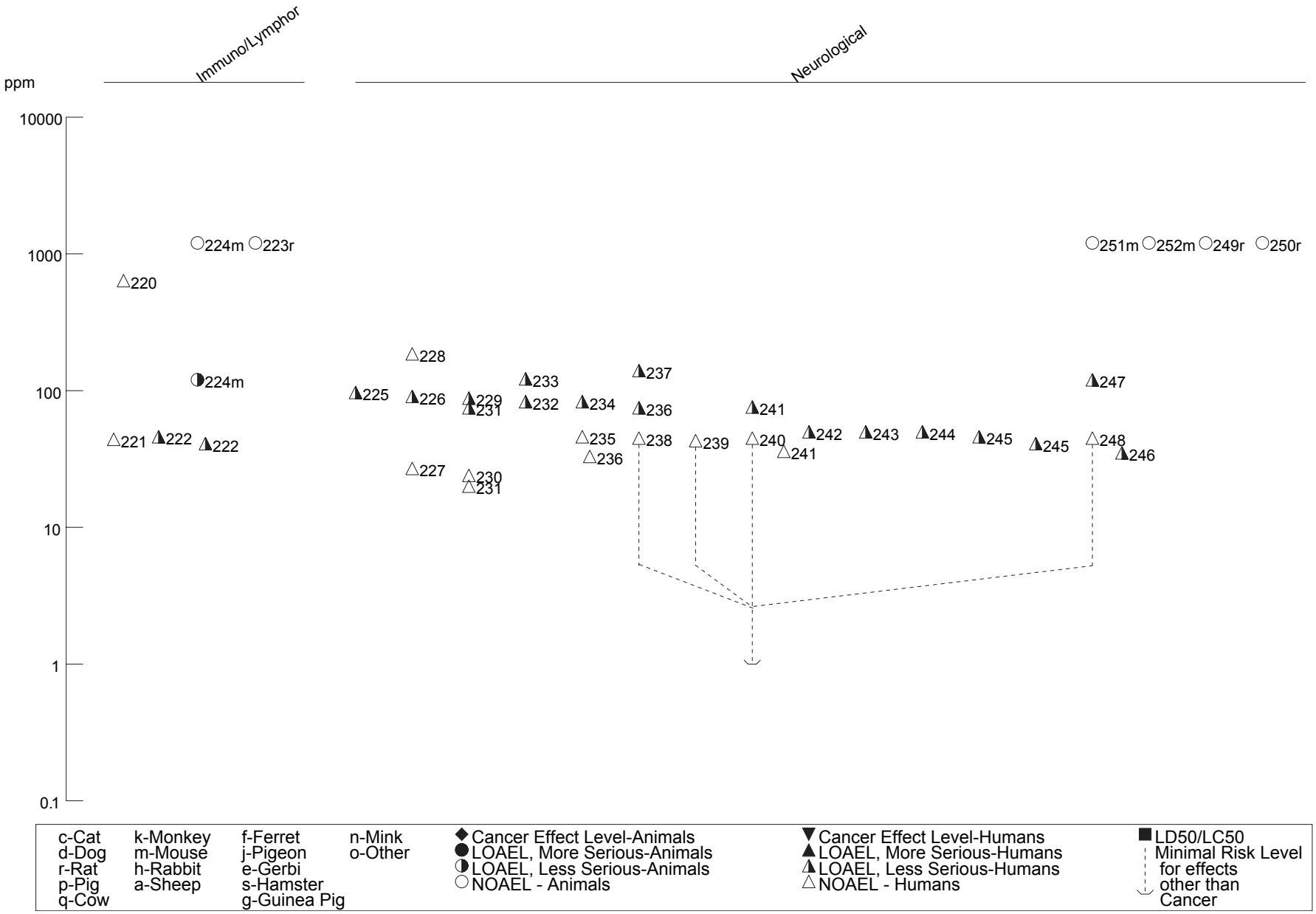
Chronic (≥365 days)



3. HEALTH EFFECTS

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

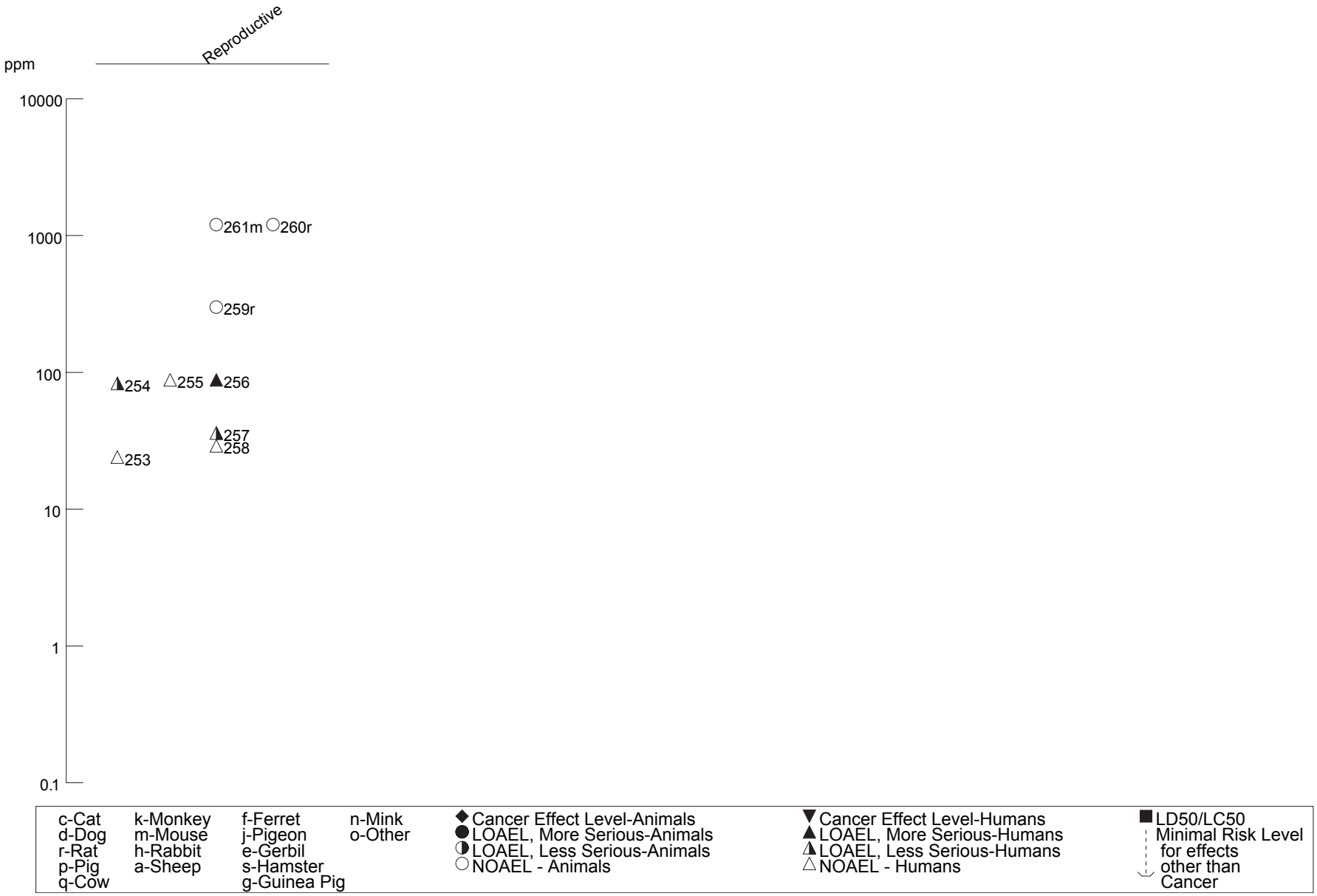
Chronic (≥365 days)



3. HEALTH EFFECTS

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

Chronic (≥365 days)



3.2.1.2 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 3-1 and plotted in Figure 3-1.

Respiratory Effects.

Overview. In humans, respiratory tract irritation has been reported from exposure to toluene under controlled and occupational exposure conditions (Andersen et al. 1983; Baelum et al. 1985; Carpenter et al. 1944; Hellquist et al. 1983; Orbaek et al. 1998; Osterberg et al. 2003; Winchester and Madjar 1986). Limited epidemiological data suggest that exposure to benzene-toluene-ethylbenzene-xylene (BTEX) solvents may increase the risk of acute asthma attacks in urban settings (Lemke et al. 2014). In animal studies, evidence for histological damage to the nose or lung has been reported following intermediate- and chronic-duration inhalation exposure to toluene, most clearly at concentrations ≥600 ppm 6 hours/day (Fujimaki et al. 2007; Jacquot et al. 2006; Kanter 2011a; NTP 1990; Poon et al. 1994; von Oettingen et al. 1942).

Controlled Exposure Human Studies. 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). 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). Scores for throat irritation were significantly increased in 20 males during a 4.5-hour exposure to 50 ppm toluene, compared with pre-exposure scores, but no significant increases were observed for nasal irritation or coughing (Muttray et al. 2005). Mucous membrane irritation of progressive severity was reported by volunteers during exposure to progressively higher toluene concentrations of 0, 3, 6, 12, 24, and 48 ppm toluene over a 90-minute period (Orbaek et al. 1998; Osterberg et al. 2003); median maximal scores for mucous membrane irritation (scale of 0–100) at 48 ppm were about 70 for subjects with multiple chemical sensitivity diagnosis and about 30 for healthy subjects (Osterberg et al. 2003). In other controlled-exposure studies, volunteers reported that they did

not experience respiratory or mucous membrane irritation during exposure to 800 ppm toluene for 3 hours (von Oettingen et al. 1942) or 1,862 ppm for 2 hours (Meulenbelt et al. 1990). 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).

Occupational Exposure Human Studies. 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. Eight workers from a print factory exposed to <200 ppm toluene for >18 months had normal chest roentgenograms and did not report breathing difficulty (Guzelian et al. 1988). In another study, increased mucosal irritation scores were reported in 72 workers exposed to toluene for an average of 20 years, compared with 61 unexposed referents (Deschamps et al. 2001). The toluene-exposed group consisted of 36 printer and machine factory workers and 36 laboratory workers exposed to toluene (but no other solvent) at toluene concentrations of 9–83 and 184-467 ppm, respectively. Since the exposed groups were analyzed together, the LOAEL for this assessment is determined to be the average of the midpoints of the exposed ranges (midpoint of 46 ppm for factory workers; midpoint of 325.5 ppm for laboratory workers; average of 186 ppm).

Environmental Exposure Human Studies. A single epidemiological study evaluated the potential associations between air pollutants and acute asthma attacks in 5–89-year-old residents in Detroit, Michigan, United States and Windsor, Ontario, Canada (Lemke et al. 2014). Air pollutants (including summed BTEX) were measured at 100 sites during two 2-week sampling periods in 2008 and 2009. Acute asthma attack rates in each city were calculated using emergency room visits and hospitalizations. Using linear regression analysis, annual 2008 asthma rates were significantly correlated with total BTEX levels in Detroit (β 6.34; 95% confidence interval [CI] 4.23–8.45), but not Windsor. Mean BTEX levels in Detroit and Windsor during the two sampling periods were 6.6–10.3 and 3.2–5.9 μg/m^3, respectively. This study suggests that exposure to BTEX solvents may increase the risk of acute asthma attacks in

urban settings; however, major limitations include coarse temporal resolution and multiple chemical exposures.

Intermediate-Duration Animal Studies. Following intermediate-duration exposure of animals to concentrations ≥600 ppm 6 hours/day, signs of pulmonary or nasal inflammation have been observed in a number of studies. Rats exposed to 600 ppm for 5 weeks, 7 hours/day showed irritation of the lungs and rats exposed to 2,500 and 5,000 ppm had pulmonary lesions (von Oettingen et al. 1942). Rats exposed to 3,000 ppm 8 hours/day 6 days/week for 12 weeks showed signs of pulmonary inflammatory responses including inflammatory cell infiltration in peribronchial and alveolar regions, alveolar edema, and interstitial fibrosis and necrosis (Kanter 2011a). Mice exposed to 1,000 ppm 5 hours/day, 5 days/week for 4 weeks showed time-dependent and reversible changes in the number of cells in, and the thickness of, the nasal olfactory epithelium that were indicative of degenerative processes followed by regenerative processes (Jacquot et al. 2006). Significantly ($p>0.05$) increased relative lung weights were reported in male and female rats and male and female mice exposed to 2,500 and 3,000 ppm (6.5 hours/day, 5 days/week for 14–15 weeks); these concentrations also induced ataxia in rats and dyspnea and increased early mortality in mice (NTP 1990). Relative lung weights were also increased at lower exposure levels (100, 625, and 1,250 ppm) in female mice, but the degree of change did not increase with increasing concentration (NTP 1990). The increased lung weights observed in the NTP (1990) studies were not accompanied with increased incidences of histological lesions in the lung, trachea, or nose in the 2500- or 3,000-ppm groups, compared with controls. 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 to 12,000 ppm for seven 10-minute periods per day separated by a 20-minute recovery period (Bruckner and Peterson 1981b). Additionally, no histological abnormalities in the lung were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation in a multigenerational study (API 1985; Roberts et al. 2003). However, these studies did not include a histological examination of the upper respiratory tract and may therefore not have observed damage to this region. In addition, the lack of pulmonary abnormalities in Bruckner and Peterson (1981b) at higher concentrations than those in the NTP (1990) studies may be explained by the shorter daily duration of exposure (3 hours/day versus 6 hours/day).

Following intermediate-duration to concentrations <600 ppm, histological lesions in the respiratory tract have not been observed consistently across studies. Sprague-Dawley rats exposed to 30 or 300 ppm toluene 6 hours/day, 5 days/week for 4 weeks showed no clear-cut evidence for exposure-related histological lesions in the nose, trachea, or lung (Poon et al. 1994). Incidences of epithelial degeneration

of the nasolacrimal ducts (with average severity scores in parentheses on a scale of 1=minimal, 2=mild, 3=moderate, and 4=marked) were 4/6 (0.5), 6/6 (1.50), and 3/6 (0.42) in control through high-concentration males, respectively, and 1/6 (0.25), 5/6 (0.38), and 3/6 (0.33) in females, respectively. In the trachea, subepithelial lymphoid proliferation was observed in 3/6 (0.15), 3/6 (0.35), and 3/6 (0.25) male rats, respectively, and 5/6 (0.29), 3/6 (1.15), and 3/6 (0.71) female rats, respectively (Poon et al. 1994). In Wistar rats, exposure to 200 ppm toluene via nose-only inhalation 2 hours/day, 5 days/week for 28 days did not induce histopathological changes in the lung or increase markers of inflammation in the lung or bronchoalveolar lavage (BAL) fluid; nasal passages were not evaluated (Jain et al. 2013). In a mouse study, no exposure-related changes were detected by light microscopy in the nose, trachea, or lung of female C3H/HeN mice exposed to 0 or 50 ppm 6 hours/day, 5 days/week for 12 weeks, but significant ($p<0.05$) changes were found in a few BAL indices of airway inflammation and a few neurotrophic factors in exposed mice, compared with control mice (Fujimaki et al. 2007). Significant changes after 12 weeks of exposure included increased numbers of inflammatory cells and macrophages (~1.95 times control values), but no changes in the number of lymphocytes or levels of 5/6 cytokines (tumor necrosis factor [TNF-α], monocyte chemoattractant protein [MCP-1], macrophage inflammatory protein [MIP-1α], interleukin-1β and interleukin-6); levels of interferon-gamma (IFN-γ) were significantly lower than control levels (~0.7 times control value). BAL fluid from exposed mice showed increased levels of neurotrophin-3 (~1.12 times control value) and decreased levels of substance P (~0.5 times control value), but no significant changes in nerve growth factor (NGF) levels (Fujimaki et al. 2007). The biological adversity of the observed changes in BAL fluid end points at 50 ppm is currently uncertain, but the histological results clearly identify 50 ppm 6 hours/day, 5 days/week for 12 weeks as a NOAEL for histological signs of respiratory irritation in mice.

Chronic-Duration Animal Studies. Inflammation of the nasal mucosa, erosion and metaplasia of the olfactory epithelium, and degeneration of the respiratory epithelium were reported in male and female rats exposed to 600 or 1,200 ppm for 15 months or 2 years (6.5 hours/day, 5 days/week) (NTP 1990). At the end of 2 years, there were significant ($p<0.05$) increases in the incidence of erosion of the olfactory epithelium (males: 0/50, 3/50, and 8/49; females: 2/49, 11/50, and 10/50 at 0, 600, and 1,200 ppm, respectively) and degeneration of the respiratory epithelium (males: 15/50, 37/50, and 31/49; females: 29/49, 45/50, and 39/50 at 0, 600, and 1,200 ppm, respectively). Exposed female rats also showed significant increases in inflammation of the nasal mucosa (27/49, 41/50, and 41/50 at 0, 600, and 1,200 ppm, respectively) and metaplasia of the olfactory epithelium (0/49, 1/50, and 5/50 at 0, 600, and 1,200 ppm, respectively). Exposed rats showed no increased incidences of lung lesions compared with controls (NTP 1990). Increased incidences of nasal or lung lesions were not observed in mice exposed to

the same concentrations for 2 years, but minimal hyperplasia of the bronchial epithelium was observed in 4/10 female mice exposed to 1,200 ppm for 15 months (NTP 1990). No histopathological lesions were observed in the upper respiratory tract or lungs of rats exposed for 2 years to 300 ppm toluene (6 hours/day, 5 days/week) (CIIT 1980; Gibson and Hardisty 1983).

Cardiovascular Effects.

Overview. Cardiac arrhythmia has been reported as a cause of death in fatal acute inhalation of solvents containing toluene (Anderson et al. 1982; Shibata et al. 1994). Following nonfatal acute exposures to high concentrations of toluene (>1,500 ppm), transient tachycardia and bradycardia have been reported (Camarra-Lemarroy et al. 2015; Einav et al. 1997; Meulenbelt et al. 1990). In addition, several cases of cardiac abnormalities associated with chronic toluene abuse have been reported (see Vural and Ogel 2006 for review). A single pregnancy cohort study suggests a weak potential association between environmental levels of toluene and increased risk of adverse cardiovascular events during delivery (Mannisto et al. 2015). However, repeated inhalation exposure studies in laboratory animals at concentrations as high as 1,200 ppm 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 of dogs to a lethal concentration of toluene (~30,000 ppm) reported the induction of arrhythmia, but the authors suggested that this was due to a predisposing arrhythmia-producing heart abnormality (Ikeda et al. 1990). Other studies of acute exposure have reported a nonsignificant increase in heart rate in rats exposed to 66,276 ppm toluene for 30 minutes (Vidrio et al. 1986) and a reduction of experimentally-induced arrhythmia in rats exposed to 6,867 ppm toluene, 10 minutes before aconitine treatment to induce arrhythmias (Magos et al. 1990).

Human Exposure Studies. 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 vapors from approximately 250 mL of thinner containing more than 50% toluene (Einav et al. 1997). In a case-series report of patients with acute toluene intoxication, 20/20 cases exhibited tachycardia upon admission; EKG alterations included ST segment depression (10/20), AV block (6/20), U-waves (4/20), and prolonged QT (4/20) (Camarra-Lemarroy et al. 2015). 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), but several cases of cardiac abnormalities associated with toluene abuse have been reported (see Vural and Ogel 2006 for review). No significant differences in prevalences of subjects with abnormally high blood pressure were found between a group of German rotogravure printers expected to have been exposed to toluene and a reference group of non-exposed paper industry workers (Gericke et al. 2001).

In the Consortium on Safe Labor pregnancy cohort in the United States (n=223,502 singleton births), environmental toluene levels 5 days prior to delivery were significantly associated with an increased risk of adverse cardiovascular events during delivery (including ischaemic heart disease, stroke, heart failure, cardiac arrest/failure, and other/unspecified cardiovascular event) after adjustment for hospital site, maternal age, race/ethnicity, insurance status, smoking during pregnancy, and pre-pregnancy body mass index (BMI) (odds ratio [OR] 1.42, 99% CI 1.02–1.97) (Mannisto et al. 2015). Toluene exposure levels were estimated using the Community Multiscale Air Quality Model, developed by the EPA (estimated air levels were not reported). Toluene exposure levels on the day of delivery or 1, 2, 3, 4, 6, or 7 days prior to delivery were not significantly associated with risk of adverse cardiovascular events during delivery. This study provides weak evidence that toluene exposure near delivery date may increase the risk of adverse cardiovascular events during delivery; however, concurrent exposure to multiple pollutants (which were not controlled for in statistical analyses) limits the conclusions that can be drawn from this study.

Animal Studies. 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 nonsignificant 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). Additionally, no histological abnormalities in the heart were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation in a multigenerational study (API 1985; Roberts et al. 2003). 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; Gibson and Hardisty 1983) or in rats or 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 or in rats and mice exposed for only 15 months. 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). Additionally, no histological abnormalities in the gastrointestinal tract were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation in a multigenerational study (API 1985; Roberts et al. 2003).

Hematological Effects. Consistent evidence for hematological effects has not been 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 some 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 1985). 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).

Human Studies. Following acute exposures, no effects on leukocyte counts were observed in volunteers exposed to 800 ppm toluene for 3 hours (von Oettingen et al. 1942), and two workers accidentally

exposed to about 1,862 ppm for 2–3 hours had normal values for hematological variables (Meulenbelt et al. 1990). White blood cell counts were below the reference range in a case-series report of 20 patients with acute toluene intoxication (4/20); no other hematological parameters were reported (Camarra-Lemarroy et al. 2015).

Consistent evidence for hematological effects has not been reported in workers chronically exposed to toluene. 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. No significant hematological effects were observed in workers engaged in shoemaking (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. Shoemakers were exposed to toluene concentrations that varied from 65 ppm (15–100 ppm) in winter to 100 ppm (10–200 ppm) in summer and printers 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. Workers involved in printing, shoemaking, and audio equipment production, and exposed to toluene at 41 ppm (females) or 46 ppm (males) had significantly decreased relative (but not absolute) lymphocyte counts when compared to controls; leukocyte counts were not different between exposed and nonexposed workers (Yin et al. 1987). 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. Lymphocyte and leukocyte counts were not significantly ($p>0.05$) different in a group of 25 nonsmoking subjects with >5 years of exposure to toluene in shoe-repair facilities, compared with 25 nonsmokers without occupational exposure to toluene (Akbas 2004).

Animal Studies. Results of animal studies do not provide consistent evidence for hematological effects following inhalation exposure to toluene.

Following acute inhalation exposure, increased hematocrit and blood glucose levels have been 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).

In another acute exposure study, decreased leukocyte counts were observed in dogs exposed acutely to ≥500 ppm toluene for 1 hour or 700 ppm for 4 hours (Hobara et al. 1984a), but intermediate-duration exposure studies provide conflicting evidence for this effect. Positive reports include concentration-related decreases in thrombocytes in mice exposed to 10–1,000 ppm for 20 days (Horiguchi and Inoue 1977), reversible decreased leukocyte counts in rats exposed to 2,500 or 5, 000 ppm for 5 weeks (von Oettingen et al. 1942), and decreased leukocytes in female rats exposed to concentrations >1,250 ppm for 15 weeks (NTP 1990). In contrast, no hematological effects were reported for male rats exposed to up to 3,000 ppm for 15 weeks or male or female mice exposed to up to 1,200 ppm for 14 weeks (NTP 1990), male rats exposed to 2,000 ppm 6 hours/day for 90 days (Ono et al. 1996), or rats exposed to 300 ppm 6 hours/day, 5 days/week for 4 weeks (Poon et al. 1994).

In chronic-duration studies, rats exposed to 100 or 300 ppm toluene had significantly reduced hematocrit levels (CIIT 1980; Gibson and Hardisty 1983), but no consistent effects on hematological variables were reported for mice or rats exposed to toluene at levels up to 1,200 ppm for 15 months or 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). Rhabdomyolysis was also diagnosed in a 48-year-old man, who was a chronic toluene abuser and had been inhaling one tube of toluene-containing glue per day in the month preceding admission (Karmakar and Roxburgh 2008). In a case-series report of patients with acute toluene intoxication, 16/20 cases presented with rhabdomyolysis; 3 of these cases were fatal (Camarra-Lemarroy et al. 2015).

No histological effects on bone were reported in mice or rats exposed to toluene at concentrations up to 1,200 ppm for 15 months or 2 years (NTP 1990). Additionally, no histological abnormalities were observed in bone or skeletal tissue from F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation in a multigenerational study (API 1985; Roberts et al. 2003). However, bone mineral density and bone mineral content were

significantly ($p<0.05$) decreased in the right femoral neck of mice exposed to 300 ppm toluene 6 hours/day for 8 weeks (Atay et al. 2005).

Hepatic Effects.

Overview. Studies of chronic toluene abusers or occupationally-exposed humans have provided inconsistent evidence for 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 AP (Guzelian et al. 1988; Svensson et al. 1992b), but others recorded no adverse effects on serum liver enzyme levels (Gericke et al. 2001; Lundberg and Hakansson 1985; Matsushita et al. 1975; Seijii et al. 1987; Ukai et al. 1993). A number of animal studies have reported increased liver sizes or histological changes in mice or rats repeatedly exposed to concentrations ranging from 300 ppm 6 hours/day, 5 days/week for 4 weeks to 3,000 ppm 6 hours/day for 15 weeks (Bruckner and Peterson 1981b; Gotohda et al. 2009; Kanter 2012; Kjellstrand et al. 1985; NTP 1990; Poon et al. 1994; Tas et al. 2011, 2013a), but other studies have recorded no effects on liver histology in rats exposed to 320 ppm for 30 days (Kyrklund et al. 1987), rats intermittently exposed to 3,000 ppm for 4–8 weeks (Dick et al. 2014), or rats or mice exposed at concentrations up to 1,200 ppm for 2 years (CIIT 1980; Gibson and Hardisty 1983; NTP 1990).

Human Studies. Evidence for toluene-induced liver toxicity from occupational studies is limited and inconsistent. Eight men from a printing factory employing 289 workers exposed to toluene at concentrations of less than 200 ppm (further information on air toluene levels not reported) 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 >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. Serum AP values were significantly greater than controls in a group of 47 rotogravure workers occupationally exposed to a TWA toluene concentration of 11–47 ppm (midpoint 29 ppm) for 3–39 years than in controls (Svensson et al. 1992b). The difference in AP values remained significant when the data were corrected to eliminate nine workers who reported consumption of alcoholic beverages. 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). Serum ALT and AST levels were not

altered in printers and non-printers exposed to median toluene levels of 24 and 4.1 ppm toluene for at least 20 years, compared with unexposed referents (Gericke et al. 2001). Women working in a shoe factory for an average of >3 years and exposed to toluene concentrations that varied from 65 ppm (15–100 ppm) in winter to 100 ppm (10–200 ppm) in summer (average of winter and summer exposure, 83 ppm) 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 eight 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. A group of 47 Swedish paint industry workers exposed for >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 with nonexposed controls (Lundberg and Hakansson 1985). 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 1985), and this is a confounding factor for this study.

Several case studies have reported effects on the liver from toluene exposure, although the exposure concentration is generally unknown. 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). No effects on blood levels of bilirubin, AP activity, serum AST activity, or serum ALT activity were reported for two workers accidentally exposed to 1,862 ppm toluene for 2–3 hours; however, the liver was enlarged upon palpation in one man (Meulenbelt et al. 1990). In a case-series report of 20 patients with acute toluene intoxication, the average AST, AP, and gamma-glutamyl transferase (GGT) values were above the reference range; half of the patients evaluated had a previous history of drug abuse, including cocaine and marijuana, in addition to toluene inhalation (Camarra-Lemarroy et al. 2015).

Animal Studies. 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 ALT and AST (Tahti et al. 1983). Exposure of rats to 4,000 ppm toluene for 6 hours resulted in 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). Increased expression of markers of fibrosis (α-smooth muscle actin, collagen, glucocorticoid receptors, and leptin receptors) was observed in livers of male rats exposed to 1,500 ppm 4 hours/day for 7 days (Gotohda et al. 2009). Rats exposed to 1,500 ppm toluene, 4 hours/day for 7 days demonstrated increased markers of oxidative stress, including 8-Oxo-2'-deoxyguanosine (8-OH-dG) and superoxide dismutase in the liver; however, no significant changes in lipid peroxidase or 4-hydroxy-nonenal levels were seen (Tokunaga et al. 2003).

Intermediate exposure of animals to inhaled toluene has been associated with changes in liver weight, histology, or biochemistry in some, but not all, studies. Increased liver weights were reported for male mice exposed to 4,000 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). In other studies, 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 or mice exposed continuously to 150–400 ppm (Ikeda et al. 1986; Kjellstrand et al. 1985; Kyrklund et al. 1987) 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) or rats exposed to 3,000 ppm toluene 1 hour/day, 3 days/week, for 4–8 weeks (Dick et al. 2014). Several other studies have reported histological or biochemical changes associated with liver damage in animals after intermediate-duration inhalation exposure to toluene. These effects include: increased serum AP activity in male rats exposed to 300 ppm for 6 hours/day, 5 days/week for 4 weeks (Poon et al. 1994); centrilobular hepatocellular hypertrophy in male mice exposed to 2,500–3,000 ppm 6.5 hours/day, 5 days/week for 14 weeks (NTP 1990); enlarged hepatic sinusoids filled with blood, minimal hepatic

fibrosis (mean severity score of 0.78 on a scale of 0 for no fibrosis through 4 for complete cirrhosis), and increased apoptotic cells in livers in male rats exposed to 3,000 ppm 8 hours/day, 6 days/week for 12 weeks (Kanter 2012); and increased serum activities of ALT and AST (but not AP), hepatocyte degeneration, mild pericentral fibrosis, and increased levels of Bax protein (a marker of apoptosis) and apoptotic cells in livers of male rats exposed to 3,000 ppm 1 hour/day for 30 days (Tas et al. 2011, 2013a). No weight changes or histological abnormalities in the liver were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation in a multigenerational study (API 1985; Roberts et al. 2003).

In chronic studies, no exposure-related increased incidences of gross or histopathological liver lesions or changes in liver weight were found in rats exposed to toluene at 300 ppm 6 hours/day, 5 days/week for 2 years (CIIT 1980; Gibson and Hardisty 1983) or rats or mice exposed to up to 1,200 ppm 6.5 hours/day, 5 days/week for 15 months or 2 years (NTP 1990).

Renal Effects.

Overview. Studies of chronic toluene abusers, occupationally exposed workers, and laboratory animals have provided little support for irreversible kidney damage due to inhaled toluene. Chronic abuse of toluene can produce acidosis, but in most cases, renal dysfunction was transient, and normal function returned when exposure ceased (Baskerville et al. 2001; Camarra-Lemarroy et al. 2015; Dickson and Luks 2009; Goodwin 1988; Kamijo et al. 1998; Meulenbelt et al. 1990; Patel and Benjamin 1986; Tang et al. 2005). Studies of workers occupationally exposed to 100–200 ppm toluene, which assessed changes in tests of kidney function, have not shown consistent effects across studies (Askergren et al. 1981a, 1981b; Gericke et al. 2001; Gonzalez-Yerba et al. 2006; Nielsen et al. 1985; Stengel et al. 1998). Animal studies indicate that inhalation of toluene caused concentration-dependent kidney damage in rats, but only after repeated exposure to concentrations ≥600 ppm for at least 6 hours/day (Bruckner and Peterson 1981b; CIIT 1980; Gibson and Hardisty 1983; NTP 1990; Ono et al. 1996; von Oettingen et al. 1942).

Human Studies. Numerous cases have been reported where toluene abuse was associated with acidosis (Baskerville et al. 2001; Camarra-Lemarroy et al. 2015; Dickson and Luks 2009; Gerkin and LoVecchio 1998; Goodwin 1988; Jone and Wu 1988; Kamijima et al. 1994; Kamijo et al. 1998; Kaneko et al. 1992; Meulenbelt et al. 1990; Patel and Benjamin 1986; Tang et al. 2005; Tsao et al. 2011). Acidosis generally reflects the inability of the kidneys to maintain the pH balance of the blood due either to saturation of kidney transport of hydrogen ions or a defect in tubular function. Associated changes with toluene-

induced acidosis and renal tubular dysfunction are low serum levels of potassium (hypokaelemia) and phosphate (hypophostaemia) (Baskerville et al. 2001; Camarra-Lemarroy et al. 2015; Tang et al. 2005; Tsao et al. 2011). Oliguric renal failure was observed in 3/20 patients who died in a case-series report of acute toluene intoxication hospitalizations; rhabdomyolysis was considered a key factor in renal failure (Camarra-Lemarroy et al. 2015). 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. Recovery of normal acid-base balance after abuse was stopped also was reported in several other cases (e.g., Baskerville et al. 2001; Dickson and Luks 2009; Tsao et al. 2011). 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. An 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 74 unexposed control subjects (Stengel et al. 1998). Multiple regression analysis indicated a slight increase in N-acetyl-β-D-glucosaminidase urinary levels with increasing cumulative toluene exposure (+2% per 100 ppm x years), but this relationship was not apparent when 17 subjects with hypertension were excluded from the analysis.

Albumin excretion levels were significantly increased in a group of 134 workers exposed to 5–23 ppm styrene (52 plastic boat manufacturers), 80–106 ppm toluene (42 printers), or unspecified levels of xylene and toluene (40 paint manufacturers), compared with 48 unexposed referents (Askergren et al. 1981a). However, when the solvent-specific subgroups were analyzed separately, only the styrene-exposed workers varied significantly from control. β-2-microglobulin excretion levels where not significantly altered compared with controls when workers were analyzed together or by solvent-specific exposure group (Askergren et al. 1981a). Similarly, no significant changes in glomerular filtration rate between a group of 107 workers exposed to styrene (33 plastic boat manufacturers), toluene (34 printers), and xylene and toluene (40 paint manufacturers), compared with 48 unexposed referents (Askergren et al. 1981b). For the studies by Askergren et al. (1981a), a NOAEL for renal function in this assessment was determined to be the midpoint of the toluene exposure range in printers (93 ppm). Glomerular filtration rate and serum creatinine levels were also not altered in printers and non-printers exposed to median toluene levels of 24 and 4.1 ppm toluene for at least 20 years, compared with unexposed referents (Gericke et al. 2001).

An increased ($p<0.05$) prevalence of abnormally high urinary activities of N-acetyl-β-D-glucosaminidase (NAG) was observed in a group of 50 toluene-exposed shoe workers (exposure level unspecified), compared with 25 control subjects (26/50 versus 4/25; Gonzalez-Yerba et al. 2006). However, the prevalences of abnormally high urinary albumin excretion, another indicator of renal damage, were not significantly different between the exposed and control groups.

Animal Studies. The only renal effects reported in an acute-duration study were increased markers of oxidative stress, including 8-OH-dG and superoxide dismutase, in the kidneys of rats exposed to 1,500 ppm toluene, 4 hours/day for 7 days; however, no significant changes in lipid peroxidase or 4-hydroxy-nonenal levels were seen (Tokunaga et al. 2003). In intermediate-duration animal studies, histological changes in the kidney have not been observed consistently across studies. Reports of toluene-induced renal histological changes include renal casts in rats exposed to 600– 5,000 ppm 7 hours/day for 5 weeks (von Oettingen et al. 1942) and slight to mild necrosis of kidney tubules (5/8 versus 0/8 in controls) with increased kidney weights in male rats exposed to 2,000 ppm toluene (but not 600 ppm), 6 hours/day for 90 days (Ono et al. 1996). No exposure-related renal histological changes were observed in mice exposed to 4,000 ppm 3 hours/day for 8 weeks showing decreased absolute, but not relative, kidney weight (Bruckner and Peterson 1981b); mice and rats showing decreased absolute kidney weights following exposure to 12,000 ppm for 70 minutes/day, 5 days/week for 8 weeks (Bruckner and Peterson

1981b); female mice showing increased relative kidney weight following exposure to 1,250 ppm 6.5 hours/day, 5 days/week for 14 weeks (NTP 1990); male or female rats showing increased relative kidney weight after exposure to concentrations between 1,250 to 3,000 ppm 6.5 hours/day, 5 days/week for 15 weeks (NTP 1990); male or female rats exposed to 30 or 300 ppm 6 hours/day, 5 days/week for 4 weeks (Poon et al. 1994); or F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation in a multigenerational study (API 1985; Roberts et al. 2003).

In chronic-duration animal inhalation studies, evidence for exposure-related renal histological changes is not consistent across studies. No exposure-related renal histological lesions were found in rats exposed to 300 ppm 6 hours/day, 5 days/week for 2 years (CIIT 1980; Gibson and Hardisty 1983) or mice exposed to up to 1,200 ppm 6.5 hours/day, 5 days/week for up to 2 years (NTP 1990). In male and female rats in the 2-year NTP (1990) study, nearly all control and exposed rats showed nephropathy at sacrifice, but the mean severity score for nephropathy was statistically significantly ($p<0.05$) increased in the high-exposure group (1,200 ppm), compared with the controls (3.2 versus 2.8 in males and 2.7 versus 2.4 in females). In addition, the incidence of renal tubule casts increased with increasing exposure level in male rats: 1/50 in controls; 2/50 at 600 ppm; and 5/50 at 1,200 ppm (NTP 1990).

Endocrine Effects.

Human Studies. Evidence for endocrine effects in humans exposed to toluene is not consistent across studies and does not clearly identify toluene as an endocrine-disrupting chemical.

Serum levels of T3 were significantly increased in printers exposed to toluene for an average of 25 years (current median exposure level, 36 ppm), compared with unexposed controls (Svensson et al. 1992a). Serum levels of T4, TSH, and prolactin were not altered between exposed and referent groups; however, TSH levels were significantly inversely related with cumulative toluene exposure. Serum levels of prolactin were not changed in workers exposed to median toluene levels of 29 ppm for 3–39 years, compared with control subjects (Svensson et al. 1992b). Hypothyroidism was diagnosed in a 29-year-old man who had been sniffing glue containing toluene (concentration not specified) for 18 years (Hong et al. 1996).

An 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). However, no exposure-related changes in salivary cortisol levels were observed in volunteers exposed to a peak exposure of 200 ppm (measured pre- and postexposure); the peak exposure condition was obtained by slowly flooding the exposure chamber with toluene starting at a concentration of 0 ppm until the peak exposure of 200 ppm was obtained at 25 minutes and held constant for an additional 15 minutes (40-minute exposure) (Kobald et al. 2015).

Several studies of blood levels of reproductive hormones in repeatedly exposed workers or acutely exposed human subjects have not provided strong and consistent evidence of exposure-related effects. These studies are discussed in Section 3.2.1.5, Reproductive effects.

Workers exposed to a mixture of styrene (0–5.9 ppm), toluene (0–2.02 ppm), and xylene (0–32.65 ppm) demonstrated an increase in fasting insulin and glucose levels compared with unexposed controls (Won et al. 2011). A homeostasis model assessment of insulin resistance levels (HOMA-IR) was significantly higher in the exposed group. After adjustment for age, smoking, alcohol consumption, waist and hip circumference, weight, and regular exercise, fasting glucose levels were still significantly higher in the exposed group, but HOMA-IR was no longer significant. Adjusted analysis was not reported for fasting insulin levels. The results of this study are confounded by mixed exposure and its significance is therefore uncertain.

Animal Studies. Evidence for endocrine effects in animals following acute- or intermediate-duration inhalation exposure to toluene is not consistent across studies and does not clearly identify toluene as an endocrine-disrupting chemical.

Serum prolactin levels were significantly ($p<0.05$) increased (by 62% compared with control values) in male Sprague-Dawley rats, 17 days after exposure to 80 ppm toluene 6 hours/day, 5 days/week for 4 weeks (Von Euler et al. 1994). A companion study found no significant changes in serum prolactin levels in rats 29–40 days after exposure to 40, 80, 160, or 320 ppm by the same exposure protocol (Hillefors-Berglund et al. 1995).

Significant ($p<0.05$) 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 was associated with significantly increased serum levels of corticosterone (about 393 or 600% increased, compared with control) (Little et al. 1998). Thymus and adrenal weights in exposed rats were not significantly different from nonexposed control values.

Male rats exposed to 1,500 ppm 4 hours/day for 7 days showed significantly ($p<0.05$) increased adrenal weight (118.2% of control) and adrenocortical cell size (150.7% of control), but not adrenocortical cell number; increased serum levels of ACTH (151.5% of control) and corticosterone (176.1% of control); increased adrenal gland levels of mRNA for a steroid metabolism enzyme, cytochrome P450 side-chain cleavage (P450scc; 131.5% of control); and decreased body weight (92.1% of control) (Gotohda et al. 2005). This exposure scenario was shown to cause, in companion studies, neuronal damage and an increase in glucocorticoid receptor in the hippocampus, suggesting a possible link between the elevated serum levels of ACTH and corticosterone and elevated hippocampal glucocorticoid receptor mRNA (Gotohda et al. 2000a, 2000b, 2002).

No statistically significant ($p<0.05$) changes in serum levels of prolactin, LH, or FSH were reported in male Sprague-Dawley rats exposed to 500 ppm toluene 6 hours/day for 3 days or to 1,000 ppm 6 hours/day for 5 days, compared with values in nonexposed controls (Andersson et al. 1980). Mean serum level of corticosterone was significantly increased (162% of control value) in rats exposed to 500 ppm, but the mean level in rats exposed to 1,000 ppm rats (79% of control value) was not significantly different from the control mean (Andersson et al. 1980).

No statistically significant ($p>0.05$) trends for changed levels of FSH, LH, corticosterone, growth hormone, or TSH with increasing exposure level were found in male Sprague-Dawley rats exposed to 80, 500, 1,500, or 3,000 ppm toluene 6 hours/day for 3 days (Andersson et al. 1983b). A significant ($p<0.01$) trend for increased serum prolactin levels with increasing exposure level was found. The mean prolactin level in rats exposed to 3,000 ppm rats was about 250% of the control value, whereas means in rats exposed to 80, 500 and 1,500 ppm were about 180, 150, and 160% of the control mean, respectively.

In pregnant Wistar rats exposed to 0 or 1,500 ppm 6 hours/day on GDs 7–20, significantly ($p<0.05$) depressed serum levels of corticosterone were found in exposed dams when measured on GD 14 (88% of control) and GD 18 (60% of control) (Hougaard et al. 2003). Other endocrine end points were not measured in this study.

Female, but not male, 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). In a multigenerational study, no histological abnormalities on the pancreas, adrenal, or thyroid glands were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation (API 1985; Roberts et al. 2003). 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.

In a multigenerational study, no effects on the skin were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation (API 1985; Roberts et al. 2003).

Ocular Effects. Humans exposed for 6–8 hours to toluene concentrations of ≥100 ppm 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 toluene (Campagna et al. 2001; Cavalleri et al. 2000; Muttray et al. 1997, 1999; Zavalic et al. 1998a, 1998b, 1998c). These studies are discussed in the Section 3.2.1.4, 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; Roberts et al. 2003). No exposure-related lesions were observed in rats exposed at concentrations up to 1,000 ppm 6 hours/day, 5 days/week for 4 or 13 weeks during ophthalmic examinations conducted 2–6 weeks or 1 year postexposure (Boyes et al. 2016). Results from studies of visual impairment in rats after acute or intermediate inhalation exposure to toluene are discussed in Section 3.2.1.4, Neurological Effects.

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

Findings regarding body weight effects in animals are inconsistent. 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); 1,500 ppm, 4 hours/day for 7 or 20 days (Gotohda et al. 2005; Ishigami et al. 2005); 2,000 ppm, 8 hours/day, 7 days/week for 11 or 23 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); 10,000 ppm 1 hour/day, 3 days/week for 4 weeks (Dick et al. 2015); 12,000 ppm 70 minutes/day, 5 days/week for 8 weeks (Bruckner and Peterson 1981b); and 2,500 ppm, 6.5 hours/day for 15 weeks (NTP 1990). Significantly decreased body weight gain was observed in male rats continuously exposed to 200 or 400 ppm for 30 days (Ikeda et al. 1986). Decreased body weights were seen in female and male mice exposed 6.5 hours/day for 14 weeks 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). In contrast, no exposure-related effects on body weights were observed in rats or mice exposed to 1,000 ppm toluene for 6 hours/day, 5 days/week, for 3–13 weeks (API 1997; Beasely et al. 2010, 2012; Boyes et al. 2016; Horiguchi and Inoue 1977), mice exposed to up to 6,000 ppm toluene 30 minutes/day for 40 days (Bowen and McDonald 2009), rats exposed to 3,000 ppm toluene 1 hour/day, 3 days/week for 4–8 weeks (Dick et al. 2014), rats exposed to up to 2,000 ppm toluene 6 hours/day for 90 or 95 days (API 1985; Ono et al. 1996; Roberts et al. 2003), or rats or mice exposed to up to 1,200 ppm toluene 6–6.5 hours/day for 15 months or 2 years (CIIT 1980; Gibson and Hardisty 1983; NTP 1990); mean body weights in exposed animals were within 10% of control means.

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

3.2.1.3 Immunological and Lymphoreticular Effects

Overview. Human studies of immunological end points in toluene-exposed subjects do not identify consistent or strong evidence for toluene effects on immune system end points such as counts of blood lymphocytes or levels of blood immunoglobulins (Little et al. 1999; Pelclova et al. 1990; Stengel et al. 1998; Yin et al. 1987). Human studies also provide conflicting evidence regarding the potential role of occupational exposure to toluene and development of autoimmune disorders (Diot et al. 2002; Chaigne et al. 2015; Marie et al. 2014). In animal studies, evidence for toluene effects on the immune system includes the finding of decreased survival following respiratory infection by *S. zooepidemicus* in a study of mice exposed for 3 hours to toluene concentrations as low as 2.5 ppm, but not 1 ppm (Aranyi et al. 1985). No evidence for exposure-related adverse changes in weight or histology of the spleen or thymus

has been reported in animals exposed by inhalation for intermediate (NTP 1990; Poon et al. 1994; von Oettingen et al. 1942) or chronic durations (NTP 1990). In a series of studies from a single laboratory, intermediate-duration inhalation exposures of normal or allergy-challenged [ovalbumin (OVA)-immunized and challenged] mice to toluene concentrations <100 ppm have been reported to modulate immune system end points, but mechanistic understanding of the observed changes is insufficient to determine their biological adversity (Fujimaki et al. 2009a, 2009b, 2010, 2011; Liu et al. 2010; Takeda et al. 2013; Win-Shwe et al. 2007a, 2010a, 2010b, 2012a, 2012b; Yamamoto et al. 2009).

Occupational Human Exposure Studies. No differences in serum IgG, IgA, or IgM values were noted between a group of 42 rotogravure printers exposed to concentrations of 104–1,170 ppm (midpoint, 637 ppm) for an average of 13 years and a group of 16 office and technical workers exposed to toluene concentrations ranging from 2.1 to 4.3 ppm 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 not significantly elevated compared to unexposed controls, but a dose-response relationship was observed between cumulative toluene exposure and IgE levels (Stengel et al. 1998). Relative lymphocyte counts in blood (but not absolute counts) were significantly decreased in a group of workers who were involved in shoemaking, printing, or audio equipment production and were predominately exposed to toluene (Yin et al. 1987). Mean toluene exposures were 41 ppm for females and 46 ppm for males over an average of 82 months.

A number of other studies have examined immune-related end points in toluene exposed workers, but the results of these studies are confounded by mixed exposure to other solvents. 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). No effects on natural killer cell activity or serum levels of IL-2 or γ-IFN were observed in shoe factory workers, compared with a control group of workers not exposed to solvents (Yucesoy et al. 1999). Mean 8-hour TWA breathing zone concentrations of solvents in shoe workplaces were about 58 ppm for

n-hexane, 27 ppm for toluene, and 11 ppm for methyl ethyl ketone. Mean concentrations of hippuric acid and 2.5-hexadione in exposed workers were about 2- and 3-fold higher than concentrations in nonexposed controls (Yucesoy et al. 1999). Multiple linear regression analysis showed significantly ($p<0.05$) decreased production of TNF, but not IL-10 or IL-12, in cultured peripheral blood mononuclear cells from a high accumulated exposure group of paint factory workers, compared with a low-exposure group (Haro-Garcia et al. 2012). Mean 8-hour TWA concentrations of solvents in the factory were about 12 mg/m^3 for benzene, 28 mg/m^3 (7 ppm) for toluene, and 19.5 mg/m^3 for xylene. Mean urinary concentrations of S-phenylmercapturic acid were about 3.4 and 2.4 µmol/mol creatinine in the high- and low-exposure groups, respectively (Haro-Garcia 2012). Workers exposed to a mixture of styrene (0–5.9 ppm), toluene (0–2.02 ppm), and xylene (0–32.65 ppm) demonstrated a significant increase in serum TNFα compared with unexposed controls (Won et al. 2011). No change was observed in IL-1β or IL-6. However, alterations in serum TNFα were significantly associated with urinary phenylglyoxylic acid levels, not urinary hippuric acid levels, indicating that alterations are associated with styrene exposure rather than toluene exposure (Won et al. 2011).

A few case-control studies have evaluated potential occupational risk factors for autoimmune diseases using retrospective exposure analysis based on work history and job-exposure matrices. Chaigne et al. (2015) reported a significantly increased risk of the autoimmune disorder primary Sjögren's syndrome (175 cases, 250 controls) with history of occupational toluene exposure (OR 4.18, 95% CI 1.41–12.43). The risk was also significantly increased using final toluene exposure scores, calculated based on the probability and intensity of exposure (estimated from a job-exposure matrix) and the reported duration of exposure (OR 4.69, 95% CI 1.42–15.45). Diot et al. (2002) similarly reported a significantly increased risk of systemic sclerosis (80 patients, 160 controls) with history of occupational toluene exposure (OR 3.44, 95% CI 1.09–10.90). However, the risk was not significantly increased with a "high" final cumulative toluene exposure score (>1), calculated based on the probability, intensity, and frequency of exposure (estimated from a job-exposure matrix) and the reported duration of exposure (OR 6.19, 95% CI 0.63–60.53). In another case-control study of systemic sclerosis (100 cases, 300 controls), the risk of systemic sclerosis was not increased with a history of any occupational exposure to toluene (OR 2.04, 95% CI 0.41–11.06) or a high cumulative toluene exposure score (OR 4.59; 95% CI 0.52–55.61) (Marie et al. 2014).

Controlled Exposure Human Studies. Serum levels of T-cell proteins specific for a benzoic acid-human serum albumin antigen were elevated following a 20-minute exposure to 15 ppm in a group of 20 subjects who were clinically sensitive to toluene, compared with a group of 16 subjects who were not sensitive to

toluene (Little et al. 1999). No significant differences in levels of IgG specific to the antigen were found between the groups following exposure to toluene. These findings may indicate sensitization to benzoic acid, a metabolite of toluene.

Animal Studies. Single 3-hour exposures of mice to 2.5, 10, 25, 50, 100, 250 or 500 ppm toluene, immediately before inhalation exposure to aerosols of *S. zooepidemicus*, significantly ($p<0.05$) decreased the ability to survive the respiratory infection (Aranyi et al. 1985). For example, the respective mortality percentages in exposed and control groups were: 500 ppm, 56.8 and 20.7%; 50 ppm, 25.0 and 6.4%; and 2.5 ppm, 26.1 and 12.6%. A 3-hour exposure to 1.0 ppm toluene once or for 5 or 20 consecutive days did not significantly change the number of animals that died following experimental exposure to *S. zooepidemicus*, compared with control mice exposed only to aerosols of *S. zooepidemicus*. In another assay, mice were exposed to varying concentrations (0,1, 2.5, 5, 10, 25, 50, 100, 250, or 500 ppm) of toluene for 3 hours, followed immediately by inhalation exposure to aerosols of ^{35}S-labeled *Klebsiella pneumonia* and determination of the percent of bacteria killed in the lungs 3 hours later (Aranyi et al. 1985). Exposure before infection to toluene at all concentrations except 5 and 50 ppm significantly ($p<0.05$) decreased pulmonary bactericidal activity, but no clear dose-response relationship was apparent. For example, the percentages of bacteria killed in 3 hours in respective exposed and control groups were: 500 ppm, 85.9 and 71.9%; 100 ppm, 87.3 and 73.4%; 50 ppm, 82.3 and 84.1%; and 2.5 ppm, 84.6 and 79.2%. A 3-hour exposure to 1.0 ppm toluene once or for 5 or 20 consecutive days before infection did not consistently produce significant ($p<0.05$) changes in pulmonary bactericidal activities against *K. pneumonia*. The results show most clearly that acute exposure to toluene concentrations ≥2.5 ppm decreased the ability to survive following respiratory infection with *S. zooepidemicus*.

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 rats and mice exposed to toluene concentrations up to 3,000 ppm for 14–15 weeks (NTP 1990). In rats and mice exposed to concentrations of toluene up to 1,200 ppm, 6.5 hours/day for 2 years, an increased incidence of pigmentation of the spleen was observed 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 GDs 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).

In a multigenerational study, no histological abnormalities were observed the thymus or spleen from F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation (API 1985; Roberts et al. 2003).

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 3-1 and plotted in Figure 3-1.

In a series of studies from a single laboratory, intermediate-duration inhalation exposures of normal or allergy-challenged (OVA-immunized and challenged) adult mice to toluene concentrations <100 ppm have been reported to modulate a number of immune system end points (Fujimaki et al. 2009a, 2009b, 2010, 2011; Liu et al. 2010; Takeda et al. 2013; Win-Shwe et al. 2007a, 2010a). Examined end points included BAL fluid cell counts, mRNA or protein levels for inflammatory cytokines, anti-microbial peptides, neurotrophins, neurotrophin receptors or other immune regulatory transcription factors in lung, BAL fluid or hippocampus, and plasma levels of antibodies. Another series of studies from the same laboratory examined immune system end points in postnatal day (PND) 21 male BALB/c mice exposed to 0, 5, or 50 ppm toluene 6 hours/day on GD 14 through 21 (Yamamoto et al. 2009) or on GDs 14–18, PNDs 2–6, or PNDs 8–12 (Win-Shwe et al. 2010b, 2012a, 2012b). Examined end points included brain, thymus, or spleen weights, plasma levels of antibodies, and mRNA levels for cytokines and transcription factors in the spleen or hippocampus. Current mechanistic understanding of the observed findings in these studies, which include evidence for non-monotonic dose-related changes in several end points (described in more detail in the following paragraphs), is insufficient to determine their possible biological adversity, and NOAEL or LOAELs from these studies are not included in Table 3-1 or Figure 3-1.

In allergy-challenged male C3H/HeN mice exposed nose-only to 0, 9, or 90 ppm 30-minutes on days 0, 1, 2, 7, 14, 21, and 28, significantly ($p<0.05$) changed numbers of macrophages were found in BAL fluid samples from mice exposed to 9 ppm (~50% increased over non-exposed control) and 90 ppm (~25% decreased over control) (Win-Shwe et al. 2007a). Significantly increased numbers of macrophages also were found in BAL fluid in normal C3H/HeN mice exposed to 9 ppm (~130% increased) and 90 ppm (~260% increased). No significant exposure-related changes were found in other cellular end points in BAL fluid from exposed normal or allergy-challenged mice: numbers of total inflammatory cells, neutrophils, lymphocytes, or eosinophils. Levels of mRNAs for certain cytokines in lungs of allergy-challenged mice were significantly changed at 9 ppm (IL-5, ~100% increased) and 90 ppm (IFN-γ, ~70%

decreased), but no exposure-related changes were noted in mRNAs for IL-4 or IL-12. In allergy-challenged mice, significant ($p<0.05$) changes were found in plasma levels of total IgE at 90 ppm (~100% increased), anti-OVA IgE at 9 ppm (~500% increased), total IgG_1 at 9 and 90 ppm (~75% increased at each concentration), and anti-OVA IgG_1 at 9 ppm (~90% increased); no significant changes were found in plasma levels of total IgG_2 and anti-OVA IgG2. In allergy-challenged mice, toluene exposure led to several significant biochemical changes in the lungs, including increased plasma NGF at 9 ppm (~200% increased) and decreased lung levels of mRNA for brain-derived nerve factor (BDNF, ~40% decreased) and tropomyosin-related kinase A (Trk A, ~50% decreased) at 9 and 90 ppm; no significant exposure-related changes were found in lung levels of mRNAs for NGF, neurotrophin-3 (NT-3), or Trk B. The neurotrophins were measured in these studies to investigate a hypothesis that low-level toluene exposure may aggravate airway inflammation responses in allergy-challenged mice by modulating signaling pathways in neurological and immune systems (i.e., modulating neuroimmune cross talk).

In another study with allergy-challenged male C3H/HeN mice exposed nose-only to 0 or 9 ppm for 30 minutes on days 0, 1, 2, 7, 14, 21, and 28, significantly ($p<0.05$) increased total cell and macrophage counts (both ~200% increased) were found in BAL fluid from mice exposed to 9 ppm, compared with controls (Fujimaki et al. 2009a). No significant exposure-related changes were found in numbers of polymorphonuclear leukocytes and lymphocytes. Levels of BDNF in BAL fluid from mice exposed to 9 ppm were also significantly increased (~130%). Levels of NGF in BAL fluid were not increased in exposed mice, but plasma levels of NGF were significantly increased by about 120% above levels in non-exposed mice. Exposure-related effects were diminished by treatment with anti-CD4 antibody. The latter result suggests that the observed toluene-induced effects may involve CD4+ cells, which have been reported to produce BDNF and NGF.

In allergy-challenged male C3H/HeN mice exposed nose-only to 0, 9, or 90 ppm 30 minutes/day on days 0, 1, 2, 7, 14, 21, 28, 35, 42, 49, and 56, significant ($p<0.05$) exposure-related changes were found in lung levels of mRNAs for NGF (~70% increased over nonexposed controls), the NGF receptor, Trk A (~100% increased), CCL2 (~140% increased), and CCL3 (~90% increased) at 9 ppm, but not at 90 ppm (Fujimaki et al. 2009b). No significant exposure-related changes were found in lung levels of mRNA for p75 neurotrophin receptor (p75NTR). Light microscopy of lungs from allergy-challenged mice exposed to 9 ppm showed peribronchial inflammation with eosinophil infiltration, but incidences of mice with this condition were not reported by the study authors. In mice that were not allergy challenged ("unchallenged" mice), exposure to 9 ppm led to slight hyperplasia of bronchial epithelia without accumulation of macrophages and neutrophils. Immunohistological examination for NGF-positive cells

showed a “few” NGF-positive bronchial epithelial cells in unchallenged mice exposed to 9 ppm toluene and “marked increase” in allergy-challenged mice exposed to 9 ppm toluene. The study report did not mention histological findings for unchallenged or allergy-challenged mice exposed to 90 ppm.

Similarly, C3H/HeN mice exposed nose-only to 0 or 9 ppm for 30 minutes/day on days 0, 1, 2, 7, and 14 or days 0, 1, 2, 7, 14, 21, 28, 35, 42, and 49 demonstrated qualitative damage to the tracheal epithelial structure in exposed groups with >50% of the epithelial cells in the trachea being “remarkably damaged” in mice exposed up to day 49 (Takeda et al. 2013). Again, incidences of mice with these findings were not reported by the study authors. The allergy-challenged group did not differ from the toluene-only group. No damage was observed with a single 30-minute exposure to 9 ppm, and no lymphocyte infiltration was observed in any group. This study also examined protein expression of biophylaxis factors in the lungs, including toll-like receptor 2 (TLR2), α defensin (NP-3), and β defensins (BD 1-4). BD-2 was significantly decreased with exposure until day 14 or 49 (~10 and 50%, respectively) while BD-4 was significantly increased (~80%) with the shorter exposure group, but significantly decreased (~35%) with longer exposure. No changes were observed in NP-3, BD-1, BD-3, or TLR2 levels. Protein expressions in allergy-challenge mice were not reported.

Thymus cells, but not spleen cells, from normal male C3H/HeN mice exposed to 50 ppm 6 hours/day, 5 days/week for 3 weeks (in inhalation chambers) showed enhanced proliferation in culture (measured by rate of thymidine incorporation into DNA) by concanavalin A (Con A), compared with cells from nonexposed mice (Liu et al. 2010). Thymocytes from toluene-exposed mice also showed increased levels of the cytokine, IL-2, in response to Con A, and enhanced DNA-binding activities of transcription factors involved in IL-2 production (NF-κB, STAT5, and NF-AT), compared with thymocytes from non-exposed mice (Liu et al. 2010).

Cultured spleen cells from male B10.BR mice exposed to 50 ppm toluene 6 hours/day, 5 days/week for 6 weeks in inhalation chambers showed significantly enhanced LPS-stimulated proliferation in normal mice and decreased LPS-stimulated proliferation in allergy-challenged, compared with spleen cells from B10.BR mice exposed to 0 or 5 ppm toluene (Fujimaki et al. 2010). In contrast, cultured spleen cells from normal or allergy-challenged male C57Bl/10 mice exposed to 0, 5, or 50 ppm by the same protocol for 6 weeks showed no significant ($p>0.05$) difference in Con A- or LPS-stimulated proliferation. Spleens from allergy-challenged B10.BR mice exposed to 5 ppm showed significantly increased mRNA levels, compared with controls, for transcription factors Foxp3, STAT5, and STAT6, but no changes in

these transcription factors were apparent in spleens from normal or allergy-challenged B10.BR mice exposed to 50 ppm or in normal or allergy-challenged C57Bl/10 mice (Fujimaki et al. 2010).

Levels of hippocampal mRNAs for two neurotrophins and their receptors (NGF, Trk A, BDNF, and Trk B) were significantly ($p<0.05$) increased in normal male C3H/HeN mice exposed to 500 ppm 6 hours/day, 5 days/week for 6 weeks in inhalation chambers, but not after exposure to 5 or 50 ppm (Win-Shwe et al. 2010a). In allergy-challenged male C3H/HeN mice, hippocampal mRNA levels for NGF were significantly increased at 50 ppm, but not at 5 or 500 ppm. Exposure of normal male BALB/c and C57BL/10 mice by the same protocol to 5, 50, or 500 ppm did not produce significant ($p>0.05$) changes in these end points, except for increased mRNA for BDNF in normal BALB/c mice exposed to 500 ppm. The results demonstrate differences among mouse strains in responsiveness of these end points to toluene exposure.

Significantly ($p<0.05$) increased number of total cells in BAL fluid samples (~50% increased over the non-exposed control value) was observed in allergy-challenged male C3H/HeN mice exposed to 50 ppm toluene 6 hours/day, 5 days/week for 6 weeks, but no significant changes were observed in allergy-challenged mice exposed to 5 or 500 ppm, or to allergy-challenged mice after 3 weeks of exposure to any of the three tested concentrations (5, 50, or 500 ppm; Fujimaki et al. 2011). No significant changes were found in numbers of other cell types found in BAL fluid (macrophages, lymphocytes, neutrophils, eosinophils) at any of the three tested concentrations in allergy-challenged mice after 3 or 6 weeks of exposure. No significant ($p>0.05$) exposure-related changes in BAL fluid cell counts were noted in normal mice (not allergy-challenged) exposed to 5, 50, or 500 ppm, compared with normal controls. Other effects reported in allergy-challenged mice exposed to 50 ppm included increased hypertrophy and hyperplasia in bronchial epithelium and mucus secretion in lungs without accumulation of alveolar inflammatory cells (incidence data not reported by the study authors) and significantly increased lung levels of fibronectin (35% increased compared with allergy-challenged mice without toluene exposure). These findings were not observed in normal mice exposed to 50 ppm, compared with normal controls. The report did not mention histological findings in lungs of normal or allergy-challenged mice exposed to 5 or 500 ppm. Significantly ($p<0.05$) increased splenic mRNA levels for immune regulatory transcription factors (STAT3, STAT4, STAT5, STAT6, GATA3, Foxp3, IL-4, and IL-12) were measured in allergy-challenged mice exposed to 50 ppm; mRNA levels for most of these transcription factors in spleens were not significantly different in allergy-challenged mice exposed to 5 or 500 ppm, compared with allergy-challenged mice without toluene exposure. The only significant ($p<0.05$) change in levels of plasma immunoglobins in exposed allergy-challenged mice was for increased total IgG_1 at 50 ppm (~90%

increased); no significant exposure-related changes were found in levels of total IgE, IgG_{2a}, or antigen-specific antibodies (anti-OVA IgE, anti-OVA IgG_1, anti-OVA $IgG_{2a.}$). The authors concluded that although exposure to 50 ppm toluene increased the expression of transcription factors in the spleen and plasma levels of total IgG_1, the overall results "did not show a strong correlation between toluene exposure and exacerbation of allergic responses."

Yamamoto et al. (2009) examined spleen weight, plasma antibody levels, and splenic mRNA levels for cytokines and transcription factors in PND 21 male mice exposed to 0, 5, or 50 ppm 6 hours/day on GD 14 through PND 21 with or without exposure to peptidoglycan (PGN, a putative suppressor of type 2 humoral allergic responses). Findings for PND 21 mice exposed to toluene without peptidoglycan exposure were:

- increased absolute (29% increased) and relative (24% increase) spleen weight at 50 ppm, compared with mice exposed to 0 or 5 ppm (see Table 3-2);
- significantly decreased plasma levels of IgE and IgG2a antibodies at 50 ppm and significantly increased levels of IgG1 antibodies at 5 and 50 ppm; and
- significantly decreased splenic mRNA levels for transcription factors T-bet, GATA-3, and Foxp, but no exposure-related changes in splenic mRNA levels of cytokines IFN-γ, IL-12, IL-4, or IL-5.

When the dams and offspring were exposed to PGN several times during gestation and through PND 21, a different pattern of findings emerged in PND 21 male offspring:

- increased absolute (≥32% increase) and relative (≥35% increase) spleen weights in all PGN+toluene groups, compared with 0-ppm toluene alone controls, but no significant differences among the PGN+toluene groups (see Table 3-2);
- no significant differences in IgE levels among PGN+ toluene groups; significantly decreased plasma levels of IgG1 at PGN+50 ppm and IgG2a at PGN+5 ppm and PGN+50 ppm, compared with PGN+0 ppm controls;
- significantly decreased splenic mRNA levels for Foxp3 at PGN+5 ppm and PGN+50 ppm, compared with PGN+0 ppm, but no exposure-related changes in splenic mRNA levels of cytokines IFN-γ, IL-12, IL-4, IL-5, T-bet or GATA-3 among PGN+toluene groups.

This study found toluene-induced increased spleen weight in offspring exposed to 50 ppm toluene alone, but no apparent response of spleen weight to toluene with co-exposure to PGN, a suppressor of type 2 humoral allergic responses. Mechanistic understanding is insufficient to determine the biological adversity of the observed pattern of changes in plasma immunoglobulins and splenic mRNA levels for transcription factors related to type 1 and type 2 immune responses in the spleen.

Table 3-2. Mean Spleen Weight (SE) for PND 21 Mice (n=6/Group) Exposed to Toluene 6 Hours/Day on GD 14 Through PND 21 With and Without Exposure to PGN

Exposure group	Spleen weight (mg)	Relative spleen weight (% spleen: body weight)
0 ppm	74.73 (3.63)	0.81 (0.043)
5 ppm	68.75 (3.10)	0.71 (0.028)
50 ppm	96.40 (5.84)[a]	1.01 (0.054)[b]
0 ppm + PGN	105.03 (5.39)[b]	1.16 (0.055)[b]
5 ppm + PGN	105.68 (3.65)[a]	1.15 (0.037)[a]
50 ppm + PGN	98.62 (5.31)	1.09 (0.058)

[a]p<0.01, compared with 5-ppm toluene group, as reported by study authors.
[b]p<0.01, compared with 0-ppm toluene group, as reported by study authors.

GD = gestation day; PGN = peptidoglycan; PND = postnatal day; SE = standard error

Source: Yamamoto et al. 2009

Win-Shwe et al. (2010b) examined weights of brain, lung, thymus, and spleen, and hippocampal mRNA levels for neurotrophic factors, inflammatory cytokines, astrocyte marker GFAP, microglia marker Iba1, and several other transcription factors in PND 21 male mice exposed to 0, 5, or 50 ppm 6 hours/day on GDs 14–18, PNDs 2–6, or PNDs 8–12. No significant ($p>0.05$) exposure-related changes were found in body weight or weights of brain, lung, thymus, or spleen. Statistically significant changes (compared with nonexposed controls) were reported in hippocampal mRNA levels for the following neuroimmune factors in PND 21 male mice:

- increased NGF at 50 ppm with PND 2–6 exposure and at 5 ppm (but not 50 ppm) with PND 8–12 exposure;
- increased BDNF at 5 and 50 ppm with PND 8–12 exposure;
- increased CCL3 at 5 ppm with PND 8–12 exposure;
- increased TNF-α at 50 ppm with PND 2–6 exposure, and at 5 ppm with PND 8–12 exposure;
- increased GFAP at 5 ppm with PND 8–12 exposure;
- increased Iba1 at 50 ppm with GD 14–18 exposure, and at 5 ppm with PND 8–12 exposure;
- increased HO-1 at 50 ppm with PND 2–6, and 5 ppm with PND 8–12;
- increased TLR4 at 5 ppm with PND 8–12 exposure; and
- increased NF-κB at 50 ppm with PND 2–6, and at 5 and 50 ppm with PND 8–12 exposure.

Most of the examined neuroimmune factors were upregulated at 5 ppm (but not 50 ppm) with PND 8–12 exposure. Of the 10 factors examined, only one (CCL2) was not upregulated. It is unclear why exposure to 50 ppm in this period did not upregulate most of the factors upregulated by 5 ppm with PND 8–12 exposure. Exposure during GDs 14–18 upregulated only GFAP at 5 ppm and Iba1 at 50 ppm, whereas exposure during PNDs 2–6 upregulated only NGF, TNF-α, HO-1, and NF-κB at 50 ppm.

Win-Shwe et al. (2012a) examined lung, spleen, and thymus weights, plasma levels of antibodies, percentage distribution of splenic T lymphocytes, and splenic mRNA levels for inflammatory cytokines and transcription factors in PND 21 male mice exposed to 0, 5, or 50 ppm 6 hours/day on GDs 14–18, PNDs 2–6, or PNDs 8–12. The only significant ($p<0.05$) exposure-related changes found in body weight or weights of thymus, left lung, or spleen were decreased weight of thymus and spleen at 5 ppm (but not 50 ppm) with PND 8–12 exposure. The magnitudes of these decreases were not reported. Statistically significant changes ($p<0.05$, compared with nonexposed controls) reported in blood and splenic immune system end points in PND 21 male mice included:

- decreased plasma levels of total IgG2a antibodies at 5 ppm (but not 50 ppm) with PND 2–6 exposure, and increased levels at 5 ppm (but not 50 ppm) with PND 8–12 exposure;
- decreased plasma levels of total IgG1 antibody at 5 and 50 ppm with GDs 14–18 and PND 8–12 exposure, and at 50 ppm with PND 2–6 exposure;
- decreased percentage CD+4 splenic T lymphocytes at 50 ppm with PND 2–6 and 8–12 exposure;
- decreased percentage CD8+ T lymphocytes at 50 ppm with PND 8–12 exposure;
- decreased IL-12 mRNA levels in spleen at 5 and 50 ppm with PNDs 2–6 and 8–12 exposure; and
- decreased T-bet and Foxp3 mRNA levels in spleen at 5 and 50 ppm with PND 2–6 and 8–12 exposure.

No significant exposure-related changes were found in IFN-γ or GATA3 mRNA levels in spleen.

Exposure during PNDs 8–12 produced changes in a greater number of examined end points in PND 21 mice than exposure during GDs 14–18 (decreased plasma total IgG1 at 5 and 50 ppm) or PNDs 2–6 (decreased IgG2a antibodies, IgG1 antibodies, and percentage of CD+4 lymphocytes in spleen at 5 ppm, and decreased splenic IL-12, T-bet, and Foxp3 mRNA at 5 and 50 ppm) (Win-Shwe et al. 2012a). End points modulated with PND 8–12 exposure were:

- decreased thymus and spleen weight at 5 ppm;
- increased IgG2a antibodies at 5 ppm;
- decreased plasma IgG1 antibodies and decreased splenic mRNA levels for IL-12, T-bet, and Foxp3 at 5 and 50 ppm; and
- decreased percentage of CD+4 and CD8+ lymphocytes in the spleen at 50 ppm.

Following the same gestational and postnatal exposure protocol, mRNA levels of neurotrophic factors (NGF, BDNF), pro-inflammatory cytokines (CCL2, CCL3, TNF-α), NGF transcription factor NF-kB, neurogenesis modulator toll-like receptor 4 (TLR4), oxidative stress marker HO-1, astrocyte marker GFAP, and microglia marker Iba-1 were determined in the hippocampus of offspring on PND 21 (Win-Shwe et al. 2012b). Significantly increased mRNA levels (~133-600%) were reported for NGF, BDNF, TNF-α, CCL3, NF-kB, TLR4, GFAP, Iba-1, and HO-1 in the 5 ppm group exposed from PND 8 to 12, NGF, TNF-α, NF-kB, and HO-1 in the 50 ppm group exposed from PND 2 to 6, and NF-kB in the 50 ppm group exposed from PND 8 to 12. No mRNA alterations were observed with exposure from GD 14 to 18, compared with controls. No changes were found in neonatal body weight or relative organ weights of the brain, thymus, lung, or spleen at any exposure in this study.

3.2.1.4 Neurological Effects

Overview. 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, permanent damage to the central nervous system, and death (Aydin et al. 2002, 2003; Byrne et al. 1991; Caldemeyer et al. 1996; Camarra-Lemarroy et al. 2015; Capron and Logan 2009; Deleu and Hanssens 2000; Devathasan et al. 1984; Filley et al. 1990; Ryu et al. 1998; Gupta et al. 2011; Hormes et al. 1986; Hunnewell and Miller 1998; Ikeda and Tsukagoshi 1990; Kamran and Bakshi 1998; King et al. 1981; Kiyokawa et al. 1999; Kucuk et al. 2000; Maas et al. 1991; Maruff et al. 1998; Meulenbelt et al. 1990; Miyagi et al. 1999; Nomura et al. 2016; Papageorgiou et al. 2009; Poblano et al. 1996; Rosenberg et al. 1988a, 1988b, 2002; Ryu et al. 1998; Suzuki et al. 1983; Uchino et al. 2002; Yamanouchi et al. 1995). Self-reported neurological symptoms, reduced ability in tests of cognitive and neuromuscular function, and hearing and color vision loss have been observed in humans occupationally exposed to average concentrations of toluene ranging from 35 to 200 ppm (Abbate et al. 1993; Boey et al. 1997; Campagna et al. 2001; Cavalleri et al. 2000; Eller et al. 1999; Foo et al. 1990; Guzelian et al. 1988; Kang et al. 2005; Matsushita et al. 1975; Orbaek and Nise 1989; Ukai et al. 1993; Yin et al. 1987; Vrca et al. 1995, 1996, 1997a, 1997b; Zavalic et al. 1998a, 1998b, 1998c); several occupational studies identify NOAELs for these effects in the range of 20–187 ppm toluene (Deschamps et al. 2001; Gericke et al. 2001; Kang et al. 2005; Nakatsuka et al. 1992; Neubert et al. 2001; Schäper et al. 2003, 2004, 2008; Seeber et al. 2004, 2005; Zupanic et al. 2002). 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; Dick et al. 1984; Echeverria et al. 1991; Kobald et al. 2015; Rahill et al. 1996; von Oettingen et al. 1942).

Numerous studies in animals have also reported clinical signs of neurotoxicity and neurobehavioral alterations following acute exposure ≥100 ppm (Bowen and Balster 1998; Bushnell et al. 1985; Conti et al. 2012; Hogie et al. 2009; Huerta-Rivas et al. 2012; Kim et al. 1998; Kishi et al. 1988;Lopez-Rubalcava and Cruz 2000; Mullin and Krivanek 1982; Paez-Martinez et al. 2003, 2008; Taylor and Evans 1985; Tomaszycki et al. 2013; Wood and Colotla 1990; Wood et al. 1983); however, studies of rodents exposed for intermediate durations to concentrations as high as 1,000 ppm have not found strong and consistent evidence for exposure-related changes for these neurological end points (API 1997; Beasely et al. 2010, 2012; Miyagawa et al. 1995; NTP 1990; Rogers et al. 1999; Tahti et al. 1983; von Euler et al. 2000; von Oettingen et al.1942; Win-Shwe et al. 2010c, 2010d; Wood and Cox 1995). Following repeated abuse-

like exposures (>1,000 ppm), neurobehavioral alterations have been observed in several animal studies (Baydas et al. 2005; Bikashvili et al. 2012; Bowen et al. 2007; Castilla-Serna et al. 1991; Dashiniani et al. 2014; Duncan et al. 2012; Lorenzana-Jimenez and Salas 1990; Mattsson et al. 1990; Miyagawa et al. 1998; Oshiro et al. 2007; Pryor 1991; Pryor and Rebert 1992). Hearing loss has also been reported in laboratory animals exposed to 250–2,000 ppm toluene (Campo et al. 1997, 1998; Davis et al. 2002; Johnson 1992; Johnson and Canlon 1994; Johnson et al. 1988; Lataye and Campo 1997; McWilliams et al. 2000; Pryor and Rebert 1992; Pryor et al. 1984a, 1984b, 1991; Waniusiow et al. 2008, 2009).

Controlled Human Exposures

Overview. Acute exposure of healthy human subjects to toluene concentrations ≤50 ppm did not result in adverse neurological effects in a number of studies (Andersen et al. 1983; Lammers et al. 2005a; Muttray et al. 2005; Osterberg et al. 2000, 2003), and concentrations ≥75 ppm resulted in subtle neurological impairments in most studies (Andersen et al. 1983; Baelum et al. 1985; Dick et al. 1984; Echeverria et al. 1991; Gamberale and Hultengren 1972; Kobald et al. 2015; Rahill et al. 1996; von Oettingen et al. 1942). In contrast, studies examining individuals who were clinically sensitive to toluene (e.g., multiple chemical sensitivities) found minor neurological deficits at concentrations as low as 12–48 ppm (Little et al. 1999; Orbaek et al. 1998; Osterberg et al. 2003).

Healthy Volunteers. No significant, dose-related increases in subjective complaints of neurological effects and/or altered performance on neurobehavioral tasks were observed in a number of studies of healthy volunteers following exposure to toluene concentrations of 10–50 ppm for 2–6 hours (Andersen et al. 1983; Muttray et al. 2005; Osterberg et al. 2000, 2003). Similarly, 11 males exposed to 40 ppm toluene for 4 hours did not attain impaired scores on six neurobehavioral measures designed to assess mood, cognitive and motor functions, compared with pre-exposure scores (Lammers et al. 2005a). However, blood toluene levels, measured at 60, 120, 240, and 360 minutes after onset of exposure, were significantly associated with impaired performance on one motor performance test (finger tapping with alternating hands). Similar results were observed when the same 11 males were exposed to 110 ppm for four 30-minute exposures over 4 hours, 1 week later (Lammers et al. 2005a). Since blood toluene levels were only correlated with 1/6 neurobehavioral measures, and there were no significant differences between pre- and post-exposure scores, 40 ppm for 4 hours and 110 ppm for four 30-minute exposures are considered NOAELs for neurological effects. In other studies, volunteers exposed to 80 ppm toluene for 4 hours did not demonstrate impairments in choice reaction time, simple reaction time, color-word vigilance, or memory reproduction (Iregren et al. 1986; Olson et al. 1985).

In repeat-measure experiments, increased subjective complaints and deficits in neurological effects were observed between 75 and 200 ppm after exposure for 7–8 hours (Andersen et al. 1983; Echeverria et al. 1991; von Oettingen et al. 1942) and 300 ppm toluene after exposure for 20 minutes (Gamberale and Hultengren 1972). Concentration-related impairments were observed during the digit span, pattern recognition, pattern memory, and one-hole tests in 42 volunteers exposed to 0, 75, and 150 ppm toluene for 7 hours on sequential days, compared with their own pre-exposure measures (Echeverria et al. 1991). There were no exposure-related changes in the results on simple reaction time, mood (profile on mood scale), visual memory, hand-eye coordination, verbal short-term memory, finger tapping, reaction time, continuous performance test, or critical tracking test (Echeverria et al. 1991). Sixteen volunteers exposed to 0, 10, 40, and 100 ppm toluene for 6 hours on sequential days reported a statistically significant increase in the occurrence of headache, dizziness, and feeling of intoxication during the 100 ppm exposure, but not during the other concentrations (Andersen et al. 1983). Statistically significant exposure-related deficits were not observed in any of the eight performance tests designed to assess concentration, vigilance, motor coordination, visual perception, and cognitive function. However, for three of the tests (screw-plate [motor coordination], Landolt's ring [visual perception], and multiplication), there was a borderline significant deficit associated with toluene exposure ($p=0.05–0.1$), and the subjects felt that the tests were more difficult and strenuous during the 100 ppm exposure (Andersen et al. 1983). Following acute exposures to toluene at concentrations of 0, 50, 100, 200, 400, and 600 ppm for 8 hours and 800 ppm for 3 hours on subsequent days, three volunteers reported drowsiness and headache at 200 ppm that became more severe with exposure duration and at higher concentrations, characterized by impaired intellectual, psychomotor, and neuromuscular effects (von Oettingen et al. 1942). Concentration-dependent increases in simple and choice reaction times were measured in 12 volunteers exposed to increasing concentrations of toluene at 0, 100, 300, 500, and 700 ppm for 20-minute intervals (Gamberale and Hultengren 1972). Compared with 0 ppm values, these increases were significant at 300 ppm. Perception speed was also impaired, but not significantly different from 0 ppm values until exposure to concentrations of 700 ppm (Gamberale and Hultengren 1972).

Single-measure studies consistently report impaired performance on neurobehavioral tasks following exposure to 100 ppm for 6–8 hours (Baelum et al. 1985; Dick et al. 1984; Rahill et al. 1996). Forty-three solvent-exposed printers and 43 referents without history of exposure were exposed to 0 or 100 ppm toluene for 6.5 hours (Baelum et al. 1985). Within each group (printers and referents), about half of subjects were exposed to 100 ppm and the other half were exposed to normal air. Both printers and referents exposed to 100 ppm had increased complaints of fatigue, sleepiness, and feelings of

intoxication, compared with mean values for unexposed control subjects. Both exposed groups also showed significant decreases in manual dexterity (peg-board task), visual perception (Landolt's ring test), and color discrimination, compared with control values. No consistent differences were observed in neurobehavioral performance between exposed printers and exposed referents (Baelum et al. 1985). 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). Additionally, exposure to 100 ppm for 8 hours led to impaired performance on the visual-vigilance task, but not choice-reaction time or pattern recognition, compared with unexposed controls (Dick et al. 1984).

Additionally, volunteers exposed to a peak exposure of 200 ppm showed impaired performance in a visual attention task when an irrelevant distractor was given (compared with pre-exposure performance); the peak exposure condition was obtained by slowly flooding the exposure chamber with toluene starting at a concentration of 0 ppm until the peak exposure of 200 ppm was obtained at 25 minutes and held constant for an additional 15 minutes (40-minute exposure) (Kobald et al. 2015). This visual attention deficit was accompanied by a significant decrease in the N1 component of the event-related potential (ERP) measured in the parieto-occipital lobe via EEG (Kobald et al. 2015).

Susceptible Populations. Individuals with multiple chemical sensitivity or toxic encephalopathy had significantly higher self-reported scores of fatigue (headache, drowsiness, decreased concentration) during exposure to increasing toluene concentrations over 2 hours (0 ppm [20 minutes], 3 ppm [10 minutes], 6 ppm [10 minutes], 12 ppm [20 minutes], 24 ppm [10 minutes], 48 ppm [20 minutes], and 0 ppm [10 minutes]), compared with healthy referents (Orbaek et al. 1998; Osterberg et al. 2003). During these studies, psychomotor tests were performed before exposure and during the 12- and 48-ppm exposure periods. Both healthy referents and individuals with multiple chemical sensitivity showed increased response time in the reaction-time test (visual stimuli) following exposure, compared with pre-exposure scores (Osterberg et al. 2003). However, the increase was not dose-related in healthy individuals, and exposure-related impairments were not observed in the reaction time-inhibition test (with auditory alarm) or digit symbol test in either group (Osterberg et al. 2003). In a companion study, there were no observed psychomotor impairments in exposed individuals with toxic encephalopathy or healthy referents using the same protocol (Osterberg et al. 2000). A LOAEL of 48 ppm for the studies conducted by Orbaek et al. (1998) and Osterberg et al. (2003) was determined for susceptible individuals based on increased self-reported fatigue. A NOAEL could not be determined, as fatigue scores were not reported at individual exposure concentrations.

Little et al. (1999) reported that 20 subjects who were clinically sensitive to toluene showed statistically significant impairments in immediate and delayed prose memory (number of items recalled decreased 31%), the digit symbol test (number of correct items decreased 11%), and the letter cancellation test (percent correct decreased 5%) following a 15-minute exposure to 15 ppm toluene, compared with their pre-exposure scores. A near-significant 15% increase in reaction time was also observed ($p=0.06$). No significant differences between pre- and post-exposure values were found for focal length or the STROOP tests. An acute inhalation MRL of 2 ppm was calculated as described in the footnote in Table 3-1 and Appendix A, based on the minimally adverse LOAEL (15 ppm) for neurological effects in susceptible populations from the study by Little et al. (1999).

The highest NOAEL values and all LOAEL values for each reliable controlled human exposure study for neurological effects are recorded in Table 3-1 and plotted in Figure 3-1.

Occupational Exposure Human Studies

Overview. Several studies of workers repeatedly exposed predominantly to toluene in workplace air at concentrations ranging from 35 to 200 ppm have found evidence for increased incidence of self-reported neurological symptoms (Guzelian et al. 1988; Matsushita et al. 1975; Orbaek and Nise 1989; Ukai et al. 1993; Yin et al. 1987); performance deficits in neurobehavioral tests (Boey et al. 1997; Eller et al. 1999; Foo et al. 1990; Kang et al. 2005; Matsushita et al. 1975; Orbaek and Nise 1989); hearing loss (Morata et al. 1997); changes in auditory-evoked potentials and/or VEPs (Abbate et al. 1993; Vrca et al. 1995, 1996, 1997a, 1997b), and color vision loss (Campagna et al. 2001; Cavalleri et al. 2000; Zavalic et al. 1998a, 1998b, 1998c). Several occupational studies identify NOAELs for these effects in the range of 20–187 ppm toluene (Deschamps et al. 2001; Gericke et al. 2001; Kang et al. 2005; Nakatsuka et al. 1992; Neubert et al. 2001; Schäper et al. 2003, 2004, 2008; Seeber et al. 2004, 2005; Zupanic et al. 2002). A series of studies investigating a number of neurological end points in German rotogravure printers support a NOAEL of 45 ppm (Schäper et al. 2003, 2004, 2008; Seeber et al. 2004, 2005; Zupanic et al. 2002), and served as the basis for the chronic-duration inhalation MRL of 1 ppm for toluene (see footnote of Table 3-1, Section 2.3 and Appendix A).

Neurobehavioral Effects and Self-Reported Neurological Symptoms. The majority of studies show that workers (e.g., rotogravure printers) with long-term occupational exposure to toluene at concentrations <50 ppm did not report increased neurological symptoms or show impaired overall performance on neuropsychological or psychomotor tests (Eller et al. 1999; Gericke et al. 2001; Kang et al. 2005; Neubert

et al. 2001; Seeber et al. 2004, 2005; Zupanic et al. 2002). One study of 89 rotogravure and offset printers exposed to toluene for an average of 14 years reported a statistically significant correlation between current exposure levels (0–27 ppm) and impaired performance for the digit span memory test after adjustment for sex, age, synonym score, history of central nervous system diseases, alcohol consumption, psycho-active drug consumption in the last day, concentration in performing tests, and computer experiments (Chouanière et al. 2002). However, no significant association was observed between current exposure levels and scores for three other memory tasks (associate learning, associate recall, pattern memory), simple reaction time tests, symbol digit substitution tests, or self-reported neurotoxic symptoms. Using work history and historical exposure levels (0–179 ppm), a cumulative exposure index for toluene exposure was calculated for each individual (0–2,353 ppm-years [mean 392 ppm-years]). There were no significant correlations between cumulative exposure indices and performance on any psychomotor task or self-reported neurotoxic symptoms (Chouanière et al. 2002). Since current exposure levels were only correlated with 1/6 neurobehavioral measures, and there were no correlations between cumulative exposure indices and performance in any of the tests, 27 ppm is considered an occupational NOAEL for neurological effects.

In contrast, the majority of studies with average exposure estimates in the range of 70–100 ppm reported subtle, but statistically significant, performance deficits in neurobehavioral and psychomotor tests (Boey et al. 1997; Eller et al. 1999; Foo et al. 1990; Kang et al. 2005). Twenty workers from oil refinery, gravure printing, and rubber boat manufacturing exposed to 70–80 ppm toluene had significant impairments on the finger tapping and selective attention tests and nonsignificant impairments in the digit span backward test, compared with 21 referent workers with exposure <10 ppm (Kang et al. 2005). However, 30 workers exposed to 10–30 ppm toluene did not differ from the referent group (Kang et al. 2005). Based on these findings, NOAEL and LOAEL levels were estimated at 20 and 75 ppm, respectively. Abnormal tendon reflexes, decreased grasping power and agility of fingers, and generalized weakness were significantly decreased in 38 female shoemakers who were exposed to toluene concentrations that varied from 65 ppm (15–100 ppm) in winter to 100 ppm (10–200 ppm) in summer for an average of 40 months, compared with 16 controls (Matsushita et al. 1975). The LOAEL for neurological effects is estimated at 83 ppm (the average between summer and winter exposures). A strong correlation between impaired neurobehavioral performance and toluene exposure was seen in 30 female workers exposed to 88 ppm toluene, compared with 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) showed performance deficits on eight neurobehavioral tests, compared with a control group (average TWA exposure of 12.2 ppm). The exposed group showed significantly impaired verbal and nonverbal memory, compared with controls, as measured by the digit span and visual reproduction tests (Boey et al. 1997). Similarly, statistically significant impairments in tests for visuospatial function, number learning, and word recognition were observed in 49 rotogravure workers exposed to <20 ppm for 12 years plus >100 ppm for 4–27 years, compared with 19 referents (Eller et al. 1999). Thirty workers in the same plant only exposed to <20 ppm for 1–12 years did not differ from the referent group. Reported exposure level information was inadequate to determine NOAEL/LOAEL levels.

Increased self-reporting of neurological symptoms has also been associated with occupational toluene exposure at concentrations ≥40 ppm (Guzelian et al. 1988; Orbaek and Nise 1989; Ukai et al. 1993; Yin et al. 1997). Forty-four men and 57 women exposed to TWA concentrations of 46 and 41 ppm toluene, respectively, during shoemaking, printing, and audio equipment production had increased complaints of headaches, dizziness, and sleep difficulties, compared with 127 control subjects (Yin et al. 1987). Increased subjective complaints of neurological symptoms both during and after work was reported in a group of 452 workers exposed predominantly to toluene (geometric mean 24.7 ppm) compared with 517 unexposed referents (Ukai et al. 1993). Statistically ($p<0.01$) increased complaints during work included headache and floating sensation, and statistically increased complaints regarding symptoms outside work over the past 3 months included inability to concentrate, hearing loss, speech difficulties, anosmia, and reduced strength. When exposed workers were analyzed by toluene exposure levels (1–20, 21–50, 51–100, and >100 ppm), workers exposed to >100 and >50 ppm demonstrated significantly increased subjective complaints during and after work, respectively, compared with workers exposed to 1–20 ppm (Ukai et al. 1993). No increase in complaints was observed between the groups exposed to 1–20 and 21–50 ppm. The NOAEL and LOAEL values for increased self-reported neurological symptoms were determined to be 36 and 75 ppm for this assessment, based on the midpoints of the 21–50 and 51–100 ppm group (Ukai et al. 1993). 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).

Complaints of fatigue, recent short-term memory problems, concentration difficulties, and mood lability were significantly ($p<0.05$) increased in 30 rotogravure printers exposed to 11.6–453 ppm toluene for 4–43 years in two plants; taking the midpoints in the ranges of concentration estimates, a representative exposure concentration of 140 ppm was determined (Orbaek and Nise 1989). However, no significant

impairments were found between printers and controls in a battery of psychometric tests. In a 20-year follow-up of 12 printers and 19 controls from the cohort, there was no longer any significant ($p>0.05$) differences in incidences of reported subjective neurological symptoms (Nordling Nilson et al. 2010). In follow-up psychometric testing, the general performance deteriorated in 10/11 tests measured in both groups, compared with the initial assessments. In two measures (reasoning and associative learning), the deterioration in the exposed group was significantly greater than the referents. When the results of the 20-year follow-up were compared between the groups, the exposed group performed significantly worse on 2/11 tests (verbal memory and sustained attention). Taken together, these two studies indicate a LOAEL of 140 ppm for increased incidence of self-reported neurological symptoms in the initial assessment and statistically significant performance deficits on neurobehavioral tests during the 20-year follow-up study.

Consistent with the data presented above, a meta-analysis of the findings from studies of workers predominantly exposed to toluene concluded that occupational toluene exposure has a negative impact on neurobehavior at exposure concentrations >89 ppm (Meyer-Baron 2005). However, one study included in the meta-analysis reported no alterations in subjective neurological complaints or performance in neurobehavioral tests in 26 printer factory workers, 10 machine factory workers, and 36 laboratory workers who were exposed exclusively to toluene for at least 5 years (average 19.9 years), compared with 61 unexposed workers (Deschamps et al. 2001). Toluene air concentrations, measured by personal air monitoring over an entire work-shift, ranged from 9 to 83 ppm in the factories and from 184 to 467 ppm in the laboratories; however, the two groups were combined for analysis. Since neurological evaluations were conducted after 2 days without exposure (toluene in expired air <0.3 ppm), the lack of observed effects may indicate that impaired neurological effects do not persist after the solvent is eliminated from the body. Since the exposed groups were analyzed together, the NOAEL for this assessment is determined to be the average of the midpoints of the exposed ranges (midpoint factory, 46 ppm; midpoint laboratory, 325.5 ppm; average, 186 ppm).

A number of studies of humans chronically exposed to mixtures of solvents containing toluene provide supporting evidence for toluene-mediated general neurological and neurobehavioral effects, but concurrent exposure to other solvents limits the conclusions that can be drawn from the results. Impaired performance on multiple tasks measuring visual intelligence, cognition, memory, and perception were observed in 100 car painters exposed to mixed solvents including toluene, xylene, and various aliphatic hydrocarbons, alcohols, esters, ketones, and terpenes for 1–40 years, compared with 101 unexposed railway workers (Hanninen et al. 1976). In a group of 325 paint factory workers exposed to benzene,

toluene, xylene, n-hexane, methyl iso-butyl ketone, n-butyl acetate, and acetone for an average of 5 years, reduced ability in pattern comparison and memory tasks was correlated with combined solvent exposure (Tsai et al. 1997). A significant reduction in the Santa Ana dexterity test and a nonsignificant reduction in visual retention were observed in 40 female shoemakers exposed to toluene, methyl ethyl ketone, *n*-hexane, cyclohexane, dichloroethylene, trichloroethylene, benzene, and xylene, compared with 28 unexposed referents (Lee et al. 1998a). Self-reported neurological symptoms (headache, sweating, palpitation, lethargy, fatigue, nausea, vomiting, etc.) were increased in 92 paint manufacturers from two factories in eastern Thailand exposed to xylene (0.1–13.1 ppm) and toluene (0.5–48.7 ppm), compared with a referent group of 100 frozen food factory workers (Thetkathuek et al. 2015). Self-reported neurological symptoms were also increased in lacquer-ware manufacturers exposed to toluene, butyl-acetate, and ethyl-acetate, compared with nine unexposed referents (Tanaka et al. 2003). Similarly, increased self-reported neurological symptoms were significantly associated with a "solvent summation index" in 637 printers exposed to one or more of the following solvents: toluene (0–90.9 ppm), n-hexane (0.12–94.5 ppm), benzene (0–8.8 ppm), and isopropyl alcohol (0–374 ppm), compared with 125 unexposed referents (Yu et al. 2004). However, self-reported neurological symptoms in a furniture factory were not increased in painters/varnishers exposed to toluene, benzene, and xylene compared to unexposed referents (Mandiracioglu et al. 2011). A case-study of a worker exposed to paint thinner (containing 60% toluene; other solvents present included xylene, ethyl acetate, and butyl acetate) for 5 years in an unventilated workspace reports chronic headache, encephalitis, and white matter lesions; however, no neurological deficits were observed during a clinical exam (Kobayashi 2014). Another neurological effect reported with occupational mixed solvent exposure is increased postural sway in U.S. Air Force workers exposed to jet fuel (mean cumulative exposure 23.8 ppm toluene) (Smith et al. 1997).

Auditory System Effects

Overview. The limited number of occupational studies assessing hearing loss suggest that effects may occur at exposure concentrations >50 ppm, consistent with findings for other neurological end points. One occupational study indicated that exposure to an average of 122 ppm toluene may lead to hearing deficits (Morata et al. 1997), and altered brainstem auditory-evoked potential (BAEP) have also been reported following occupational exposure to 50–100 ppm (Abbate et al. 1993; Vrca et al. 1996, 1997a). However, no evidence for hearing loss was found in workers exposed to 45 ppm (Schäper et al. 2003, 2008). Hearing loss produced by toluene exposure may not be due solely to neurological damage, as animal studies indicate that solvent exposure damaged the OHCs in the cochlea that are responsible for amplifying incoming sound waves prior to signal transduction (Campo et al. 1997, 1998; Johnson and

Canlon 1994; Lataye and Campo 1997; Lataye et al. 1999; Waniusiow et al. 2008). However, a battery of audiological tests performed for a case-series report indicated that hearing loss observed in seven workers exposed to toluene and/or xylene was due to retrocochlear or central abnormalities, rather than cochlear damage (Gopal 2008).

Hearing Loss. 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 OR estimates for hearing loss were 1.76 times greater for each gram of hippuric acid per gram creatinine (95% CI 1.00–2.98).

Schäper et al. (2003, 2008) found no evidence of hearing loss in a longitudinal 5-year study in rotogravure printers exposed to TWA levels of 45 ppm (n=181), compared with a low-exposure group of end-process workers exposed to TWA levels of 10 ppm (n=152). Both groups had average noise exposure levels of 82 dB(A). Additionally, a stepwise regression analysis of a subgroup of 80 workers with toluene exposure ranging from 1 to 69 ppm for 3–38 years did not find a correlation between hearing loss and toluene exposure levels, urinary hippuric acid concentration, or length of employment (Schäper et al. 2003, 2008).

Multiple studies of humans chronically exposed to mixtures of solvents containing toluene provide supporting evidence for toluene-mediated hearing loss, but concurrent exposure to other solvents limits the conclusions that can be drawn about toluene toxicity. Reduced upper limit of hearing was observed in workers currently exposed to styrene, toluene, and methanol for at least 5 years during the production of plastic buttons or bathtubs (Morioka et al. 1999). In a large study of 1,319 aluminum workers, multivariate analysis identified exposure to solvents (toluene, xylene, and methyl ethyl ketone) as a risk factor for high-frequency hearing loss (Rabinowitz et al. 2008). High-frequency hearing loss was also associated with cumulative total solvent exposure in paint and varnish workers exposed to ethylbenzene, xylene, trimethylbenzene, and toluene (Sulkowski et al. 2002). Similarly, paint and lacquer workers exposed to solvent mixtures including toluene had increased incidences of hearing loss, compared to unexposed controls (Juárez-Pérez et al. 2014; Zamyslowska-Szmythke et al. 2011). Workers from

various industries exposed to a mixture of *n*-hexane and toluene and noise demonstrated increased mean hearing thresholds, compared with workers exposed to noise-only and unexposed referents (Sliwinska-Kowalska et al. 2005). Further analysis showed that workers exposed to the mixture of *n*-hexane and toluene and noise had a 5.3-fold increased risk of developing hearing loss (single hearing threshold in either ear exceeding 25 dB HL) compared with unexposed referents, after adjusting for age, gender, and noise exposure. A group of workers exposed to mixed solvents (white spirits, thinner, toluene, and xylene) who were 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).

BAEPs. Studies reporting altered BAEP following occupational exposure provide supporting evidence that toluene exposure may lead to hearing loss. Occupational exposure to an average of 97 ppm toluene for 12–14 years had an apparent effect on hearing in 40 rotogravure workers when 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 another study, BAEPs in 49 printers exposed to toluene for an average 20 years were found to be affected, with a significant decrease in all wave amplitudes and a significant increase in all wave latencies, compared with 59 unexposed referents (Vrca et al. 1996). Average toluene blood levels in printers and controls were 0.036 and 0.00096 mg/L, respectively. Based on blood levels, companion studies estimated averaged toluene exposure levels to be between 40 and 60 ppm (Vrca et al. 1995, 1997a). In the 49 exposed printers, increased wave latencies were significantly correlated with increased duration of exposure, except for the P2 wave (Vrca et al. 1997a).

Additionally, BAEPs in 77 paint factory workers exposed to a mixture of 14 solvents (including toluene) for an average of 10 years were found to be affected, with differences observed between right and left ear

mean latencies in waves I, III, and V and intervals I-V, I-III, and III-V, compared with 84 unexposed referents (Juárez-Pérez et al. 2014). Multiple linear regression models for waves and interpeak interval latencies for both ears showed a significant increase in latencies in the exposed group. The TWA median toluene exposure was 3.5 ppm and industrial noise was <85 dBA; however, concurrent exposure to other solvents limits the conclusions that can be drawn about toluene toxicity from this study.

Visual System Effects

Overview. Occupational studies indicate that long-term exposure to toluene may result in color vision loss (Campagna et al. 2001; Cavalleri et al. 2000; Muttray et al. 1997; Zavalic et al. 1998a, 1998b, 1998c) and altered VEPs (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 other parts of the eye. 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. 1996, 1999), but no studies examining the eyes for structural damage following chronic toluene exposure were located.

Color Vision. Multiple studies indicate that long-term occupational exposure to toluene can cause color vision loss. The color confusion index (CCI) was statistically significantly increased by 14% in 32 printers exposed to geometric mean toluene concentrations of 156 ppm, compared with 83 unexposed controls on Monday morning prior to their work shift (Zavalic et al. 1998a). The CCI in 41 shoemakers exposed to geometric mean toluene concentrations of 35 ppm was not significantly elevated when compared with controls. When alcohol consumers were excluded, the CCI in 27 shoemakers and 10 printers was significantly increased by 4 and 11%, respectively, compared with 36 controls. When adjusted for age and alcohol consumption, CCIs were significantly higher in both shoemakers and printers (adjusted mean CCI values were not reported). Individual adjusted CCIs were significantly correlated with individual exposure estimates (air, blood, or urine) in printers, but not shoemakers. Further analysis of color vision loss in these groups of workers demonstrated that total dychromatopsia (combined incidence of blue-yellow and red-green color confusion [dyschromatopsia type II] and just blue-yellow color confusion [dyschromatopsia III]) was significantly increased in printers, but not shoemakers, compared with unexposed workers, (Zavalic et al. 1998c). Dyschromatopsia type I (red-green color confusion only) was not observed in any exposed or unexposed workers. Taken together, these studies indicate a clear LOAEL of 156 ppm for color vision loss in printers, based on increased CCIs significantly associated with individual estimates of toluene exposure and increased prevalence of dyschromatopsia. A NOAEL of 35 ppm was identified, as it is unclear if the statistically significant

findings for increased CCI in the small sample of non-alcohol consuming workers or adjusted CCIs in all workers represents an adverse effect, especially since the magnitude of change was small and individual CCIs in this group were not associated with toluene exposure estimates in the air, blood, or urine.

Statistically significant increases in color confusion and total confusion indices were also reported in 33 toluene-exposed rubber workers, compared with 16 unexposed controls of similar age (Cavalleri et al. 2000). The indices were positively correlated with cumulative toluene exposure indices calculated from urinary toluene levels and duration of employment; however, air concentration levels were not reported.

Additional studies indicate that observed color vision impairments result from chronic, rather than acute, exposure. Color vision was assessed on Monday and Wednesday mornings in 45 male printers exposed to mean concentrations of ~120 ppm toluene (Zavalic et al. 1998b). Compared with unexposed controls, printers demonstrated a statistically significant increase in CCI, but results did not differ between examinations on Monday and Wednesday. Similarly, in 59 male rotogravure workers occupationally exposed to unspecified levels of toluene for periods of 1 month to 36 years (mean of 10 years), results of color vision testing did not differ between the beginning and the end of the workweek (Muttray et al. 1995). 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 nonsignificant decrease in color vision for the printers, which may indicate a chronic effect of toluene exposure on color vision (Muttray et al. 1999).

In contrast, no alterations were observed in red/green or blue/yellow color vision in 74 workers who were exposed to solvents (primarily [>90%] toluene: 46 ppm geometric mean), compared with 120 age-matched controls from clerical sections of the same factories (Nakatsuka et al. 1992). Likewise, a 5-year longitudinal study in 333 rotogravure workers exposed to “high” or “low” TWA toluene levels of 43 or 9 ppm, respectively, did not find an increase in CCI associated with toluene exposure levels or duration using multiple regression analysis (Schäper et al. 2004). The analysis was adjusted for exposure level and duration, age, eye (right/left), occupational qualification, and examination year.

Color vision impairment has also been reported in humans chronically exposed to mixtures of solvents containing toluene, but concurrent exposure to other solvents limits the conclusions that can be drawn

concerning toluene toxicity in these particular studies. Campagna et al. (2001) reported statistically significant increased incidence of dyschromatopsia and increased CCI in 72 printers (high-exposure) and 34 non-printers (ambient exposure) in a French photogravure plant for an average of 18–19 years, compared with 19 unexposed bookbinding workers of similar age from the same town. Geometric means of current toluene exposure levels, past cumulative toluene exposure, and past cumulative hydrocarbon exposure (e.g., toluene, xylene) were 136 mg/m^3 (36 ppm), 1,299 mg/m^3 x years, and 1,793 mg/m^3 x years in printers and 32 mg/m^3 (8.5 ppm), 299 mg/m^3 x years, and 534 mg/m^3 x years in non-printers, respectively. After adjusting for age and alcohol consumption, CCIs were significantly correlated ($p<0.05$) with current toluene exposure, past cumulative toluene exposure, and past cumulative hydrocarbon exposure (Campagna et al. 2001). Therefore, the significant effects observed may not be attributable specifically to toluene exposure, especially in the ambient-exposure group in which toluene exposure only accounted for 56% of past cumulative hydrocarbon exposure. Similarly, workers exposed to mixed solvents (including toluene, xylene, ethyl benzene, propyl benzene, ethyl toluene, methyl ethyl ketone, methyl isobutyl ketone, and perchloroethylene) during a spraying process showed a significant impairment of color vision with errors of the blue-yellow type, compared with unexposed referents similar in age, consumption of alcohol, and smoking habits (Muttray et al. 1997).

VEPs. Occupational exposure to toluene may also affect VEPs. A series of studies evaluated VEPs (P300, N75, N145, and P100 waves) in printers occupationally exposed to average concentrations of 50 ppm toluene for an average of 20 years and unexposed controls (Vrca et al. 1995, 1997b). 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) and the latency of the P100 wave were significantly increased in exposed subjects compared with controls (Vrca et al. 1995). In the exposed printers, wave amplitudes decreased significantly with the duration of exposure (Vrca et al. 1997a).

Visual Perception. Toluene exposure may also alter visual perception; however, the available evidence is limited to one study. The frequency at which individual flickers of light appeared to be a steady light source (flicker fusion frequency) was assessed in a large cohort of 1,290 printers occupationally exposed to toluene for an undefined number of years (Neubert et al. 2001). In a subgroup of 47 workers exposed to toluene concentrations of 70–80 ppm, 4 and 5% decreases in flicker fusion frequency before and after their 6-hour work shift, respectively, were found, compared with 200 unexposed referents. This small decrease was only statistically significant after the acute work-shift exposure. There was no change in flicker fusion frequency in the remaining workers exposed to 4–70 ppm, compared with unexposed

referents. From these findings, NOAEL and LOAEL values for impaired visual perception were estimated at 33 and 75 ppm, respectively, based on the midpoint of the exposure ranges for the high- and low-exposure groups.

Nerve Conduction Impairment. There is limited evidence that occupational exposure to toluene may alter nerve conduction in the peripheral and autonomic nervous systems. 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).

In support, altered nerve conduction has been observed in workers exposed to solvent mixtures. Compared with unexposed referents, bone glue factory workers exposed to an undefined solvent mixture had an increased incidence of paresthesia and abnormal electromyography readings (Al-Batanony et al. 2012), and paint and lacquer workers exposed to white spirit, toluene, butyl acetate, ethyl acetate, and xylene had altered peripheral nerve conduction (Jovanovic et al. 2004).

The highest NOAEL values and all LOAEL values for each reliable human occupational exposure for neurological effects following exposure predominantly to toluene are recorded in Table 3-1 and plotted in Figure 3-1. Supporting studies reporting neurological effects following exposures to solvent mixtures are not included in Table 3-1.

Accidental and Intentional High-Dose Human Exposure

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, muscle weakness, temporal lobe epilepsy, paranoid psychosis, personality changes, hallucinations, nystagmus (involuntary eye movement), altered brain chemistry and impaired speech, intelligence, hearing, and vision (Aydin et al. 2003; Byrne et al. 1991; Camarra-Lemarroy et al. 2015; Capron and Logan 2009; Devathasan et al. 1984; Gupta et al. 2011; Hunnewell and Miller 1998; King et al. 1981; Kiyokawa et al. 1999; Maas et al. 1991; Maruff et al. 1998; Meulenbelt et al. 1990; Miyagi et al. 1999; Papageorgiou et al. 2009; Peralta and Chang 2012; Poblano et al. 1996; Ryu et al. 1998; Suzuki et al. 1983; Uchino et al. 2002).

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 (Aydin et al. 2002, 2003; Caldemeyer et al. 1996; Filley et al. 1990; Ikeda and Tsukagoshi 1990; Kamran and Bakshi 1998; Kucuk et al. 2000; Nomura et al. 2016; Rosenberg et al. 1988a, 2002; Ryu et al. 1998; Uchino et al. 2002; Yamanouchi et al. 1995). Cerebral, cerebellar, and brainstem atrophy were also present (Deleu and Hanssens 2000; Ryu et al. 1998; Gupta et al. 2011; Kamran and Bakshi 1998; Papageorgiou et al. 2009; 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; Kiyokawa et al. 1999; Rosenberg et al. 1988b, 2002; Uchino et al. 2002). 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).

Animal Studies

Neurobehavior

Overview. Numerous studies have evaluated neurobehavior in animals following toluene exposure using tests to evaluate locomotor activity, exploratory behavior, learning and memory, and anxiety-like behaviors. Acute-duration studies consistently demonstrated increased, followed by decreased, locomotor activity at exposure levels ≥500 ppm, cognitive deficits have been reported at concentrations as low as 125 ppm, and anti-anxiety-like effects at higher concentrations (≥2,000 ppm). In contrast, studies of rodents exposed for intermediate durations to concentrations as high as 1,000 ppm have not found strong and consistent evidence for exposure-related changes in neurobehavioral end points.

Acute-Duration Studies. Most acute exposure studies have reported a biphasic response with low-concentration stimulation and high-concentration depression of locomotor activity. Single or repeated exposure to toluene exposure for 30–72 minutes at concentrations of 500–3,000 ppm produced increased locomotor activity in mice; however, initial increases in locomotion were followed by a decrease in activity at concentrations of 3,000–10,000 ppm or repeated exposure to lower concentrations (Bowen and Balster 1998; Bowen et al. 2010; Bushnell et al. 1985; Conti et al. 2012; Kim et al. 1998; Lopez-Rubalcava and Cruz 2000; Tomaszycki et al. 2013; Wood and Colotla 1990). In adolescent and young adult rats exposed to 0, 1,000, 2,000, 4,000, or 6,000 ppm for 30 minutes, increased locomotor activity compared with controls was found only at concentrations ≥4,000 ppm, although exploratory behavior was significantly reduced in the 6,000 ppm group (Huerta-Rivas et al. 2012). Exploratory behavior during the conditioned defensive burying task was increased in mice exposed to 4,000 or 6,000 ppm, but not 500–2,000 ppm (Paez-Martinez et al. 2003).

In several animal studies, acute exposure to toluene also diminished cognitive ability, but the lowest exposure levels required to impair learning and/or memory ranged from 125 to 4,000 ppm, depending upon the species, task, and duration of exposure. Monkeys exposed to concentrations of 0, 100, 200, 500, 1,000, 2,000, 3,000, or 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 ≥2,000 ppm, compared with controls (Taylor and Evans 1985). 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, compared with pre-exposure performance, but recovery was complete 2 hours later (Kishi et al. 1988). 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). Statistically significant impaired learning and memory in the object recognition task and a decline in the conditioned step-through inhibition avoidance response were found in adolescent and young adult rats exposed to 1,000, 2,000, 4,000, or 6,000 ppm for 30 minutes, compared with unexposed controls (Huerta-Rivas et al. 2012). These effects were also present in adolescent and young adult rats exposed to 6,000 ppm for 30 minutes 2 times/day for 10 days (lower exposure levels were not evaluated). Similarly, impaired learning coupled with decreased anxiety-like behavior during the conditioned defensive burying task were reported in mice exposed to 4,000 or 6,000 ppm for 30 minutes, compared with unexposed controls, but not in mice exposed to 500, 1,000, or 2,000 ppm (Paez-Martinez et al. 2003). Decreased anxiety was also observed during the elevated-plus

maze in male mice exposed to ≥2,000 ppm for 30 minutes, compared with unexposed controls, but not in mice exposed to 1,000 ppm (Bowen et al. 1996).

Intermediate-Duration studies. Studies of rodents exposed for intermediate durations to concentrations as high as 1,000 ppm have not found strong and consistent evidence for exposure-related changes in neurobehavioral end points. In rats exposed to 0, 10, 100, or 1,000 ppm 6 hours/day 5 days/week for 4 weeks, statistically significant decreased accuracy was observed in a signal detection task in 1,000-ppm rats, but no exposure-related changes were found in motor activity, anxiety-like behavior, or acquisition of a visual discrimination task or lever-press response at any exposure level (Beasley et al. 2012). In contrast, rats similarly exposed for 13 weeks were delayed in their acquisition of the lever-press response at 1,000 ppm, compared with unexposed controls, but no impairments were observed in a signal detection task, motor activity, anxiety-like behavior, acquisition of a visual discrimination task, or fear conditioning (Beasley et al. 2010). When rats exposed to 100–1,000 ppm toluene for 13 weeks were challenged with acute exposures of 1,200–2,400 ppm toluene 33–42 weeks later, previous exposure did not alter performance in the signal detection task (Beasely et al. 2010). In another study, prior exposure to 80 ppm for 4 weeks (5 days/week, 6 hours/day) did not influence the performance of rats 2 weeks later in a lever-press task following acute challenges to 20 daily "trigger" exposures of 10 ppm toluene for 1 hour (Rogers et al. 1999). Trigger-exposed animals, with or without prior exposure, showed statistically significant ($p<0.05$) increased lever presses and increased incorrect lever presses during training, compared with unexposed rats, indicating that these effects are mediated by the acute 10-ppm triggers rather than prior 80-ppm exposures. Similarly, no adverse effects were observed in spatial learning, open-field activity, and/or passive or active avoidance tasks in mice exposed to 0 or 50 ppm for 6 weeks (Win-Shwe et al. 2010c), mice exposed to 0, 9, or 90 ppm toluene for 4 weeks (Win-Shwe et al. 2010d), rats exposed to 0, 25, 100, or 250 for 4 weeks (Wiaderna and Tomas 2002), rats exposed to 0 or 80 ppm for 4 weeks (von Euler et al. 2000), or young rats exposed to 0 or 600 ppm toluene for 50 days (Miyagawa et al. 1995). No statistically significant changes in general locomotor activity were observed in rats exposed to 40 ppm toluene for 16 weeks, compared with controls; however, there was a statistically significant decrease in rearing behavior (Berenguer et al. 2003, 2004). Nose-poking exploratory behavior was increased in rats exposed to 178, 300, or 560 ppm toluene for 3 weeks (2 times/week, 2 hours/day), compared with their pre-exposure behavior (Wood and Cox 1995).

Impaired Motor Coordination and Reflexes

Overview. Impaired motor coordination and decreased reflexes have been observed in animals following acute- and intermediate-duration exposures to toluene; however, acute-duration studies are limited and findings from intermediate-duration studies are inconsistent.

Acute-Duration Studies. Acute exposure in monkeys and rats to concentrations ≥3,000 ppm caused overt signs of neurological motor impairments such as ataxia, tremors, and inability to walk (Mullin and Krivanek 1982; Taylor and Evans 1985). Concentration-related impairments in motor coordination and reflexes were observed in rats following a single 4-hour exposure to concentrations of 0, 810, 1,660, 3,100, or 6,250 ppm, resulting in decreased lift and pinna reflexes and impaired performance during vertical bar placing and horizontal rod grasping (Mullin and Krivanek 1982). The impairment observed at 810 ppm was borderline, but the majority of rats in the 1,600 group failed reflex testing. Overt signs of neurotoxicity, including ataxia, tremors, and inability to walk, were observed at ≥3,100 ppm (Mullin and Krivanek 1982). A few rats failed reflex testing and demonstrated tremors in the 810 ppm group. Impaired motor coordination on the rotarod was found in mice exposed to 6,000 ppm toluene for 30 minutes, compared with unexposed controls, but lower exposures (500–4,000 ppm) did not change motor coordination (Paez-Martinez et al. 2003). In another study, no exposure-related impairment of rotarod test performance was reported for mice exposed to toluene at concentrations up to 8,000 ppm for 30 minutes (Cruz et al. 2001).

Rats exposed to 100 ppm for 4 hours or 3 hours/day for 5 days exhibited an altered opticokinetic response, displaying slower and more irregular eye movements while tracking a moving object, compared with pre-exposure values (Hogie et al. 2009). Following exposure, rats also demonstrated unsteady eye positions at rest (nystagmus). These findings indicate potential disturbances in the vestibular and opto-oculomotor systems, suggesting that the cerebellum may be a target site for toluene.

Intermediate-Duration Studies. Overt signs of neurological motor impairments, such as ataxia, tremors, and inability to walk, were observed in rats, but not in mice, exposed to ≥2,500 ppm for 5–15 weeks, compared with unexposed controls (API 1997; NTP 1990; von Oettingen et al. 1942). In rats exposed to 80 ppm for 4 weeks, impaired motor skills in beam walking were found, requiring a significantly wider beam to maintain balance (von Euler et al. 2000). The study authors reported that the impairment was predominantly due to improper placement of hindlimbs. In contrast, motor impairments in the tilting

plane or rotarod test were not found in rats exposed to 1,000 ppm toluene for 8 hours/day, 7 days/week for 13 weeks (Tahti et al. 1983).

Nociception. Toluene exposure can alter pain perception in animals; however, alterations in nociception may be species-, concentration-, and duration-dependent. Mice exposed to toluene at 500–8,000 ppm for 30 minutes exhibited a dose-related increase in nociception in the hotplate test (Cruz et al. 2001; Paez-Martinez et al. 2008). In both studies, mice exhibited decreased latencies for paw-licking that were significantly shorter at 1,000 ppm, compared with unexposed controls. Cruz et al. (2001) also reported statistically significant decreases in latencies for the antialgesic flexor reflex and escape responses at 2,000 and 6,000 ppm, respectively. In rats exposed to 0, 25, 100, or 250 ppm 6 hours/day, 5 days/week for 4 weeks, increased escape behavior in the hot-plate test in the 100- and 250-ppm groups was observed, which likely accounts for the observed increased paw-lick latencies (rather than decreased nociception) (Wiaderna and Tomas 2002). A clearer demonstration of decreased nociception was found in rats exposed to 6,000 ppm toluene for 30 minutes that required significantly increased shock intensity to elicit flinch, jump, or vocalization responses, compared with unexposed controls (Huerta-Rivas et al. 2012). No change in nociception was observed at 1,000, 2,000, or 4,000 ppm, and this effect was not observed in rats exposed to 6,000 ppm for 30 minutes, 2 times/day for 10 days (Huerta-Rivas et al. 2012).

Auditory System

Overview. Hearing loss in animals has been observed following acute- and intermediate-duration exposure to toluene at concentrations in ≥250 ppm in guinea pigs (McWilliams et al. 2000) and ≥1,000 ppm in rats (Johnson 1992; Johnson et al. 1988; Pryor et al. 1984b). Observed hearing loss may not be solely due to neurological damage, as exposure to 500–2,000 ppm damages the outer hair cells (OHCs) in the cochlea that are responsible for amplifying incoming sound waves prior to signal transduction (Campo et al. 1997; McWilliams et al. 2000). This type of damage is referred to as sensorineural hearing loss.

Hearing loss was examined in rats exposed to 0, 400, 700, or 1,000 ppm 14 hours/day for 16 weeks using both behavioral and electrophysiological methods (Pryor et al. 1984b). Diminished auditory responses in a conditioned pole-climb avoidance response (CAR) task using a 20-kHz tone and increased BAER thresholds were measured in rats exposed to 1,000 ppm, compared with unexposed controls (Pryor et al. 1984b). Similarly, diminished auditory brainstem responses were measured in rats exposed to 1,000 ppm toluene for 16 hours/day for 10 days or 2 weeks, compared with controls (Johnson 1992; Johnson et al.

1988). The effects persisted at least 4–6 months post-exposure and were compounded by post-exposure high noise levels. However, following shorter daily durations (6 hours/day), diminished brainstem auditory responses or distortion product otoacoustic emissions (DPOAE) were only observed in rats exposed to up to 1,500 ppm for 10 days when exposure was combined with wide-band or impulse noise (92 dB SPL; peak level for impulse 130 dB), compared with unexposed and noise-only exposed groups (Lund and Kristiansen 2008). Toluene exposure for 6 hours/day, 5 days/week for 90 days did not impair hearing at levels up to 500 ppm, with or without noise exposure at 90 dB SPL (Lund and Kristiansen 2008).

Sensitivity to toluene-induced hearing loss may be species-specific. Guinea pigs may be more sensitive than rats to toluene-induced hearing loss. Transient, dose-related impairments in mid- to high-frequency hearing were observed in guinea pigs exposed to 250, 500, or 1,000 ppm toluene 8 hours/day for 5 days, compared with controls, as measured by brainstem auditory responses or DPOAE (McWilliams et al. 2000). When guinea pigs in the 500 ppm group were exposed for an additional 3 weeks, hearing loss was more pronounced (McWilliams et al. 2000). However, chinchillas may be less sensitive than rats. While toluene led to decreased auditory evoked potentials in rats exposed to 2,000 ppm toluene for 10 days (8 or 12 hours/day), compared with unexposed controls, auditory evoked potentials were not altered in similarly exposed chinchillas (Davis et al. 2002).

Toluene had a more pronounced 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 following exposure to 1,000 ppm toluene for 12 hours/day for 7 days (Li et al. 1992). Thus, the severity of toluene-induced hearing loss appears to be influenced by genetic susceptibility.

Visual System. FEP responses were abnormal in rats exposed to single 30-minute exposures to 500–16,000 ppm toluene (Rebert et al. 1989a, 1989b). 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. Similarly, F2 wave amplitudes of VEPs were decreased 40–65% in rats exposed to 1,000–4,000 ppm toluene for 1–4 hours in a dose-related manner (Boyes et al. 2007). Two hours after exposure ended, partial recovery was found in the 4,000-ppm group but not in the 3,000 ppm group.

In a series of intermediate-duration experiments, Boyes et al. (2016) reported subtle, but persistent, changes in visual function in rats exposed to 1,000 ppm toluene 6 hours/day, 5 days/week for 13 weeks,

but not 4 weeks; no visual system effects were observed at ≤100 ppm. Specifically, significant decreases were observed in the maximal b-wave amplitude induced by a scotopic flash, measured by electroretinogram (ERG) at 5–6 weeks and 1 year after the 13-week exposure period (but not 2–3 weeks after the 4-week exposure period). Reduction of the b-wave amplitude indicates altered firing patterns of non-bipolar cells. No exposure-related effects were noted in the ultraviolet (UV) or green flicker data or the photopic flash data from ERGs, and no exposure-related changes were observed in VEPs measured 2–3 weeks after the 13-week exposure period (not measured in the 4-week study). Additionally, the density of rod and M-cone photoreceptors were comparable between exposed and control animals.

Olfactory System. Decreased avoidance of a toluene-containing arm in a T-maze was observed in female mice exposed following exposure to 1,000 ppm toluene 5 hours/day, 5 days/week for 4 weeks, compared with pre-exposure measures (Jacquot et al. 2006). Decreased olfactory sensitivity continued for 2 weeks following cessation of exposure. This behavior was accompanied by reversible olfactory inflammation and decreased numbers of olfactory epithelial cells, perhaps indicating that olfactory sensitivity was decreased by exposure to toluene (Jacquot et al. 2006). However, this could instead indicate tolerance to toluene odor with repeat exposures and/or toluene-seeking behavior (see the section on Animal Studies Modeling High-Concentration Solvent Abuse).

Sleep Patterns. 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 hours/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).

Brain Weight, Volume, and Histology. Altered brain weight and volume have been reported in rats, but not in mice, following intermediate-duration exposure to toluene. In rats exposed to 80 ppm 6 hours/day, 5 days/week for 4 weeks, the area of the cerebral cortex measured by MRI and D_3 receptor autoradiography was decreased significantly by 4 and 10%, respectively, compared with controls (von

Euler et al. 2000). MRI analysis indicated that the main effect was in the parietal cortex, which was significantly decreased in area by 6%. However, no change was observed in total brain weight or volume (von Euler et al. 2000). Similarly, no change in total brain weight was reported in rats up to 320 ppm using the same protocol (Hillefors-Berglund et al. 1995). In contrast, rats exposed continuously to 320 ppm for 30 days, both cerebrocortical and total brain weight were significantly decreased compared with control by 4 and 8%, respectively (Kyrklund et al. 1987). There was also 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. Following exposure to toluene at 0, 100, 625, 1,250, 2,500, or 3,000 ppm 6.5 hours/day, 5 days/week, for 15 weeks, relative brain weights were increased significantly in rats exposed to 2,500 or 3,000 ppm, compared with controls (NTP 1990). However, no changes in brain weights were observed in mice similarly 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). The mechanism(s) underlying the apparent species difference in the intermediate-duration NTP study is unknown. In a multigenerational study, no changes in brain weight were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation (API 1985; Roberts et al. 2003). No gross or microscopic brain tissue changes were observed in any of the mouse or rat studies described above.

GFAP Levels. Changes in brain levels of 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 levels in the cerebrum were found (Matsuoka et al. 1997).

Oxidative Stress Markers in Brain Tissue. Increased oxidative stress has been observed in the rat brain following acute- or intermediate-duration inhalation exposure. Increased glutathione peroxidase activity

and H_2O_2-induced chemiluminescence in cortical brain tissue were reported for rats exposed to 500 ppm toluene for 4 hours/day, 5 days/week for 1 month, compared with controls, indicating toluene-induced free radical processes (Burmistrov et al. 2001). These effects were not seen at 50 ppm. However, no changes in free or total malondialdehyde levels, another marker of oxidative stress, were observed in rats exposed to 1,000 ppm, 6 hours/day for 10 days, compared with controls (Chalansonnet et al. 2013). Similarly, no significant changes in brain levels in markers of oxidative stress, including lipid peroxidase, superoxide dismutase, or 4-hydroxy-nonenal, or total brain DNA levels of 8-OH-dG were observed in rats exposed to 1,500 ppm, 4 hours/day for 7 days (Tokunaga et al. 2003). The biological adversity of these findings are unclear, therefore NOAELs and LOAELs for changes in brain oxidative stress markers are not included in Table 3-1.

Brain Tissue Changes in Levels of Neurotransmitters, Synthesis, and Binding Sites

Overview. Changes in the levels of brain neurotransmitters and their precursors and metabolites have been observed in rodents exposed by inhalation to toluene for acute and intermediate durations. Alterations in neurotransmitter synthesis and binding have also been reported. Current mechanistic understanding is inadequate to determine the biological adversity of the observed changes and findings from these studies are not included in Table 3-1.

Following exposure to 1,000–4,000 ppm toluene for 20 minutes, dopamine (DA) levels were increased in the cerebellum and striatum of rats, while norepinephrine (NE) and serotonin (5-HT) were significantly increased in the cerebellum and cortex (Kim et al. 1998). Increased concentrations of DA in the striatum, NE in the medulla and midbrain, and 5-HT in the cerebellum, medulla, and striatum were observed in rats exposed to 1,000 ppm, but not 100 or 300 ppm, for 8 hours, compared with controls (Rea et al. 1984). In other studies, increased levels of DA and noradrenaline were observed in several brain regions in rats exposed to 80–3,000 ppm, 6 hours/day for 3–5 days (Andersson et al. 1980, 1983b); however, decreased DA levels and rates of turnover were observed 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). Altered DA turnover was also reported in rats exposed to 40 ppm toluene for 16 weeks, compared with controls, as demonstrated by significant localized increases in DA levels, as well as decreases in the levels of its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and its precursor dihydroxyphenylalanine (DOPA) (Berenguer et al. 2003, 2004). No statistically significant exposure-related changes were observed in the 5-HT neurotransmitter system. Altered levels of noradrenaline and DA were observed in select brain areas of male rats continuously exposed to 400 ppm toluene for 30 days, compared with controls (Ikeda et al.

1986). The directionality and magnitude of change varied across brain regions, and no effects were observed at 200 ppm. The biological adversity of region-specific neurotransmitter changes in the absence of behavioral effects is unknown.

Several statistically 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. Exposure to 400 ppm toluene 7 hours/day for 10 days produced a statistically significant increase in the total dehydrogenase activity in the brains of female mice (Courtney et al. 1986). Minimal, but statistically significant, alterations in monoamine neurotransmitter synthesis (tyrosine and tryptophan hydroxylase enzyme activities) were reported in the brainstem and hypothalamus of rats exposed to 40 ppm toluene 104 hours/week for 16 weeks, but not in other brain regions (Soulage et al. 2004). The direction and magnitude of changes (17–44%) differed between brain regions and sexes. Due to the lack of concentration-related effects and/or variability of the responses between brain regions and sexes, NOAEL/LOAEL values for these studies could not be determined for this assessment.

Toluene exposure at 80 ppm for 4 weeks was found to affect DA D_2 agonist binding in the rat caudate-putamen (Hillefors-Berglund et al. 1995; von Euler et al. 1993). Von Euler et al. (1994) also reported that rats exposed to 80 ppm had increased serum prolactin levels, which could be related to a possible interaction between toluene and the pituitary DA D_2 receptor since this receptor normally inhibits the release of prolactin into serum. However, in another study, no significant exposure-related changes in serum prolactin levels were reported with 4-week exposures to concentrations up to 320 ppm (Hillefors-Berglund et al. 1995). There were also no changes in DA D_3 receptor binding 4 weeks after cessation of a 4-week exposure to 80 ppm toluene (von Euler et al. 2000). There is some evidence of changes in glutamate and gamma amino butyric acid (GABA) binding 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). Binding increased in most of the brain areas studied, but decreased in some areas. Because of the variability in response, the biological adversity of the observed changes cannot be determined.

Gene Expression Changes in Brain Tissue

Overview. Numerous gene expression changes have been observed in brain tissue of rats and mice following acute or intermediate inhalation exposure. Results of these studies vary greatly, and indicate that gene expression changes are species-, strain-, dose-, duration-, and lifestage-dependent, and that immune status may affect gene expression changes in the brain. Current mechanistic understanding is inadequate to determine the biological adversity of the observed changes in gene expression, and findings from these studies are not included in Table 3-1.

In rats, microarray analysis 6 and 18 hours following an acute 6-hour exposure to 1,000 ppm identified 226 and 3,352 differentially expressed genes in the striatum ($p<0.05$), respectively, compared with controls (Hester et al. 2011). Pathway analysis identified synaptic transmission and plasticity as the predominate pathways affected by acute toluene exposure (Hester et al. 2011). However, gene expression changes from the acute study by Hester et al. (2011) were not predictive of gene expression changes in rats exposed to 0, 10, 100, or 1,000 ppm toluene for 6 hours/day, 5 days/week for 64 days (Hester et al. 2012). In the intermediate-duration study, microarray analysis 18 hours after the final exposure identified 22, 57, and 94 differentially expressed genes in the striatum ($p<0.05$) in the 10-, 100-, and 1,000-ppm groups, respectively, compared with control. However, only 57 differentially expressed genes were found in both the acute study (Hester et al. 2011) and the intermediate-duration study (Hester et al. 2012), and the direction of change (up- or down-regulation) was inconsistent. Additionally, altered gene expression in the intermediate-duration study did not demonstrate consistent dose-related changes in gene expression (Hester et al. 2012).

Win-Shwe et al. (2007a) reported upregulation of memory-related genes in the hippocampus in BALB/c mice following 30-minute exposures to 9 ppm toluene for 3 consecutive days followed by 1 day/week for 4 weeks. These changes may be mediated by T-cell activation, as nude mice similarly exposed did not demonstrate memory-gene upregulation (Win-Shwe et al. 2007a). However, when the study was repeated in C3H/HeN mice, changes in expression of memory-related genes in the hippocampus were not statistically significant in mice exposed to 9 or 90 ppm, compared with unexposed C3H/HeN mice (Win-Shwe et al. 2010c). Gestational and prenatal exposure studies found that exposure to toluene during development significantly altered hippocampal memory-related gene expression with exposure to 5 or 50 ppm from PND 8 to 12 and 50 ppm from PND 2 to 6, but not with exposure to 5 or 50 ppm from GD 14 to 16 (Win-Shwe et al. 2010b).

When C3H/HeN, BALB/c, and C57BL/10 mice were exposed to 0, 5, 50, or 500 ppm toluene for 6 hours/day, 5 days/week for 6 weeks, changes in mRNA levels of neurotrophins (NGF, BDNF) and their receptors (TrkA, TrkB) in the hippocampus differed between strains (Win-Shwe et al. 2010a). In C3H/HeN mice, mRNA levels for NGF, BDNF, TrkA, and TrkB were all significantly upregulated ($p<0.01$) in the 500-ppm group, compared with controls. However, only BDNF was significantly upregulated in BALB/C mice exposed to 500 ppm, and no significant changes were observed in C57BL/10 mice (Win-Shwe et al. 2010a). There were no significant, dose-related gene expression changes in marker genes for NGF signal-transduction or dopamine signaling pathways; however, HO-1 mRNA levels (a marker for oxidative stress) were significantly upregulated in the 500-ppm C3H/HeN group. The immune system may have a role, as neurotrophin mRNA levels were increased in allergy-challenged C3H/HeN mice exposed to 50 ppm, compared with controls, but not in allergy-challenged mice exposed to 500 ppm (Win-Shwe et al. 2010a). Similarly, in a separate study, the same exposure protocol led to upregulation of mRNA levels of neuroinflammatory genes TLR4 and NF-kB in the hippocampus following exposure to 50 ppm, but not 5 or 500 ppm, in C3H/HeN mice (Win-Shwe et al. 2011).

Following exposure to 0 or 50 ppm, 6 hours/day, 5/days week for 6 or 12 weeks, mRNA levels for synaptic plasticity genes were measured in the hippocampus of C3H/HeN mice (Ahmed et al. 2007). Significant upregulation ($p<0.05$) was found in mRNA levels of N-methyl-D-aspartate (NMDA) receptor subunit 2B, CaMKIV, CREB-1, and FosB/ΔFosB in mice exposed to 50 ppm for 12 weeks, compared with controls. No changes were found in NMDA receptor NR2 or CREB-2 after 12 weeks, and no significant changes in any mRNA levels for these genes were observed after exposure for 6 weeks (Ahmed et al. 2007).

Neurodevelopment. Studies examining neurotoxic effects of gestational and/or neonatal exposure during critical periods of neurodevelopment are discussed in Section 3.2.1.6, Developmental Effects.

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

Animal Studies Modeling High-Concentration Solvent Abuse

Overview. Numerous animal studies model human solvent abuse using single or repeat exposure to high concentrations of toluene (most concentrations >1,000 ppm). Examined end points in these studies were

the same as those examined in animal studies at lower exposure levels or included abuse-related behaviors (e.g., reward-seeking, tolerance) or brain systems (e.g., mesolimbic system). Due to adequate dose-response information from lower-dose studies, LOAELs from these studies are not included in Table 3-1.

Adverse Neurological Effects. Effects observed in these high-exposure studies (>1,000 ppm) include overt signs of neurological impairment (e.g., ataxia, tremors, loss of consciousness) (Beyer et al. 2001; Bruckner and Peterson 1981a, 1981b; Davis et al. 2002; Lorenzana-Jimenez and Salsas 1990; NTP 1990; Ono et al. 1999; Pryor 1991; Pryor and Rebert 1992), neurobehavioral alterations (Apawu et al. 2014; Batis et al. 2010; Baydas et al. 2005; Bikashvili et al. 2012; Bowen et al. 2007; Bushnell et al. 2007; Castilla-Serna et al. 1991; Dashniani et al. 2014; Duncan et al. 2012; Galliot et al. 2012; Gmaz et al. 2012; Harabuchi et al. 1993; Hinman 1987; Huerta-Rivas et al. 2012; Lammers et al. 2005b; Lorenzana-Jimenez and Salas 1990; Mattsson et al. 1990; Miyagawa et al. 1998; Oshiro et al. 2007, 2011; Paez-Martinez et al. 2013; Pryor 1991; Pryor and Rebert 1992; Samuel-Herter et al. 2013), impaired motor coordination (Gmaz et al. 2012; Lorenzana-Jimenez and Salas 1990; Samuel-Herter et al. 2013; Tahti et al. 1983), altered pain perception (Lopez-Rubalcava and Cruz 2000; Paez-Martinez et al. 2008), altered sleep patterns (Alfaro-Rodriguez et al. 2011), auditory effects (Campo et al. 1998; Johnson and Canlon 1994; Lataye and Campo 1997; Lataye et al. 1999; Mattsson et al. 1990; Pryor 1991; Pryor and Rebert 1992; Pryor et al. 1984a, 1984b; Waniusiow et al. 2008, 2009), decreased FEPs (Bale et al. 2007; Mattsson et al. 1990), nystagmus (Larsby et al. 1986; Tham et al. 1982), altered neurotransmitters and receptors in nervous system tissues (Alfaro-Rodriguez et al. 2011; Apawu et al. 2014; Gerasimov et al. 2002c; Koga et al. 2007; O'Leary-Moore et al. 2007, 2009; Ono et al. 1999; Paez-Martinez et al. 2008; Tsuga and Honma 2000; Williams et al. 2005), increased GFAP levels in brains (Baydas et al. 2003; Gotohda et al. 2000a, 2000b, 2007), damage to olfactory and hippocampal neurons (Gelazonia et al. 2006a, 2006b), reduced brain weight (Edelfors et al. 2002), increased markers of oxidative stress (Baydas et al. 2003, 2005; Coskun et al. 2005), and gene expression changes in the brain (Gotohda et al. 2000b; Ikematsu et al. 2007; Sanchez-Serrano et al. 2011).

Abuse-Specific Behaviors. Since toluene is often intentionally inhaled to become intoxicated, multiple animal studies have been conducted assessing dependence, reward-seeking behavior, and tolerance following toluene exposure. Removal of mice following exposure to 250 ppm toluene continuously for 4 days resulted in chemical withdrawal, as evidenced by increased handling-induced convulsions (Wiley et al. 2003). Multiple studies report that mice and rats show conditioned place preference after acute exposure to toluene concentrations of ≥700 and ≥1,895 ppm, respectively, preferring the toluene-filled

chamber over an air-filled chamber (Funada et al. 2002; Gerasimov et al. 2003; Lee et al. 2006; Schiffer et al. 2006). When rats were evaluated in a wait-for-reward task 22–23 hours after daily 30-minute exposures to 0, 1,000, 3,600, or 6,000 ppm for 40 days, a higher response-to-reinforcer ratio and decreased number of rewards received was observed in the 3,600- and 6,000-ppm groups, compared with controls (Bowen and McDonald 2009). Altered performance persisted during the 40-day recovery period. Tolerance to toluene and toluene-induced effects with repeated exposure to >1,000 ppm has been noted in various neurobehavioral tests, including the righting-reflex (Lorenzana-Jimenez and Salas 1990), pain perception (Huerta-Rivas et al. 2012), visual signal detection (Oshiro et al. 2007, 2011), and locomotor activity (Bowen et al. 2007). However, intermittent exposure to 3,000 ppm toluene during adolescence in rats did not increase ethanol-seeking behaviors in adulthood (Dick et al. 2014).

Mesolimbic System. Consistent with observed patterns of abuse in humans, acute exposure to high toluene levels of 4,000–10,000 ppm in animals can affect the mesolimbic system, which plays an important role in drug dependence. This system contains both multiple neurotransmitter pathways, and includes several brain structures such as the frontal cortex, hippocampus, basal ganglia, nucleus accumbens, specific areas of the brainstem, and the white matter tracts connecting them. Effects observed include increased c-Fos activity (neural activity) in catacholaminergic cells of the nucleus accumbens shell (Tomaszycki et al. 2013), increased c-Fos activity in the forebrain and midbrain structures implicated in reward, emotion, and olfactory stimulation (Perit et al. 2012), persistent alterations in excitatory synaptic strength of mesolimbic dopamine (DA) neurons (Beckley et al. 2013), increased activation of DA neurons in the ventral tegmental area of the midbrain (Riegel and French 2002), increased DA and noradrenaline levels in the medial prefrontal cortex and nucleus accumbens (Koga et al. 2007), and altered glutamatergic signaling in corticostriatal circuitry (Dick et al. 2015). Effects on the dopaminergic system may be changed with continued abuse, and may indicate potential compensatory mechanisms following repeat exposure. Mice exposed once to 2,000–4,000 ppm showed significant increases in DA release in the caudate-putamen and nucleus accumbens; however, repeated exposures to the same concentrations over 7 days resulted in a significant decrease in DA release in the nucleus accumbens (Apawu et al. 2014). Toluene abuse may also lead to cross-sensitization to other drugs, as acute exposure to toluene increased diazepam-induced locomotion (Wiley et al. 2003), apomorphine-induced locomotion (Wiaderna and Tomas 2002), cocaine-induced locomotion (Beyer et al. 2001), and cocaine-induced increases in DA levels in the nucleus accumbens (Gerasimov et al. 2002c).

Effects observed in the mesolimbic system may result from permanent brain injury. Numerous animal studies report injury to multiple regions of the brain following acute or repeated exposure to

concentrations of toluene ranging from 1,500 to 6,000 ppm, including degenerative changes and ultrastructural damage in the hippocampus, frontal cortex, and brain stem (Kanter 2008a, 2008b, 2011c, 2013), decreased or shrunken cells in the hippocampus (Gotohda et al. 2002; Korbo et al. 1996; Zhvania et al. 2012), impaired dendritic outgrowth in the frontal cortex (Pascual and Bustamante 2010), and white matter damage in anterior commissure (Duncan et al. 2012).

3.2.1.5 Reproductive Effects

Overview. Current data do not provide convincing evidence that acute or repeated inhalation exposure to toluene may cause reproductive effects in humans. Limited evidence in humans indicates that occupational exposure to toluene may lead to an increased incidence of spontaneous abortion (Lindbohm et al. 1992; Ng et al. 1992b; Taskinen et al. 1989) or decreased fecundity in female workers (Plenge-Böenig and Karmaus 1999). One population-based cohort study reported increased risk of preterm birth with increasing environmental toluene exposure (Poirier et al. 2015); however, concurrent exposure to multiple pollutants (which were not controlled for in statistical analyses) limits the conclusions that can be drawn from this study. A few studies in animals exposed to toluene via inhalation at concentrations ≥2,000 ppm reported effects on male and female reproductive tissues, including abundant vacuoles, lytic areas, and mitochondrial degeneration in the antral follicles of the ovaries of female rats (Tap et al. 1996) and reduced sperm count, motility, and quality and altered reproductive organ weight and histology in male rats (Kanter 2011b; Ono et al. 1996, 1999). However, changes in sperm count and epididymis weight were not accompanied by any change in indices of reproductive performance (e.g., fertility) in male rats exposed to 2,000 ppm for 60 days before mating (Ono et al. 1996). The majority of animal studies provided little evidence for toluene reproductive toxicity. Studies in rats exposed repeatedly by inhalation to toluene, including a 2-generation reproductive toxicity study, have shown no evidence of adverse effects on mating or fertility at tested concentrations as high as 1,200–2,000 ppm (API 1981, 1985; Ono et al. 1996; Roberts et al. 2003; Thiel and Chaboud 1997). In addition, the majority of gestational exposure studies reported no exposure-related changes in reproductive indices (see citations below).

Occupational Exposure Human Studies. 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). Increased incidence of dysmenorrhea was reported in 38 female shoemakers who were exposed to toluene concentrations that varied from 65 ppm (15–100 ppm) in winter to 100 ppm (10–200 ppm) in summer for an average of 40 months, compared with 16 controls (Matsushita et al. 1975). No other reproductive end points were assessed by Matsushita et al. (1975). The concentration for reproductive effects is estimated at 83 ppm (the average between summer and winter exposures). The incidence of spontaneous abortions exceeded population norms among five female workers (Lindbohm et al. 1992) and among the wives of small groups of 28–48 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.

Fecundity (probability of conception) was decreased in female workers, but not in male workers, in German printing facilities for periods when they were employed in printing facilities and exposed to toluene, compared with periods when they were employed in other jobs without toluene exposure (Plenge-Böenig and Karmaus 1999). Toluene exposure was divided into three groups based on work history and exposure measurements from previous years (conducted by industrial hygienists of the Employer's Liability Insurer): low exposure (e.g., stacking and book binding; <10 ppm), medium exposure (cylinder preparation, galvanisers; 10–30 ppm), and high exposure (printers; <200 ppm before 1984, <100 ppm in 1984–1994, and <50 ppm after 1994). Actual air concentrations of toluene were not reported. Reproductive and work history data collected from workers were analyzed to determine fecundability ratios based on time to pregnancy and periods of unprotected intercourse during exposed and unexposed employment. The analysis included adjustments for age, ethnicity, smoking, parity, pelvic inflammatory diseases, and frequency of sexual intercourse. In women, the fecundability ratio was significantly reduced for periods of toluene exposure (0.47, 95% CI 0.29–0.77), whereas fecundity was not significantly affected in toluene-exposed periods in male workers and their partners. Female workers in this study were exclusively employed in areas of printing facilities (stacking and book binding), with air concentrations expected to be lower than areas of high (operating printing machines) and medium (preparing cylinders) toluene exposure. Men were employed in all three areas.

Several studies of blood levels of reproductive hormones in repeatedly exposed workers have not provided strong and consistent evidence of exposure-related effects. No statistically significant changes were observed in serum FSH, LH, or testosterone levels in 1,225 male rotogravure workers exposed to

median toluene concentrations of 24 ppm (printers) or 4.5 ppm (non-printers) for at least 20 years, compared with 109 unexposed referents (Gericke et al. 2001). Significantly decreased serum levels of LH, FSH, and testosterone were found in 20 male toluene-exposed rotogravure printers, compared with 44 unexposed referents (Svensson et al. 1992a). TWA air concentration estimates for the exposed workers during the period when blood was sampled ranged from 8 to 111 ppm, with a median of 36 ppm. Increasing workplace air concentrations were not significantly ($p>0.05$) associated with plasma concentrations of LH, FSH, testosterone, or prolactin, after adjustments for age, in a study of 47 male toluene-exposed printers from two factories (Svensson et al. 1992b). Median serum levels of these hormones in exposed workers were not significantly different from median levels in 46 unexposed referents (Svensson et al. 1992b). TWA air concentrations for the exposed workers during the period when blood was sampled ranged from 1 to 108 ppm (median 11 ppm) in one factory and from 1 to 142 ppm (median 47 ppm) in the second factory (Svensson et al. 1992b). In female U.S. Air Force personnel with mixed exposure to fuel (primarily JP-8 jet fuel) and several solvents, pre-ovulatory LH levels were significantly reduced compared with unexposed female U.S. Air Force personnel (Reutman et al. 2002). No air exposure levels were reported; instead, mean breath concentrations of aromatic and aliphatic hydrocarbons were measured. While mean toluene breath concentrations in the unexposed and exposed group were reported (1.3 and 9 ppb, respectively), analysis was only conducted for the combined concentrations of BTEX (3.8 and 73.5 ppb, respectively). Since no toluene-specific analysis was conducted, and toluene only accounted for 12% of the BTEX breath concentration in the exposed group, it cannot be determined if toluene exposure influenced preovulatory LH levels in this study.

Environmental Human Exposure. In a population-based cohort study, the potential associations between exposure to air pollutants and adverse birth outcomes (preterm birth, term low birth weight, and small for gestational age) were evaluated in 15,284 mothers who gave birth between 2008 and 2012 in Halifax, Nova Scotia (Poirier et al. 2015). The median (range) toluene concentration in Urban Halifax during this time period, determined using land-use regression-modeled air pollution data, was 0.4 (0.2–1.1) $\mu g/m^3$ (0.0001 [0.00005–0.003] ppm). A significant increased risk of preterm birth was observed in mothers exposed to the 2nd, 3rd, or 4th quartile of toluene, compared with mothers exposed to the 1st quartile of toluene exposure, before and after adjustment for mother's age, parity, and smoking. However, after adjustment for age, parity, smoking, and neighborhood income, the risk was only significantly elevated in mothers in the 2nd quartile (OR 1.35, 95% CI 1.12–1.63). Interquartile ranges were not reported; however, following piecewise logistic regression breakpoint analysis for exposure to toluene and preterm birth, a cut point of 0.36 $\mu g/m^3$ (95% CI 0.33–0.40) was determined. Mothers exposed to toluene levels above the cut point were at a significantly higher risk of preterm birth after

adjustment for age, parity, smoking, and neighborhood income (OR 1.19, 95% CI 1.01–1.39). No significant increases in risk of low birth weight or small for gestational age were observed with increasing toluene exposure. This study suggests that low-level toluene exposure may increase the risk of preterm birth; however, the likelihood of concurrent exposure to multiple pollutants (which were not controlled for in statistical analyses) limits the conclusions that can be drawn from this study.

Controlled Human Exposure. LH, FSH, and testosterone levels were determined in blood samples collected at 20-minute intervals from five men, five women in the luteal phase of the menstrual cycle, and five women in the follicular phase, before, during, and after a 3-hour exposure to 0 or 50 ppm (Luderer et al. 1999). Analysis of variance in data for men and women indicated no statistically significant direct effects of exposure on any hormone end point, with the exception of a significant interaction between exposure and sampling period for LH levels in men, indicating a greater LH decline during toluene exposure than clean-air exposure. Interpretation of these reproductive hormone data from a biological adversity perspective is constrained by the lack of data on reproductive function and the variance across studies in applying adjustments for confounding factors such as age, smoking and alcohol consumption.

Intentional Human Exposure. A single case report of testicular atrophy and aspermia involving chronic solvent abuse was located (Suzuki et al. 1983).

Acute-Duration and Gestational Exposure Animal Studies. 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). However, the majority of gestational exposure studies in rodents did not report exposure-related changes in reproductive end points (e.g., number of litters, gestational age, implantations, pre- or post-implantation loss, number of live and dead pups, sex ratio) at concentrations ranging from 50 to 12,000 ppm (API 1978, 1991, 1992; Bowen and Hannigan 2013; Bowen et al. 2005, 2007, 2009a, 2009b; Courtney et al. 1986; Dalgaard et al. 2001; Hougaard et al. 2003; Jones and Balster 1997; Klimisch et al. 1992; Ladefoged et al. 2004; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997), despite evidence for maternal toxicity (decreased maternal weight gain) at concentrations as low as 1,200 ppm (API 1991; Dalgaard et al. 2001; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997). However, 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 (Ungvary and Tatrai 1985). Additionally, rats exposed to 5,000 ppm 6 hours/day during days 6–15 of pregnancy resulted in increased post-implantation loss and complete fetal resorption in 6/9 litters (API 1992). These effects were not observed at 133 ppm in rabbits or

≤3,500 ppm toluene in rats (API 1992; Ungvary and Tatrai 1985). Another rat study found no exposure-related effects on mating, fertility, or pregnancy indices for F1 rats that had been exposed *in utero* to 0, 300, 600, 1,000, or 1,200 ppm toluene for 6 hours/day during GDs 9–21 (Thiel and Chahoud 1997).

Other studies examining reproductive effects in offspring following exposure to toluene during gestation are discussed in Section 3.2.1.6, Developmental Effects.

Intermediate-Duration Animal Studies. 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 epididymides 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 to 3,000 ppm toluene 8 hours/day, 6 days/week for 12 weeks resulted in a statistically significant 23% decrease in mean seminiferous tubule diameter, decreased immunohistological staining for proliferating cell nuclear antigen (PCNA) in spermatogonia and early-stage spermatocytes, decreased spermatogenesis, increased apoptosis in testes, and various histological and ultrastructural abnormalities in testes (Kanter 2011b). In contrast, no statistically significant changes in numbers of spermatogenic cells at various stages in seminiferous tubules, testes weight, testicular histology, or serum hormone levels were found in rats exposed to 4,000 or 6,000 ppm 2 hours/day for 5 weeks, compared with controls, but significant changes in sperm parameters were found in rats exposed to 6,000 ppm (Ono et al. 1999). Changes included a 66% decrease in sperm head counts, a 78% decrease in motility, and a 76% decrease in ovum penetration (Ono et al. 1999). In rats exposed to 0 or 1,500 ppm 4 hours/day for 20 days, no exposure-related effects were revealed by histological examination of testis and epididymis sections, by immunohistochemical analysis of testis and epididymis for heat shock protein 70 (HSP70), c-Fos protein, and PCNA, or on testis or epididymis weights, although body weights of exposed rats were significantly lower than controls (Ishigami et al. 2005). In rats exposed to paint thinner (66% toluene) at toluene concentrations of 1,500 ppm for 2 hours for up to 30 days, significantly reduced seminiferous tubule diameter, testicular weight, and serum and testicular testosterone levels were found (Yilmaz et al. 2006). Other components in paint thinner (acetone, isobutyl acetate, butyl glycol, and isobutanol) may have contributed to these effects.

Exposure of male mice to concentrations of 100 or 400 ppm for 8 weeks did not induce dominant lethal mutations or cause pre- and post-implantation losses after they were mated with nonexposed females (API

1981). There were no treatment-related histopathological lesions in the testes of rats or mice exposed to up to 3,000 ppm toluene for 14–15 weeks although rats showed a 15% increase in testes weight (NTP 1990). In 2-generation reproduction studies in rats, exposure of males and females to concentrations of 100, 500, or 2,000 ppm 6 hours/day for up to 95 days did not adversely affect reproductive parameters (e.g., fertility) or offspring survival compared with unexposed controls (API 1985; Roberts et al. 2003). No effects on reproductive performance or offspring survival were found when males only or females only were exposed to 2,000 ppm by a similar protocol and mated to unexposed partners (Roberts et al. 2003). Additionally, no effects on pregnancy outcomes or offspring survival were observed following exposure to 1,200 ppm 6 hours/day from GD 7 to PND 18 (Dalgaard et al. 2001; Hass et al. 1999).

Chronic-Duration Animal Studies. Toluene did not cause altered weight or histopathological lesions of the ovaries or testes in rats exposed to toluene concentrations up to 300 ppm toluene for 24 months (CIIT 1980) or in rats or mice at concentrations up to 1,200 ppm for 2 years (NTP 1990).

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 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

Overview. A number of published reports have described birth defects, similar to those associated with fetal alcohol syndrome, in children born to women who intentionally inhaled large quantities of toluene or other organic solvents during pregnancy (Arnold and Wilkins-Haug 1990; Arnold et al. 1994; Erramouspe et al. 1996; Goodwin 1988; Hersh 1988; Hersh et al. 1985; Lindemann 1991; Pearson et al. 1994; Wilkins-Haug and Gabow 1991a). These reports suggest that exposure to high concentrations of toluene during pregnancy can be toxic to the developing fetus. 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, which suggested that solvent exposure may increase risk for central nervous system anomalies and neural tube closure defects (Holmberg 1979). A number of developmental toxicity studies with rats, mice, and rabbits involving toluene exposure by inhalation during gestation have been conducted to further describe developmentally toxic effects from toluene and exposure-response relationships. The results indicate that toluene did not cause maternal or developmental toxic effects in animals at exposure levels <1,000 ppm administered for 6–7 hours/day during gestation (API 1978, 1991, 1992; Jones and Balster 1997; Klimisch et al. 1992; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997; Tsukahara et al. 2009; Win-Shwe et

al. 2012a, 2012b; Yamamoto et al. 2009). Predominant effects reported at concentrations ranging from 1,000 to 3,000 ppm included retarded fetal growth and skeletal development, altered development of behavior in offspring, and were almost always accompanied by signs of maternal toxicity (API 1991, 1992; Dalgaard et al. 2001; Hass et al. 1999; Hougaard et al. 2003; Jones and Balster 1997; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997). Other animal studies reported that continuous, 24-hour/day exposure during gestation caused maternal body weight depression and effects on fetuses including depressed body weight and delayed skeletal ossification at toluene concentrations as low as 133–399 ppm in rats, mice, and rabbits (Hudak and Ungvary 1978; Ungvary and Tatrai 1985). Performance deficits in a few neurobehavioral tests were observed in one study of offspring of pregnant mouse dams exposed by inhalation to 2,000 ppm, but not to 200 or 400 ppm, for 60 minutes 3 times/day on GDs 12–17 (Jones and Balster 1997). Performance deficits were not observed in offspring of pregnant rat dams exposed by inhalation to concentrations up to 2,000 ppm for 6 hours/day during gestation (Hougaard et al. 2003; Ono et al. 1995; Thiel and Chahoud 1997). Impaired learning and memory, increased malformations, and fetal death have been observed when animals are exposed during gestation to higher concentrations modeling solvent abuse (8,000–16,000 ppm, 15–30 minutes/day) (Bowen and Hannigan 2013; Bowen et al. 2005, 2009a; Callan et al. 2015).

Human Studies. 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).

Studies of women exposed during pregnancy to much lower concentrations of toluene in the workplace are restricted to a single study. A retrospective study of 14 women in Finland occupationally exposed to mixed solvents (some of which included toluene), as well as 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).

While these findings suggest that occupational exposure to solvents may be toxic to the developing fetus, the small sample size coupled with multiple chemical exposures cannot support definitive conclusions regarding developmental toxicity of exposure to low toluene levels in humans.

Standard Developmental Toxicity Studies in Animals. Numerous studies in rats, mice, and rabbits with inhalation exposure to concentrations <1,000 ppm toluene for 1–8 hours/day during various gestational periods have found no exposure-related effects on maternal and developmental end points (API 1978, 1991, 1992; Jones and Balster 1997; Klimisch et al. 1992; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997). Exposure to 1,000–3,500 ppm during gestation resulted in decreased fetal/pup weight and/or decreased neonatal growth in a number of studies (API 1991, 1992; Dalgaard et al. 2001; Hass et al. 1999; Hougaard et al. 2003; Ladefoged et al. 2004; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997). Significantly decreased maternal body weight gain was observed at exposure levels causing decreased weight or growth in offspring, except in studies by Hass et al. (1999), which reported no significant change in maternal body weight gain at 1,200 ppm and Thiel and Chahoud (1997), which reported fetal body weight effects at 1,000 ppm and maternal body weight effects at 1,200 ppm. Additional developmental effects observed in these studies include delayed ossification in rat fetuses following exposure to 3,000 ppm for 6 hours/day from GD 6 to 15 (API 1991; Roberts et al. 2007), increased postnatal/preweaning mortality in rat pups following exposure to 1,200 ppm for 6 hours/day from GD 9 to 21 (Thiel and Chahoud 1997), and total resorption of rat litters with exposure to 5,000 ppm for 6 hours/day from GD 6 to 15 (API 1992). In another study, exposure of pregnant mice to 200 or 400 ppm, 7 hours/day on GDs 7–16 produced significantly increased litters with fetuses with enlarged renal pelvises 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). Similarly, exposure of pregnant mice to 133 or 266 ppm, 3–4 hours/day on GDs 6–15, did not significantly affect maternal or fetal survival, or incidences of fetuses with visceral or skeletal anomalies or malformations. However, incidences of fetuses with decreased body weight and skeletal retardations were significantly increased in the group exposed to 266 ppm (Ungvary and Tatrai 1985).

A series of studies indicate that continuous, 24-hour/day exposure during gestation can cause maternal and developmental toxicity at toluene concentrations as low as 133–399 ppm in rats, mice, and rabbits (Hudak and Ungvary 1978; Ungvary and Tatrai 1985). In pregnant rats exposed to 399 ppm, 24 hours/day on GDs 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 exposure on GDs 1–8, and 2/21 dams died and increased incidences of skeletal anomalies (extra ribs, fused sternebrae) were found with exposure on GDs 9–14 (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 GDs 1–21, but significantly increased incidence of fetuses with skeletal retardation occurred (Hudak and Ungvary 1978). In mice exposed to 133 ppm, 24 hours/day on GDs 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 GDs 6–13 (Hudak and Ungvary 1978). In rabbits exposed to 133 ppm, 24 hours/day on GDs 7–20, no significant effects were found on maternal or fetal survival, fetal body weight, or incidences of fetuses with skeletal retardation, minor anomalies, skeletal or visceral malformations (Ungvary and Tatrai 1985). Following exposure to 266 ppm by the same protocol, 2/8 rabbit dams died, 4/8 dams aborted, and no live fetuses were found at sacrifice (Ungvary and Tatrai 1985).

Studies of Reproductive System Development in Animals. Studies of inhalation exposure of rats to concentrations as high as 1,800 ppm for 6 hours/day during gestation and/or early postnatal periods have provided little evidence of adverse effects on reproductive performance in adulthood. Female offspring of dams exposed to 1,200 ppm toluene, 6 hours/day on GDs 9–21 showed a significant delay in reproductive development (vaginal opening) compared with unexposed controls, but no significant exposure-related effects on mating, fertility, or pregnancy indices were observed in groups of male and female F1 offspring exposed during gestation to 300, 600, 1,000, or 1,200 ppm (Thiel and Chahoud 1997). In another study, no exposure-related changes were observed in the appearance of sexual maturation landmarks in male or female rat offspring of dams exposed to 1,200 ppm toluene, 6 hours/day on GD 7 to PND 18 (Hass et al. 1999). No significant changes in the percent of motile sperm or any parameters of sperm motility on PND 100 were observed in male offspring of dams exposed to 1,200 ppm toluene, 6 hours/day from GD 7 to PND 18 (Dalgaard et al. 2001). In male offspring of dams exposed to 1,800 ppm toluene, 6 hours/day from GD 7 to 20, no significant changes in testicular weight or histology at PND 11, 21, or 90 were found, compared with controls (Dalgaard et al. 2001). Additionally, no

significant changes in serum prolactin or LH were observed in 8-week-old male rats following neonatal exposure to 80 ppm for 6 hours/day from PND 1 to 7, compared with controls (von Euler et al. 1989). However, Tsukahara et al. (2009) reported that nose-only exposures of 0.09–9 ppm for 90 minutes/day in dams from GD 14.5 to 18.5 resulted in statistically significant decreased fetal serum testosterone and 3β-HSD steroidogenic enzyme immunoreactivity in fetal testes in the 0.9 and 9 ppm groups, and significantly decreased 3β-HSD mRNA levels in the 0.9 ppm group only. These effects were more pronounced at 0.9 ppm than 9 ppm and were not accompanied by any histological changes in the fetal testes; mechanistic understanding is inadequate to determine biologically adversity of the observed effects.

Studies of Neurological Development in Animals. Gestational exposure by inhalation to toluene concentrations as high as 1000–1,500 ppm did not affect the performance of offspring in neurobehavioral tests in several animal studies (Jones and Balster 1997; Ladefoged et al. 2004; Ono et al. 1995; Thiel and Chahoud 1997); at 2,000 ppm, one study reported some statistically significant performance deficits in mouse offspring (Jones and Balster 1997), whereas another found no statistically significant deficits in rat offspring (Ono et al. 1995).

Mouse pups from dams exposed to 2,000 ppm for 60 minutes, 3 times/day on GDs 12–17 gained less weight between PND 2 and 8 compared with unexposed controls, and showed statistically significant increased latency of righting reflex on PNDs 1, 5, and 6, decreased forelimb grip strength on PNDs 5–7 and 9–11, and increased latency to climb in the inverted screen test on PNDs 14–17 (Jones and Balster 1997). No statistically significant effects on these end points were observed after exposure to 200 or 400 ppm by the same protocol, and no effects on times to reach developmental landmarks such as incisor eruption and eye opening were noted in any of the exposure groups (Jones and Balster 1997).

In another study, offspring of rat dams exposed to 600 or 2,000 ppm, 6 hours/day on GDs 7–17 showed no statistically significant differences from control rats in postnatal viability or physical development through PND 21, tests of reflexes on PNDs 6–10, locomotor activity during postnatal week 4, balance on a rotating rod during postnatal week 7, or learning ability in the Biel water maze test during postnatal week 6 (Ono et al. 1995).

No consistent, concentration-dependent performance deficits were found in tests of reflexes on PND 3, balance on a rotating rod on PND 18, locomotor activity on PNDs 31–34, or discrimination learning on

PNDs 70–81 in rat offspring of dams exposed to 300, 600, 1,000, or 1,200 ppm, 6 hours/day on GDs 9–21, compared with controls (Thiel and Chahoud 1997).

No changes were observed in Morris water maze spatial learning and memory in female offspring following exposure to 1,500 ppm for 6 hours/day from GD 7 to 20, compared with controls (Ladefoged et al. 2004).

Exposure of pregnant rats to 1,200 ppm toluene, 6 hours/day from GD 7 to PND 18 produced statistically significant decreased postnatal growth (PNDs 0–10), delayed ontogeny of reflexes between PND 2 and 13, and increased open-field locomotor activity on PND 28 in male and female offspring, compared with unexposed controls (Hass et al. 1999). Significantly increased latency to find the hidden platform in the Morris water maze test after platform relocation was found in13-week-old exposed female offspring, compared with nonexposed female offspring, when data were analyzed on an individual basis, but not on a litter basis. No consistent significant effects were observed in rotarod performance on PNDs 22–26, auditory brainstem responses at 4 months, or in physical development (e.g., eye opening) on PND 14 (Hass et al. 1999).

Decreased neonatal growth, impaired auriculonasocephalic reflex (reflexive movement to thermal and olfactory stimuli, e.g., “dummy dam”), impaired ability to locate a visible platform in the Morris water maze, and impaired learning in the passive avoidance task were reported for neonatal rats exposed by inhalation to 500 ppm on PNDs 7–30 (Museridze et al. 2010). However, the available report did not indicate the daily exposure duration used in this study, provided no indication that air concentrations were measured during the exposure periods, and used mongrel rats.

Significant localized changes in dopamine and noradrenaline neurotransmitter levels and utilization were observed in 8-week-old adult male rats that were exposed to 80 ppm for 6 hours/day from PND 1 to 7, compared with controls (von Euler et al. 1989). Neurotransmitter levels were increased in some areas of the brain, were decreased in some areas, and remained the same in other areas. Localized changes in dopamine and noradrenaline neurotransmitter levels and utilization were also observed in 8-week-old rats exposed both neonatally on PNDs 1–7 and again for 3 days during postnatal week 8 to 80 ppm (6 hours/day), compared with previously unexposed 8-week-old rats (von Euler et al. 1989). The biological adversity of region-specific neurotransmitter alterations in the absence of neurobehavioral changes is unknown.

Biochemical and cellular changes in brain tissues have been reported in several studies of animals exposed to toluene by inhalation during gestation or during early postnatal periods. In rats, offspring of dams exposed to 1,500–1,800 ppm during gestation had elevated levels of cerebellar apoptosis during postnatal development (Dalgaard et al. 2001; Ladefoged et al. 2004) and increased *in vitro* generation of reactive oxygen species in cultured synaptosomes (Edelfors et al. 2002), and neonatal rats exposed to 100–500 ppm had reversible decreases in the volume of the granular cell layer in the dentate of the hippocampus (Slomianka et al. 1990). In mice, offspring of dams exposed to 400 ppm had elevated LDH activity on PND 21 (Courtney et al. 1986). Mechanistic understanding is inadequate to determine the biological adversity of these effects.

Animal Studies Modeling High Concentration Solvent Abuse. A series of studies in rats were designed to model human solvent abuse during pregnancy to determine if repeated brief exposures to very high concentrations during gestation could cause adverse developmental effects. These studies found that exposure to 8,000–16,000 ppm for 15–30 minutes twice daily from GD 8 to 20 caused decreased maternal body weight gain; decreased pup/fetal weight, length, and postnatal growth; decreased placental weight; increased number of litters with malformed, runted, or dead pups; impaired motor coordination in offspring; impaired learning and memory in offspring, and altered reward-seeking behavior with increased impulsivity in offspring (Bowen and Hannigan 2013; Bowen et al. 2005, 2007, 2009a, 2009b; Callan et al. 2015; Jarosz et al. 2008). No changes were found in the achievement of physical landmarks (Bowen and Hannigan 2013; Bowen et al. 2005).

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 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Human Studies. Numerous 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. Five cohort studies involved occupationally exposed workers exposed predominantly to toluene (Antilla et al. 1998; Lehman and Hein 2006; Svensson et al. 1990; Walker et al. 1993; Wiebelt and Becker 1999), 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; Gao et al. 2014; Gérin et al. 1998; Heck et al.

2013, 2014, 2015; Lundberg and Milatou-Smith 1998; Olsson and Brandt 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 mortality ratio, 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 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. In a follow-up study of this cohort, an excess of lung cancer deaths persisted with additional follow-up through 1999 (SMR 1.36, 95% CI 1.19-1.54, n=248), but the relative risk of lung cancer still did not increase with increasing duration of employment (Lehman and Hein 2006). No other cancers showed excess risk in this follow-up.

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 nonsignificant 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, three cases). Antilla et al. (1998) noted, however, that these workers may also have been exposed to benzene.

Wiebelt and Becker (1999) examined cancer incidence and mortality in a cohort of 6,830 German males employed in rotogravure printing plants for at least one year between 1960 and 1992, and compared them to mortality and cancer incidence for West Germany. Workers were divided into three work areas: printing/proof printing (<100 ppm after 1985, <200 ppm 1960–1985), printing cylinder preparation (<30 ppm) and finishing (<30 ppm). When the three groups were examined together, no statistically significant increases in overall cancer risk or risk for cancers at specific tissue sites were associated with exposure to toluene. When analyzed by work area, a strong increase in mortality from bone and connective tissue cancers was found in the workers from the highest exposure group (printers) based on a small number of observed cases. SMRs for bone and connective tissue cancers were 8.1 (95% CI 1.4–32.4; three cases) and 6.3 (95% CI 1.2–25.9; three cases), respectively. Wiebelt and Becker (1999) noted that the significance of these findings is unclear as they have not been previously associated with toluene exposure. Nonsignificant increases in mortality from lung cancer were observed in printers (SMR 1.3, 95% CI 0.7–2.5) and finishers (SMR 1.8, 95% CI 0.8–4.4); however, the risk was greater in the group with the lower toluene exposure levels.

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; Gao et al. 2014; Gérin et al. 1998; Heck et al. 2013, 2014, 2015; 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 report possible associations between Hodgkin’s disease in Swedish patients and controls (Olsson and Brandt 1980), childhood leukemia in Chinese or Californian patients and controls (Gao et al. 2014; Heck

et al. 2014), childhood retinoblastoma (but not neuroblastoma) in Californian patients and controls (Heck et al. 2013, 2015), and esophageal and rectal cancer in Canadian patients and controls (Gérin et al. 1998).

Animal Studies. 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 B6C3F1 mice exposed to toluene concentrations up to 1,200 ppm for 6.5 hours/day, 5 days/week for 2 years (Huff 2003; 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; Gibson and Hardisty 1983). 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.

3.2.2 Oral Exposure

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

3.2.2.1 Death

Human Case Study. 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.

Acute-Duration Animal Studies. 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).

Intermediate-Duration Animal Studies. 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 3-3 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

Overview. Human data pertaining to the systemic effects of oral exposure to toluene are limited to four case studies (Ameno et al. 1989; Caravati and Bjerk 1997; Einav et al. 1997; Malingre et al. 2002). Animal data are also limited, but include evidence for cardiovascular, hematological, hepatic, renal, and body weight effects in rats and mice exposed orally to toluene at dosage levels ranging from 312 to 2,500 mg/kg/day for 13 weeks (NTP 1990), hepatic effects in mice exposed to 105 mg/kg/day for 28 days (Hsieh et al. 1989), and hepatic effects in rats exposed to 5,200 mg/kg/day for up to 45 days (Kamel and Shehata 2008). However, no cardiovascular, hematological, hepatic, or renal effects were reported in rats exposed to 590 mg/kg/day for 6 months (Wolf et al. 1956). Acute exposure to single doses ≥1,000 mg/kg have resulted in cardiovascular and hepatic effects (Ayan et al. 2013; Gordon et al. 2010; Tas et al. 2013b). 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 3-3 and plotted in Figure 3-2.

Respiratory Effects.

Human Case Studies. Lung congestion and hemorrhage were reported in one case report involving lethal ingestion of approximately 625 mg/kg toluene by an adult male (Ameno et al. 1989). A 15-month-old girl who accidently ingested paint thinner was intubated after presenting with severe central nervous system depression (Malingre et al. 2002). Extubation the following day resulted in serious respiratory stridor and bronchoscopy showed mucosal lesions and pronounced edema. The patient was re-intubated for an additional 8 days. No residual damage was observed following extubation (Malingre et al. 2002). No additional studies were located regarding respiratory effects in humans after oral exposure to toluene.

Animal Studies. 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 590 mg/kg/day for 6 months (Wolf et

Table 3-3 Levels of Significant Exposure to Toluene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Human	once				625 (death in 30 minutes)	Ameno et al. 1989	Case report.
2	Rat (Sprague-Dawley)	NS (G)				5568 (LD50 young adult rat)	Kimura et al. 1971	
3	Rat (Sprague-Dawley)	NS (G)				6438 (LD50 adult rat)	Kimura et al. 1971	
4	Rat (Sprague-Dawley)	NS (G)				2610 (LD50 14-day-old rat)	Kimura et al. 1971	
5	Rat	NS (G)				7300 (LD50)	Smyth et al. 1969	
6	Rat	once (G)				5580 M (LD50 adult rats)	Withey and Hall 1975	
7	Rat (Wistar)	once (G)				7000 (LD50 young adult rats)	Wolf et al. 1956	

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
8	Human	once	Resp			625 M (lung congestion and hemorrhage)	Ameno et al. 1989	Case report.
			Cardio			625 M (necrosis of myocardial fibers)		
			Gastro	625 M				
			Hepatic			625 M (enlarged liver)		
			Renal			625 M (acute tubular necrosis)		
9	Rat (Wistar)	once	Hepatic		5200 M (Slight degeneration of hepatocytes and mononuclear cell infiltration; increased serum AST, ALT; increased apoptosis)		Ayan et al. 2013	
10	Rat (Long- Evans)	once (GO)	Cardio	1200 M			Gordon et al. 2007	Observed tachycardia and hypertension attributed to increased locomotion; no change in ECG.
11	Rat (Brown Norway)	once (GO)	Cardio	600 M	1000 M (6, 8, and 13% decrease in relative heart weight in young, middle aged, and aged rats)		Gordon et al. 2010	
			Bd Wt	1000 M				

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
12	Rat (Sprague-Dawley)	Gd 6-19 1x/d (GO)	Bd Wt			520 F (24% decrease in maternal body wt gain)	Gospe et al. 1994	
13	Rat (Wistar)	once (G)	Cardio		5200 M (congestion, edema and apoptosis in cardiac tissue)		Tas et al. 2013b	
14	Rat (Sprague-Dawley)	Gd 16-19 (GO)	Hepatic	1250 F			Warner et al. 2008	Hepatic NOAEL is for maternal liver weight and histology.
			Renal		1250 F (swollen tubules, tissue adhesion to Bowman's capsule, areas of solidification within glomeruli)			
			Bd Wt	1250 F				

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Mouse (B6C3F1)	14 d (GO)	Resp	600 F			Burns et al. 1994	Endpoints examined: organ weight and histology, clinical chemistry, hematology.
			Hemato		600 F (increased reticulocytes)			
			Hepatic	600 F				
			Renal	600 F				
			Bd Wt	600 F				
Immuno/ Lymphoret								
16	Mouse (B6C3F1)	14 d (GO)		600 F			Burns et al. 1994	Endpoints examined: spleen and thymus weight and histology, bone marrow cell count, immune function. assays, host-resistance assays
Neurological								
17	Human	once				625 M (severe central nervous system depression)	Ameno et al. 1989	Case report.
18	Rat (Long- Evans)	once (GO)			250[b] M (decrease in amplitude in FEP N3 peak)		Dyer et al. 1988	
19	Rat (Long- Evans)	once (GO)		400 M	800 M (increased locomotor activity)		Gordon et al. 2007	

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
20	Rat (Brown Norway)	once (GO)		300 M	650 M (increased locomotor activity)		Gordon et al. 2010	
21	Rat (Sprague-Dawley)	once (G)			2610 M (increase in motor activity, lacrimation and salivation)		Mehta et al. 1998	
22	Mouse (B6C3F1)	14 d (GO)		600 F			Burns et al. 1994	Endpoints examined: brain weight and histology.
Reproductive								
23	Rat (Sprague-Dawley)	Gd 6-21 (GO)		650 F			Gospe and Zhou 2000	Endpoints evaluated: litter size.
24	Rat (Sprague-Dawley)	Gd 16-19 (GO)		1250 F			Warner et al. 2008	Endpoints evaluated: number of corpora lutea, implantations, pre- and post-implantation loss, resorptions, and live fetuses/litter.
25	Mouse (CD-1)	Gd 7-14 1x/d (GO)		2350 F			NIOSH 1983	Endpoints evaluated: number viable and totally resorbed litters.

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
26	Mouse (ICR)	Gd 8-12 5 d (G)		1800 F			Seidenberg et al. 1986	Endpoints evaluated: litter size.
Developmental								
27	Rat	Gd 6-19				650 (delayed brain development of fetus)	Gospe and Zhou 1998	Gd 19: decreased brain volume, cell size, number of nuclei, and myelination; Pnd 21: decrease myelination (no standard dev't endpoints assessed).
28	Rat (Sprague-Dawley)	Gd 6-21 (GO)				650 (decreased neurogenesis and altered migration of cortical neurons during development)	Gospe and Zhou 2000	Endpoints assessed: litter means for body and brain weight, generation and migration of cortical neurons.
29	Rat (Sprague-Dawley)	Gd 6-19 1x/d (GO)			520 (9.4% reduction in fetal weight)		Gospe et al. 1994	
30	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	

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
31	Rat (Sprague-Dawley)	Gd 16-19 (GO)			1250 (increased incidence of grade 3 or 4 left renal pelvis dilation)		Warner et al. 2008	
32	Mouse (CD-1)	Gd 7-14 1x/d (GO)		2350			NIOSH 1983	Endpoints evaluated: total litter weights, number of viable and totally resorbed litters, number of live and dead pups per litter.
33	Mouse (ICR)	Gd 8-12 5 d (G)		1800			Seidenberg et al. 1986	Endpoints evaluated: litter size, litter weight, external malformations of dead neonates.
INTERMEDIATE EXPOSURE								
Death								
34	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)				2500 (8/10 males and 1/10 females died)	NTP 1990	
35	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)				2500 M (4/10 died) 1250 F (1/10 died)	NTP 1990	

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
36	Rat (albino)	45 d (G)	Hepatic		650 M (increased levels of markers for hepatic apoptosis and oxidative stress)		Kamel and Shehata 2008	Organ weight and histology were not assessed.
			Renal		650 M (increased levels of markers for renal oxidative stress)			

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
37	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)	Resp	2500			NTP 1990	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	1250 M 625 F	2500 M (38% increase in relative heart weight) 1250 F (11% 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)			

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
38	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				
39	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				
40	Mouse (CD-1)	28 d (W)	Hemato	84 M			Hsieh et al. 1990a	Hepatic and renal NOAELs are for organ weight and gross pathology.
			Hepatic	84 M				
			Renal	84 M				
			Bd Wt	84 M				

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Mouse (CD-1)	28 d (W)	Bd Wt	105 M			Hsieh et al. 1990b	
42	Mouse (CD-1)	28 d (W)	Endocr	22 M	105 M (increased serum corticosterone and ACTH)		Hsieh et al. 1991	
			Bd Wt	105 M				

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
43	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)	Resp	2500			NTP 1990	Endpoints evaluated: body and organ weight, organ histology, hematology, clinical chemistry, urinalysis.
			Cardio	2500		5000 (myocardial degeneration)		
			Gastro	2500				
			Hemato	2500				
			Musc/skel	2500				
			Hepatic	625 M	1250 M (10% increase in relative liver weight)			
					312 F (7% increase in relative liver weight)			
			Renal	2500				
			Endocr	2500				
			Bd Wt	625 M 2500 F	1250 M (body weight 16% lower than controls)			
Immuno/ Lymphoret								
44	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)		2500			NTP 1990	Endpoints evaluated: spleen and thymus weight and histology.

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
45	Mouse (CD-1)	28 d (W)		22[c] M	105 M (decreased thymus weight; diminished response in immune assays)		Hsieh et al. 1989	
46	Mouse (CD-1)	28 d (W)		22[c] M	84 M (diminished response in immune assays)		Hsieh et al. 1990a	
47	Mouse (CD-1)	28 d (W)		22[c] M	105 M (decreased interleukin-2 immune response)		Hsieh et al. 1991	
48	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)		2500			NTP 1990	Endpoints evaluated: spleen and thymus weight and histology.
Neurological								
49	Rat (albino)	15, 30, or 45 d (G)			650 M (increased levels of markers for cortical and cerebellar apoptosis and oxidative stress)		Kamel and Shehata 2008	
50	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)		625		1250 (brain necrosis)	NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
51	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)		625 M 1250 F	1250 M (12% increase in relative brain weight)	2500 (ataxia, hypoactivity, prostration)	NTP 1990	Endpoints assessed: brain weight and histology, clinical signs.
Reproductive								
52	Rat (albino)	15, 30, or 45 d (G)			650 M (increased levels of markers for testicular oxidative stress)		Kamel and Shehata 2008	Reproductive organ weight and histology were not assessed. Reproductive function was not assessed.
53	Rat (Fischer- 344)	13 wk 5 d/wk 1x/d (GO)		2500			NTP 1990	Endpoints evaluated: organ weight and histology; reproductive function not assessed.
54	Mouse (B6C3F1)	13 wk 5 d/wk 1x/d (GO)		625 M 2500 F	1250 M (7% increase in relative testicular weight)		NTP 1990	Endpoints evaluated: organ weight and histology; reproductive function not assessed.

Table 3-3 Levels of Significant Exposure to Toluene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Developmental								
55	Mouse (Hybrid)	Gd 0-21 + Ppd 0-55 (W)		21	106 (increased open-field activity; lack of habituation)		Kostas and Hotchin 1981	

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

b Used to derive an acute oral minimal risk level (MRL); dose (250 mg/kg/day) 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

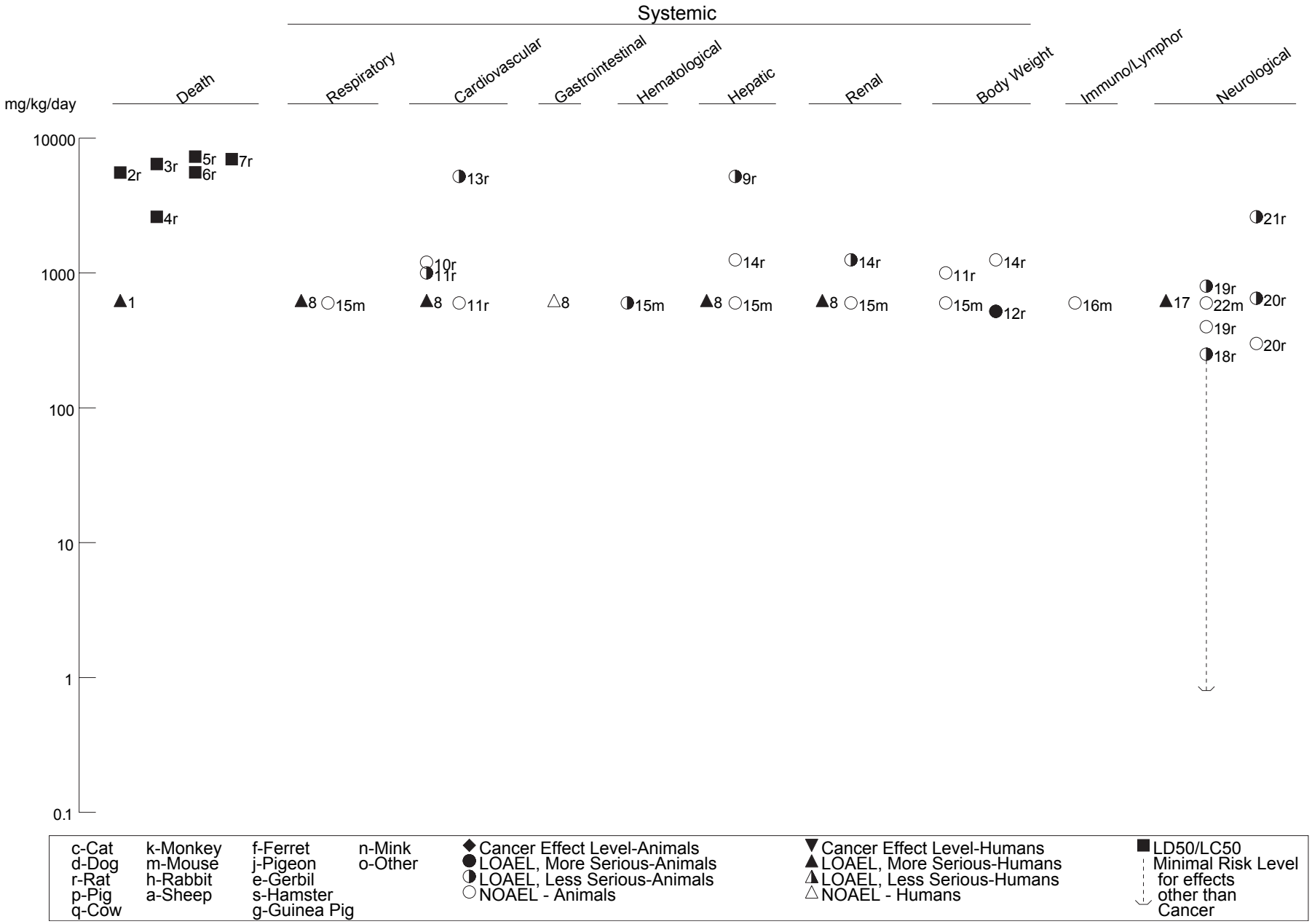
c Used to derive an intermediate oral minimal risk level (MRL) along with 2 companion studies supporting a NOAEL of 22 mg/kg/day for immunological effects; dose divided by an uncertainty factor of 100 (10 for interspecies differences in response and 10 for human variability), resulting in an MRL of 0.2 mg/kg/day

ACTH = adrenocorticotropic hormone; ALT = alanine amino transferase; AST = aspartate aminotransferase; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); ECG = electrocardiogram; Endocr = endocrine; F = Female; FEP = flash evoked potential; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Ppd = post-parturition day; Resp = respiratory; x = time(s); (W) = drinking water; wk = week(s)

3. HEALTH EFFECTS

Figure 3-2 Levels of Significant Exposure to Toluene - Oral

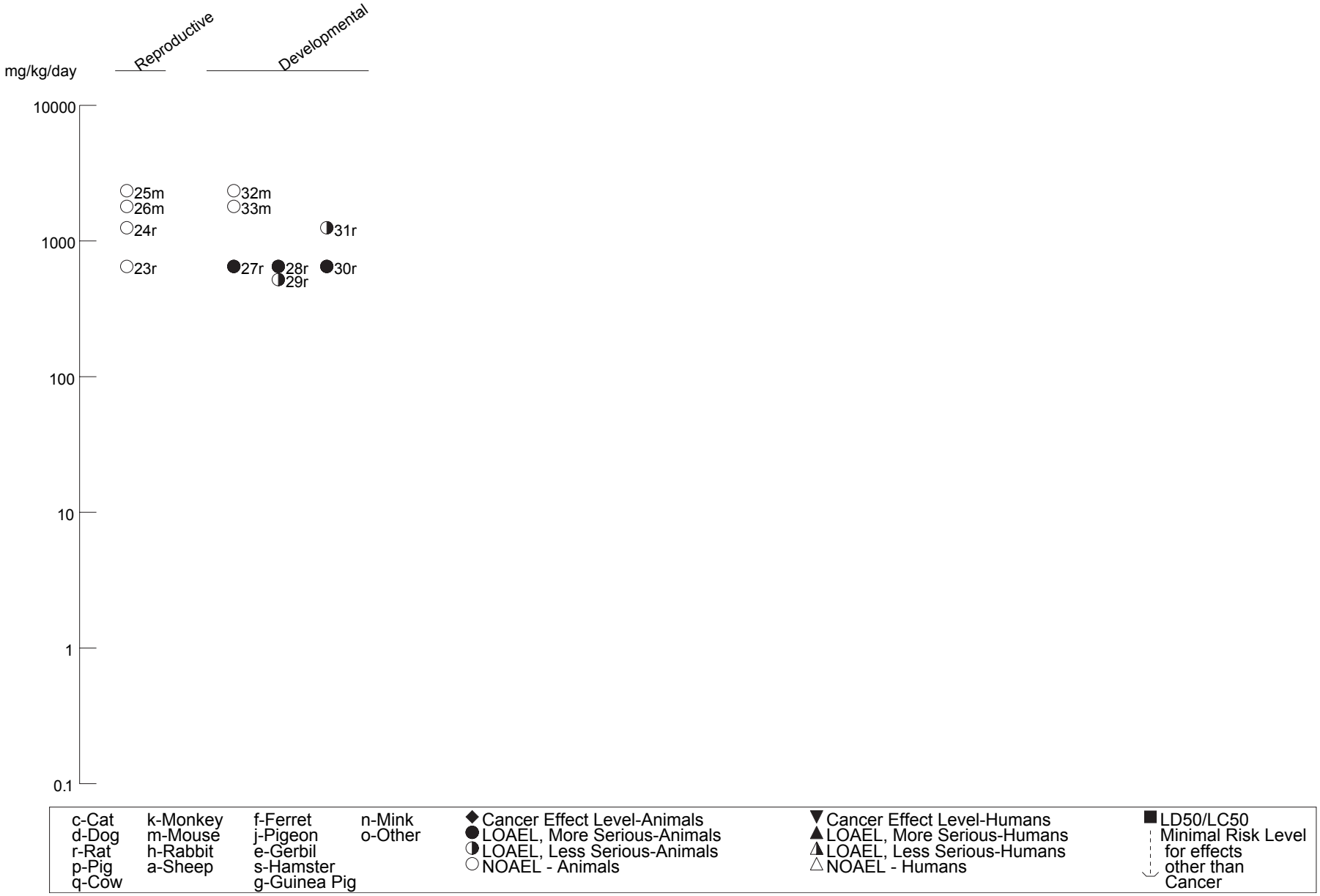
Acute (≤14 days)



3. HEALTH EFFECTS

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

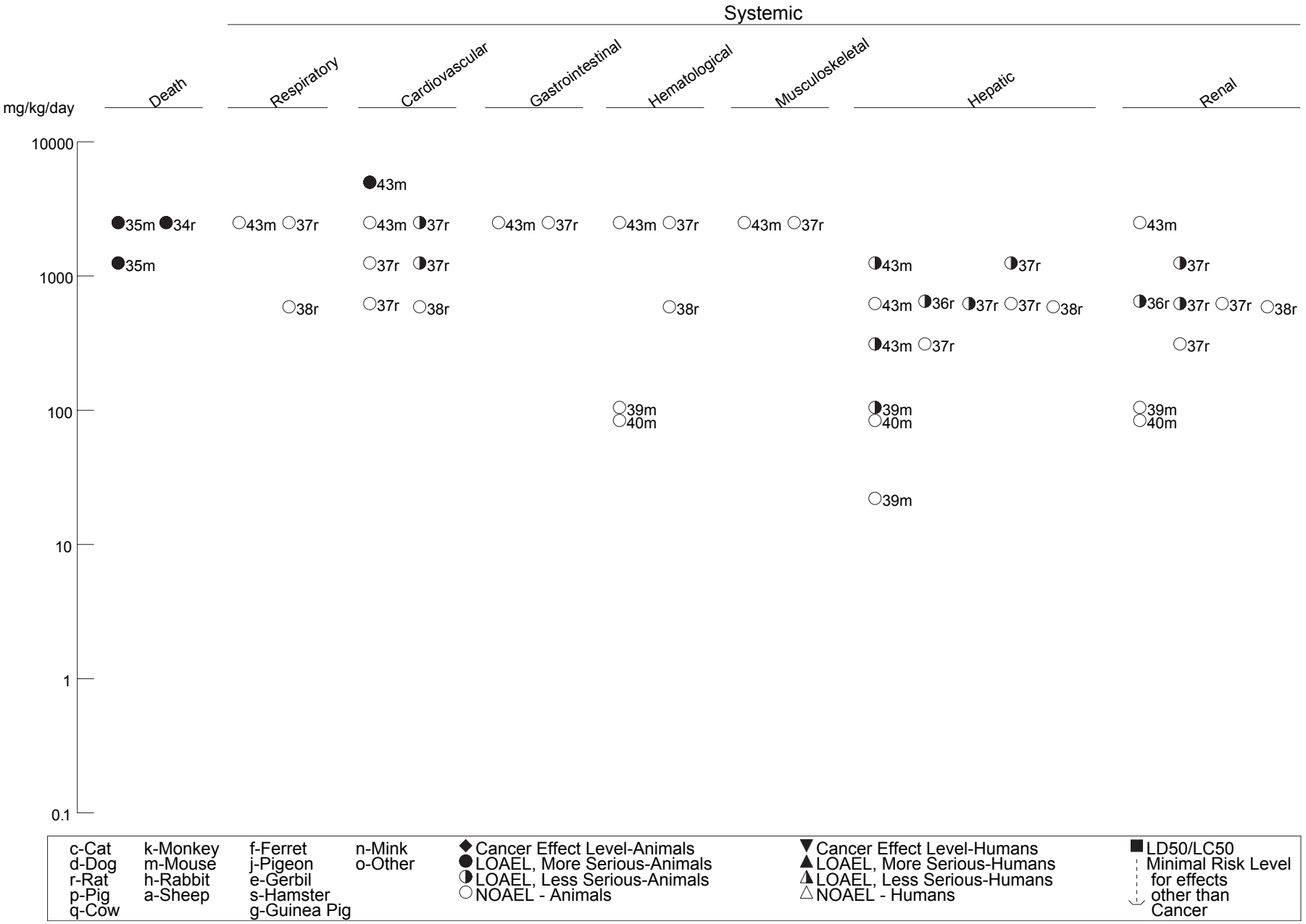
Acute (≤14 days)



3. HEALTH EFFECTS

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

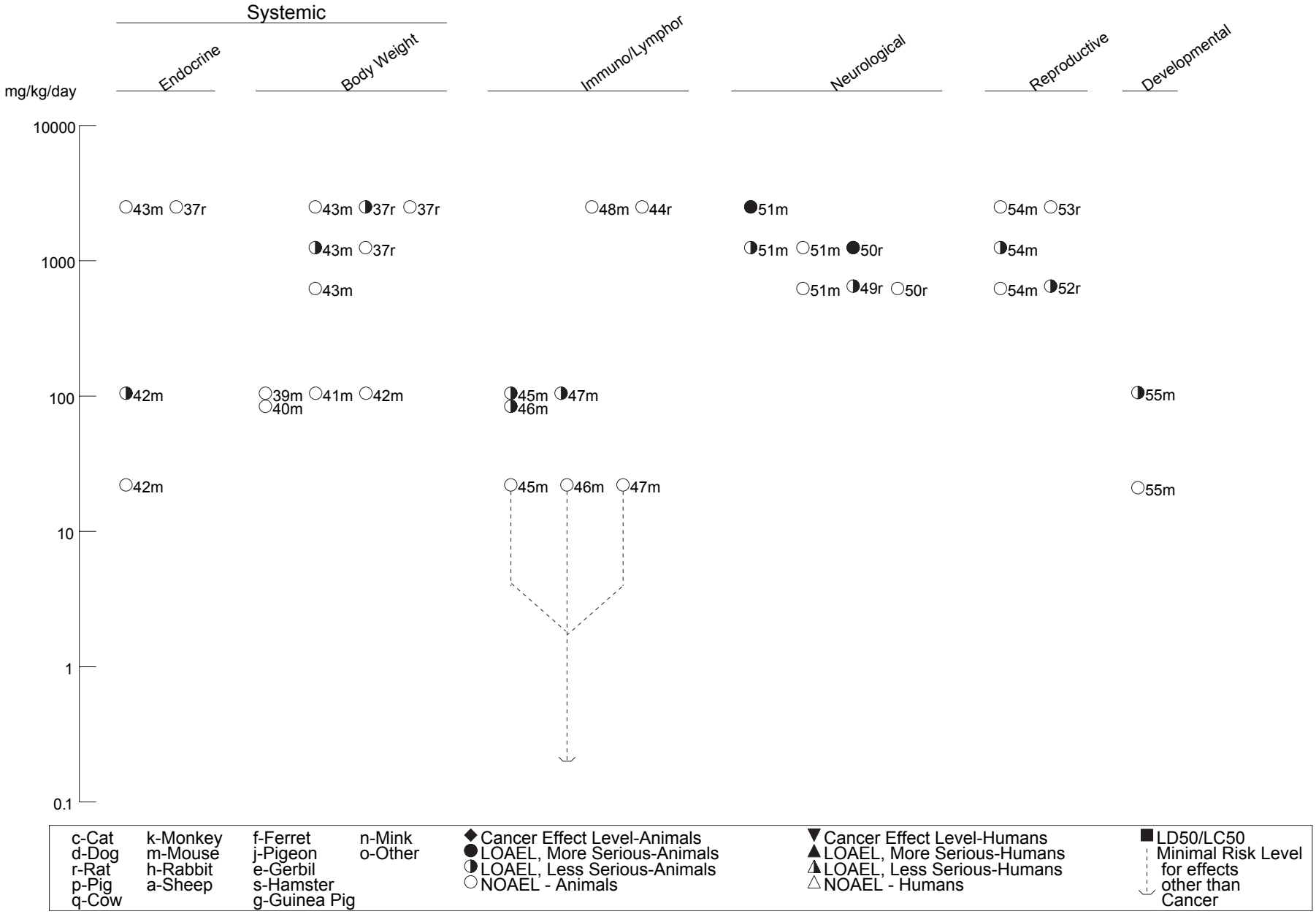
Intermediate (15-364 days)



3. HEALTH EFFECTS

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

Intermediate (15-364 days)



al. 1956). No changes in lung weight or histology were reported in female mice exposed to 600 mg/kg/day via gavage for 14 days, compared with controls (Burns et al. 1994).

Cardiovascular Effects.

Human Case Studies. 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 (Einav et al. 1997). No cardiovascular effects were reported in a 15-month-old girl following ingestion of paint thinner (Malingre et al. 2002).

Acute-Duration Animal Studies. Cardiac edema and congestion were observed in rats given single gavage doses of 5,200 mg/kg, compared with controls (Tas et al. 2013b). The number of TUNEL-labeled cardiac cells were significantly increased by ~7-fold in treated rats and semi-quantitative caspase-3 labeling was increased, compared with controls, indicating increased apoptosis in heart tissue. Blood levels of troponin T, a marker for ischemic heart damage, were also significantly increased 14-fold in toluene-exposed rats, compared with controls. No significant changes in heart rate or blood pressure were observed (Tas et al. 2013b).

In a multi-dose study, rats were given single oral doses of 0, 400, 800, and 1,200 mg/kg in random order, with 72 hours between each dose (Gordon et al. 2007). Heart rate and blood pressure were significantly elevated following the 800 and 1,200 mg/kg doses for ~1 hour, compared with the vehicle control dose; however, these findings coincided with significant, dose-related increases in motor activity. Therefore, they likely do not represent adverse cardiac effects of toluene exposure. No significant treatment-related findings were observed in electrocardiograms (Gordon et al. 2007), indicating a 1,200 mg/kg NOAEL for cardiac effects.

Transient increases in heart rate and motor activity were also observed in young, middle aged, and aged rats following single gavage doses of 0, 300, 650, or 1,000 mg/kg, and no treatment-related histopathological changes were observed in cardiac tissue in any age group (Gordon et al. 2010). However, relative heart weights of young, middle aged, and aged rats given 1,000 mg/kg were statistically significantly reduced by 6, 8, and 13%, respectively, compared with controls, indicating adverse cardiac effects. Several changes in iron content, enzyme activities and mRNA levels for genes involved in thrombosis, vasoconstriction, and inflammation were also noted in cardiac tissue by Gordon

et al. (2010). Activity of aconitase was significantly decreased in the young and middle aged rats at 1,000 mg/kg, activity of ferritin was significantly increased in the middle aged rats at 1,000 mg/kg, and activity of mitochondrial ubiquinone reductase was significantly increased in the middle-aged rats at 650 and 1,000 mg/kg. No exposure-related changes in other cardiac biochemical end points (superoxide dismutase, glutathione transferase, glutathione, mitochondrial aconitase, mitochondrial isocitrate dehydrogenase, or mitochondrial ferritin) were found. Additionally, cardiac mRNA levels for genes involved in thrombosis, oxidative stress, vasoconstriction, and inflammation were assessed in cardiac tissue from rats in the control and 1,000-mg/kg groups. Statistically significant alterations in mRNA levels, compared with controls, included: decreased tissue factor (TF, a thrombosis marker) in exposed young rats; increased macrophage inflammatory protein-2 (MIP-2, an inflammatory marker) and decreased endothelin-1 and endothelin receptor-B (ET-1, ET-B, vasoconstriction markers) in exposed middle-aged rats; and decreased MIP-2 and ET-1 in exposed aged rats. Overall, exposure-induced changes in relative heart weight and in biochemical end points and gene expression in cardiac tissue did not reveal clear age-related effects.

Intermediate-Duration Animal Studies. 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. Gastric pain was reported by a man who accidently ingested 30 mL of an organic solvent containing toluene and other chemicals (Einav et al. 1997). Gastrointestinal effects were not reported in other case studies of oral exposure (Ameno et al. 1989; Malingre et al. 2002).

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 erythrocytes, hemoglobin, hematocrit, leukocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC) in female mice exposed to 600 mg/kg/day via gavage for 14 days, compared with controls (Burns et al.

1994). However, reticulocytes were significantly increased by 87%, compared with controls (Burns et al. 1994). 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 significant changes were found in the concentrations of erythrocytes, leukocytes, lymphocytes, or neutrophils in male mice administered 22 or 84 mg/kg/day toluene in their drinking water for 28 days, compared with controls (Hsieh et al. 1990a). 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.

Human Case Studies. 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). Clinical chemistry did not reveal abnormal liver function in a 15-month-old girl following accidental ingestion of paint thinner (Malingre et al. 2002).

Acute-Duration Animal Studies. Slight degeneration of hepatocytes and mononuclear cell infiltration were observed in rats given single gavage doses of 5,200 mg/kg, compared with controls (Ayan et al. 2013). The number of TUNEL-labeled hepatic cells were significantly increase by ~6-fold in exposed rats and semi-quantitative Bax and caspase-3 labeling was increased, compared with controls, indicating increased apoptosis in hepatic tissue. Serum levels of AST and ALT were significantly increased in toluene-treated rats by 119 and 60%, respectively, compared with controls (Ayan et al. 2013). However, no alterations in liver weight or histology were reported in pregnant rats exposed to 1,250 mg/kg/day toluene via gavage from GD 16 to 19 (Warner et al. 2008) or female mice exposed to 600 mg/kg/day toluene via gavage for 14 days (Burns et al. 1994), compared with controls.

Intermediate-Duration Animal Studies. In male 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). In a separate study, 22 or 84 mg/kg/day in drinking water for 28 days did not alter liver weight in exposed male mice, compared with controls (Hsieh et al. 1990a). Relative liver weights increased significantly over control levels in mice administered toluene by gavage for 13 weeks with doses ≥312 mg/kg/day in females and ≥1,250 mg/kg/day in males (NTP 1990). In female rats, the liver weights were increased by exposure to doses ≥1,250 mg/kg/day and in male rats by exposure to doses ≥625 mg/kg/day. No treatment-related gross or histopathological lesions of the liver were reported by NTP (1990) in these 13-week studies. 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).

Increased hepatic cell apoptosis, indicated by increased caspase-3 activity, and changed markers of oxidative stress were observed in the liver of rats exposed to 650 mg/kg/day via gavage for 45 days, compared with controls (Kamel and Shehata 2008). Exposed rats showed significant increases in hepatic levels of thiobarbituric acid reactive substances (end products of lipid peroxidation), glutathione disulfide, and glutathione-S-transferase activities, as well as significantly decreased reduced glutathione levels and glutathione reductase activities. Effects were more pronounced at 45 days than after 15 or 30 days of exposure. No exposure-related changes were seen in hepatic activities of superoxide dismutase, glutathione peroxidase, or the inflammation marker COX-2 (Kamel and Shehata 2008).

Renal Effects.

Human Case Studies. Acute tubular necrosis was reported after a lethal exposure to 625 mg/kg (Ameno et al. 1989), and acidosis was noted in a nonlethal case report of thinner consumption (Caravati and Bjerk 1997). Clinical chemistry did not reveal abnormal kidney function in a 15-month-old girl following accidental ingestion of paint thinner (Malingre et al. 2002).

Acute-Duration Animal Studies. Evidence for renal pathology was reported in dams exposed to 1,250 mg/kg/day toluene via gavage from GD 16 to 19 (Warner et al. 2008). Kidneys from toluene-exposed dams demonstrated swollen tubules, tissue adhesion to Bowman’s capsule, and areas of solidification within glomeruli that were not observed in control dams. No exposure-related changes were observed in kidney weight. No changes in kidney weight or histology were reported in female mice exposed to 600 mg/kg/day via gavage for 14 days, compared with controls (Burns et al. 1994).

Intermediate-Duration Animal Studies. There were no changes in kidney weight for male mice administered doses of 5–105 mg/kg/day in drinking water for 28 days (Hsieh et al. 1989, 1990a) 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 ≥625 mg/kg/day 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 6 months (Wolf et al. 1956).

Minor changes in several markers of oxidative stress were observed in kidneys of rats following exposure to 650 mg/kg/day for 15, 30, or 45 days via gavage (Kamel and Shehata 2008). Exposure resulted in significant increases in renal levels of thiobarbituric acid reactive substances and glutathione disulfide and significantly decreased glutathione reductase activity, compared with controls. Effects were most pronounced after 45 days of exposure. No exposure-related changes were seen in renal levels of reduced glutathione or activities of superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, COX-2 or caspase-3 (Kamel and Shehata 2008).

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

Serum corticosterone and ACTH levels were significantly elevated in male mice exposed to 105 mg/kg/day toluene in drinking water for 28 days, compared with controls (Hsieh et al. 1991). Levels were not significantly elevated following exposure to 5 or 22 mg/kg/day. 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.

Acute-Duration Animal Studies. There were no dose-related changes in body weight for male rats following single gavage administration of 0, 300, 650, or 1,000 mg/kg (Gordon et al. 2010). No body weight effects were reported in female mice exposed to 600 mg/kg/day via gavage for 14 days, compared with controls (Burns et al. 1994). There was also no change in maternal body weight gain in rat dams exposed to 1,250 mg/kg/day toluene via gavage from GD 16 to 19, compared with controls (Warner et al. 2008). However, maternal weight gain was 24% lower in rats given 520 mg/kg/day toluene by gavage from GD 6 to 19, compared with control rats (Gospe et al. 1994).

Intermediate-Duration Animal Studies. 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, 1990a, 1990b, 1991). 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 (NTP 1990). 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).

3.2.2.3 Immunological and Lymphoreticular Effects

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

A series of studies investigated the effects of exposure to toluene in drinking water for 28 days on the immune system end points in male mice (Hsieh et al. 1989, 1990a, 1991). Thymus weights, mixed lymphocyte culture responses, and antibody PFC responses were decreased at doses of 105 mg/kg/day, and mitogen-stimulated lymphocyte proliferation and IL-2 immunity were depressed by doses of 22 and 105 mg/kg/day, compared with controls (Hsieh et al. 1989). A dose of 5 mg/kg/day had no effect upon any of these indicators of immune system function. In another study, IL-2 immune response was significantly decreased only at 105 mg/kg/day (no other immune end points were evaluated) (Hsieh et al. 1991). In another study, antibody PFC responses were depressed at doses of 84 mg/kg/day, and mixed lymphocyte culture responses were depressed at 22 or 84 mg/kg/day (Hsieh et al. 1990a). No significant changes were observed in thymus weight, mitogen-stimulated lymphocyte proliferation, IL-2 immunity, or cell-mediated cytotoxicity at doses up to 84 mg/kg/day (Hsieh et al. 1990a). Collectively, these studies

support a NOAEL of 22 mg/kg/day for immune effects, as consistent immune effects were not observed until dose levels of 84–105 mg/kg/day. 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 NOAEL of 22 mg/kg/day for immune effects based on the studies by Hsieh (1989, 1990a, 1991) was used as the basis for the intermediate-duration oral MRL (0.2 mg/kg/day) (see Section 2.3 and Appendix A).

In an acute-duration 14-day study, neither diminished *in vitro* immune responses nor impaired host-resistance was observed in female mice exposed to 600 mg/kg/day toluene via gavage, compared with vehicle controls (Burns et al. 1994). Spleen and thymus weights and histology were also not altered in exposed animals.

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 3-3 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Human Case Studies. 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). A man who accidently ingested 30 mL of an organic solvent containing toluene and other chemicals was drowsy and complained of dizziness (Einav et al. 1997). In a nonlethal case study, depressed consciousness, lethargy, hypotonia, and nystagmus were observed in a 15-month-old girl following accidental ingestion of paint thinner (Malingre et al. 2002).

Acute-Duration Animal Studies. 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).

Dose-related increases in motor activity were observed in rats given single oral doses of 0, 400, 800, and 1,200 mg/kg in random order, with 72 hours between each dose (Gordon et al. 2007). Changes were transient in nature, lasting ~60 minutes, and were statistically significant at 800 and 1,200 mg/kg, compared with the vehicle control. Similarly, transient dose-related increases in motor activity were observed in young (4 months), middle-aged (12 months), and aged (24 months) male rats given single gavage doses of 0, 300, 650, or 1,000 mg/kg (Gordon et al. 2010). Changes were only statistically significantly increased in young rats given 650 or 1,000 mg/kg and aged rats given 1,000 mg/kg.

In another study, 24-month-old rats showed more pronounced increased horizontal activity in response to single gavage doses of 650 or 1,000 mg/kg toluene than younger rats (1, 4, or 12 months of age) (MacPhail et al. 2012).

Two studies have examined changes in oxidative stress markers (Kodavanti et al. 2011) and gene expression (Royland et al. 2012) in rats following single gavage doses of 0, 650, or 1,000 mg/kg toluene, but mechanistic understanding is inadequate to determine the biological adversity of the observed changes. Kodavanti et al. (2011) measured levels of reactive oxygen species (NQO1, UBIQ-RD), total antioxidant substances, enzymes involved in antioxidant homeostasis (super oxide dismutase, gamma-glutamylcysteine synthetase, glutathione transferase, glutathione peroxidase, and glutathione reductase) and markers of oxidative damage (total aconitase and protein carbonyls) in the frontal cortex, cerebellum, striatum, and hippocampus of young, middle-aged, and aged rats 4 hours after single gavage doses of 0, 650, or 1,000 mg/kg toluene. Multiple indicators of oxidative stress were found to increase with toluene exposure, but findings varied greatly between brain regions and age groups. Multivariate analysis indicated that 12-month-old “middle-aged” rats were more susceptible to oxidative damage in the frontal cortex and cerebellum than younger or older age groups (Kodavanti et al. 2011). Microarray analysis of the hippocampi of young, middle-aged, and aged rats following single gavage doses of 0, 650, or 1,000 mg/kg indicated that only two genes reached a significant threshold (>1.25-fold, dose-related change) with toluene exposure, but 56 genes demonstrated a significant age-toluene interaction (Royland et al. 2012). Toluene-related genes were *Lrpap1* and *Ralgps2*, which are both associated with dementia and Alzheimer’s. Age-toluene interaction genes include those involved in immune response, cytoskeleton, protein and energy metabolism, and oxidative stress.

No changes in brain weight or histology were reported in female mice exposed to 600 mg/kg/day via gavage for 14 days, compared with controls (Burns et al. 1994).

Outer hair cell loss was observed in the area of the cochlea responsive to medium frequencies (10–25 kHz) of rats exposed to 780 mg/kg toluene via gavage 5 days/week for 2 weeks (Gagnaire and Langlais 2005). Apical and basal portions of the cochlea were spared. Cell counts were determined with cochleograms and using electron microscopy, but the available report did not specify whether or not unexposed controls were assessed in this study.

Single doses of 250–1,000 mg/kg administered by gavage to male rats caused a decrease in the FEP wave pattern amplitudes (Dyer et al. 1988). This suggests that toluene may have an effect on the visual system at high doses. The minimally adverse LOAEL of 250 mg/kg for the effects of FEP waveform was used as the basis for the acute-duration oral MRL (0.8 mg/kg) (see Section 2.3 and Appendix A).

Intermediate-Duration Animal Studies. 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 six 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. An additional study confirmed increased NE, but not VMA, in the hypothalamus using the same protocol (Hsieh et al. 1991). Again, 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 (no other neurotransmitters were examined). Due to the lack of dose response, lack of information on persistence of changes, and unclear association with neurobehavior, it cannot be determined if these changes are adverse. Therefore, a NOAEL/LOAEL was not established for the brain biochemical changes reported in this study.

Exposure to 1,250 and 2,500 mg/kg/day for 13 weeks resulted in increased relative brain weights in male mice (NTP 1990). Cellular necrosis was present in the hippocampus and cerebellum of 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 (NTP 1990).

In a study of rats exposed to 0 or 650 mg/kg/day for 15, 30, or 45 days, exposed rats showed increased caspase-3 activity and changed markers of oxidative stress in the cerebellum and cerebral cortex, compared with unexposed controls (Kamel and Shehata 2008). Altered markers of oxidative stress included increased levels of thiobarbituric acid reactive substances and glutathione disulfide, increased activity of superoxide dismutase, and decreased levels of reduced glutathione and activities of glutathione reductase and glutathione peroxidase. No changes were seen in the activities of glutathione-S-transferase or the inflammation marker COX-2 (Kamel and Shehata 2008).

Studies examining neurotoxic effects of gestational exposure during critical periods of neurodevelopment are discussed in Section 3.2.2.6, Developmental Effects.

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

3.2.2.5 Reproductive Effects

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

There was no significant difference in the mean number of implantations per dam, corpora lutea per dam, live fetuses per litter, the total number of resorptions per dam, or pre- or post-implantation loss in pregnant rats exposed to 1,250 mg/kg on GDs 16–19, compared with controls (Warner et al. 2008). Similarly, there was no effect on the number of mice producing viable litters following oral administration of 2,350 mg/kg on GDs 7–14 (NIOSH 1983). Litter size was not changed in mice administered 1,800 mg/kg on GDs 8–12 (Seidenberg et al. 1986) or rats administered 650 mg/kg on GDs 6–21 (Gospe and Zhou 2000).

Increased relative testicular weights were reported in male mice exposed to 1,250 and 2,500 mg/kg/day by gavage for 13 weeks (NTP 1990). However, no effects on the weight of the prostate, testes, uterus, or ovaries were observed in rats or female mice exposed to 312–2,500 mg/kg/day (NTP 1990). Reproductive performance was not evaluated in these 13-week studies.

Changes in several biochemical markers of oxidative stress were observed in testes of rats following exposure to 650 mg/kg/day for 15, 30 or 45 days via gavage (Kamel and Shehata 2008). Testicular levels of glutathione disulfide were increased and levels of reduced glutathione and glutathione reductase activity were significantly decreased, compared with control values. No exposure-related changes were seen in activities of superoxide dismutase, glutathione peroxidase, or glutathione-S-transferase, levels of thiobarbituric acid reactive substances or activities of COX-2 or caspase-3 (Kamel and Shehata 2008).

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 3-3 and plotted in Figure 3-2.

3.2.2.6 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 in two developmental screening studies (NIOSH 1983; Seidenberg et al. 1986). No changes were observed in litter size, litter weight, or external malformations of dead neonates following exposure to 1,800 mg/kg/day via gavage on GDs 8–12, compared with vehicle controls (Seidenberg et al. 1986). Similarly, no changes were observed in total litter weights, numbers of animals producing litters and with totally resorbed litters, or number of live and dead pups per litter following exposure to 2,350 mg/kg/day via gavage on GDs 7–14, compared with vehicle controls (NIOSH 1983).

In a comprehensive developmental toxicity study in rats, a statistically significant increase in the incidence of a dilated renal pelvis in the left kidney was observed in fetuses from dams exposed to 1,250 mg/kg/day on GDs 16–19 via gavage, compared with controls (Warner et al. 2008). Renal pelvis dilation was graded from 1 to 4, with grade 1 representing a normal kidney and grade 4 showing >50% dilation. The incidence of fetuses with grade 3 or 4 dilated left and right renal pelvis in treated fetuses was 14.6 and 12.4%, respectively, compared with respective control incidences of 5.5 and 8.9%. Incidences of litters with fetuses with grade 3 or 4 renal pelvis dilation were 7/8 for left kidney for exposed versus 2/8 in controls and 6/8 for right kidney in exposed versus 5/8 in controls. No changes were observed in any other soft-tissue anomalies, malformations, skeletal variations, ossification, or fetal body weight. Warner et al. (2008) also reported accelerated development of the cochlea in treated fetuses accompanied by increased numbers of apoptotic cells in the cochlea, compared with controls. The

biological significance of this finding is unknown. Based on the result of apoptosis analysis in the cochlea, the investigators suggested that toluene may induce excessive cell death resulting in premature maturation of the cochlea.

Following exposure of pregnant rats to gavage doses of 520 or 650 mg/kg/day toluene in corn oil on GDs 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 PND 21 (Gospe and Zhou 1998). Cortical cell proliferation and migration were also altered in offspring following exposure of pregnant rats to gavage doses of 650 mg/kg/day toluene in corn oil on GDs 6–19 (Gospe and Zhou 2000). Cortical cell density was significantly decreased by 12.5% in all layers of the cerebral cortex in toluene-exposed pups on PND 21, compared with controls. The greatest decrease (26.8%) was observed in layer IV. Decreased density was attributed to altered neurogenesis, as neurons labeled with bromodeoxyuridine (BrdU) from injections on GDs 13–21 were decreased in numbers and exhibited altered migration patterns (Gospe and Zhou 2000).

Neurological development was assessed in mice exposed to 0, 4, 21, or 106 mg/kg/day toluene from GD 0 through PND 55 (via their dams during gestation and lactation and drinking water thereafter) (Kostas and Hotchin 1981). No exposure-related changes were observed in neonatal survival or growth, attainment of physical landmarks (eye opening, pinnae detachment), surface righting, or startle-response. Significantly decreased habituation (i.e., less activity with time in chamber) during a 20-minute open-field activity assessment was observed in mice receiving 106 mg/kg/day toluene, compared with controls. In controls, activity counts during the last 5 minutes of the trial were reduced by ~45% compared with counts during the first 5 minutes; however, activity counts were only decreased by ~17% in mice exposed to 106 mg/kg/day. Habituation was not altered in other exposure groups. Additionally, rotorod performance was impaired during the first two of four consecutive trials in all exposed mice when measured on PNDs 45–55, compared with controls. However, the effect on rotorod performance diminished with increasing dose. Time spent on the rod in the 4, 21, and 106 mg/kg/day groups was decreased by approximately 20, 16, and 8%, respectively, in trial 1 and by 27, 16, and 9%, respectively, in trial 2, compared with controls. Although time spent on the rod was statistically significantly decreased compared with controls at 4 and 21 mg/kg/day for trial 1, and at all exposures for trial 2, the changes are interpreted to be of questionable exposure-related adversity because the magnitude of effect diminished with increasing dose. The lack of impairments in the later trial may be due to compensation by the mice, as suggested by the study authors. The NOAEL and LOAEL values for neurodevelopmental effects in

this study are 21 and 106 mg/kg/day, respectively, based on increased open-field activity and lack of habituation in high-dose mice.

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 3-3 and plotted in Figure 3-2.

3.2.2.7 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.

3.2.3 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 3.2.1). Toxicokinetic data (Section 3.4) indicate that humans and animals can absorb toluene across the skin. Studies of dermal exposure to toluene in humans and animals are discussed below.

3.2.3.1 Death

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

3.2.3.2 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 3-4.

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.

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. Increased complaints of itching and dermatitis of the hands was reported in in 38 female shoemakers who were exposed to toluene vapor concentrations that varied from 65 ppm (15–100 ppm) in winter to 100 ppm (10–200 ppm) in summer for an average of 40 months, compared with 16 controls (Matsushita et al. 1975). 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/day for 3 days, resulted in redness and an increase in epidermal thickness (Anderson et al. 1986). In mice, application of 25 µL undiluted toluene once weekly for 5 weeks to the dorsal surface of the ear lobe resulted in significant ear swelling that peaked at 1 hour post application starting on week 2 (Saito et al. 2011). After the 5-week

Table 3-4 Levels of Significant Exposure to Toluene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
ACUTE EXPOSURE								
Systemic								
Gn Pig (albino)	3 d 3x/d	Dermal		10 M uL	(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		0.1 mL	(eye irritation)		Exxon Chemical Co. 1962	
INTERMEDIATE EXPOSURE								
Systemic								
Mouse (BALB/c)	5 wk 1x/wk	Dermal		25 F uL	(swelling at application site [ear lobe], inflammatory cell invasion)		Saito et al. 2011	No effects were observed with diluted preparations (25 or 50% in acetone).

d = day(s); F = Female; Gn pig = guinea pig; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; x = time(s); wk = weeks(s)

exposure, microscopic examination of the ears revealed marginal inflammatory cell invasion. No significant dermal changes were observed following five applications of diluted toluene (25 or 50% in acetone) (Saito et al. 2011). Collectively, these data suggest that undiluted 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 (Exxon Chemical Co. 1962; Mobil Oil Corp. 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:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 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.

3.3 GENOTOXICITY

Overview. There is no conclusive evidence to support that toluene is a genotoxic agent. Results from human occupational exposure studies are inconsistent, and are limited by small cohort size (15–45/group), lack of historical exposure monitoring, and (in some cases) concurrent exposure to other chemicals. Most short-term *in vivo* tests in laboratory animals have not found genotoxic effects. Similarly, genotoxic effects were not induced in the majority of *in vitro* assays. Results of *in vivo* studies and *in vitro* genotoxicity studies are summarized in Table 3-5 and Table 3-6, respectively.

Human Occupational Studies. Multiple studies have assessed genotoxic end points in printers with occupational exposure predominately to toluene; however, cohort sizes are small and the results are inconsistent between studies. Chromosomal abnormalities (e.g., aberrations, breaks, gaps) have been reported in peripheral lymphocytes of printers exposed to a median time-weighted air level of 150 mg/m^3 (40 ppm) toluene per week (Nise et al. 1991), printers exposed to 104–1,170 ppm toluene and office and technical workers exposed to 2.1–4.3 ppm plus 0–2 hours/day in the rotogravure workshop (Pelclová et al. 1990), printers exposed to 30–1,550 mg/m^3 (8–410 ppm) (Pelclová et al. 2000), and printers exposed to 200–300 ppm toluene (Bauchinger et al. 1982), compared with unexposed referents. Examination of former printers 4 months to 2 years 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). In other studies, exposure-related changes in chromosomal abnormalities were not induced in printers exposed to 7–112 ppm toluene (Maki-Paakkanen et al. 1980) or printers exposed to 56–824 ppm toluene (Forni et al. 1971). Increased sister chromatid exchanges have been reported in peripheral lymphocytes of printers exposed to 200–300 ppm toluene (Bauchinger et al. 1982), printers exposed to a median air toluene concentration of 252 mg/m^3 (67 ppm) (Hammer 2002; Hammer et al. 1998), and paint workers (toluene air levels not quantified; mean urinary hippuric acid levels were 2.5-fold higher in workers compared with unexposed referents) (Priya et al. 2015), compared with unexposed referents. In printers, the number of sister chromatid exchanges was significantly correlated with urinary *para*-cresol levels and the urinary cresol/hippuric acid ratio (Hammer 2002); weak correlations with urinary hippuric acid and *ortho*-cresol were not significant ($0.05<p<0.1$). When smokers were excluded, only the urinary cresol/hippuric acid ratio was significantly associated with the number of sister chromatid exchanges (Hammer 2002). In other studies, exposure-related changes in sister chromatid exchanges were not induced in peripheral lymphocytes from printers exposed to 7–112 ppm toluene (Maki-Paakkanen et al. 1980), or printers exposed to 104–1,170 ppm toluene and

Table 3-5. Genotoxicity of Toluene *In Vivo*

Species (test system)	End point	Results	Reference
Non-mammalian cells:			
Grasshopper (vapor)	Mitotic arrest in embryos (intact chorion)	+	Liang et al. 1983
Mammalian cells:			
Rats (inhalation)	Chromosomal aberrations in bone marrow cells	+	Dobrokhotov and Enikeev 1977
Human[a]	Chromatid breaks and gaps in peripheral lymphocytes	+	Bauchinger et al. 1982
Human[a]	Chromosome changes in peripheral lymphocytes	–	Forni et al. 1971
Human[b]	Chromosomal aberrations in peripheral lymphocytes	–	Haglund et al. 1980
Human[a]	Chromosome changes in peripheral lymphocytes	–	Maki-Paakkenen et al. 1980
Human[a]	Chromosome gaps in peripheral lymphocytes	+	Nise et al. 1991
Human[a]	Aberrant cells and chromosome breaks in peripheral lymphocytes	+	Pelclova et al. 1990
Human[a]	Aberrant cells in peripheral lymphocytes	+	Pelclova et al. 2000
Human[a]	Chromosome aberrations in peripheral lymphocytes	+	Schmid et al. 1985
Human[a]	Chromatid exchanges in peripheral lymphocytes	+	Bauchinger et al. 1982
Human[b]	Sister chromatid exchange in peripheral lymphocytes	–	Haglund et al. 1980
Human[a]	Sister chromatid exchange in peripheral lymphocytes	+	Hammer 2002; Hammer et al. 1998
Human[a]	Sister chromatic exchange in peripheral lymphocytes	–	Maki-Paakanen et al. 1980
Human[b]	Sister chromatid exchange in peripheral lymphocytes	–	Pitarque et al. 2002
Human[a]	Sister chromatid exchange in peripheral lymphocytes	–	Pelclova et al. 1990
Human[a]	Sister chromatid exchange in peripheral lymphocytes	+	Priya et al. 2015
Human[c]	Sister chromatid exchange in peripheral lymphocytes	–	Richer et al. 1993
Human[a]	Sister chromatid exchange in peripheral lymphocytes	–	Schmid et al. 1985
Rats (inhalation)	DNA damage and repair in lymphocytes	–	Martinez-Alfaro et al. 2010
Mice (inhalation)	DNA damage in blood, bone marrow and liver	–	Plappert et al. 1994
Human[d]	DNA damage in peripheral lymphocytes	+	Cok et al. 2004
Human[b]	DNA damage in whole blood	+	Heuser et al. 2005
Human[b]	DNA damage in leukocytes	+	Heuser et al. 2007
Human[b]	DNA damage in whole blood	+	Moro et al. 2012
Human[b]	DNA damage in leukocytes	–	Pitarque et al. 1999
Mice (inhalation)	Micronuclei in erythrocytes	–	Bird et al. 2010
Mice (inhalation)	Micronuclei in bone marrow cells	–	Wetmore et al. 2008

Table 3-5. Genotoxicity of Toluene *In Vivo*

Species (test system)	End point	Results	Reference
Human[b]	Micronuclei in buccal cells	+	Gonzalez-Yebra et al. 2009
Human[b]	Micronuclei in peripheral lymphocytes and buccal cells	–	Heuser et al. 2005
Human[b]	Micronuclei in peripheral lymphocytes and buccal cells	–	Heuser et al. 2007
Human[b]	Micronuclei in buccal cells	–	Moro et al. 2012
Human[a]	Micronuclei in peripheral lymphocytes	+	Nise et al. 1991
Human[b]	Micronuclei in peripheral lymphocytes	+	Pitarque et al. 2002
Mice (inhalation)	Dominant lethal mutations in sperm cells	–	API 1981
Human[c]	Cell cycle delay, cell mortality in peripheral lymphocytes	–	Richer et al. 1993[f]

[a]Occupational exposure to predominantly toluene (printers).
[b]Occupational exposure to mixed solvents (e.g., shoe makers, paint manufacturers).
[c]Controlled exposure in healthy volunteers.
[d]Intentional exposure in chronic glue sniffers.

+ = positive result; – = negative result

Table 3-6. 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
Human HL-60 cells	DNA damage	No data	+	Sarma et al. 2011
Human lymphocytes	Micronuclei	–	–	Zarani et al. 1999

+ = positive result; – = negative result

office and technical workers exposed to 2.1–4.3 ppm plus 0–2 hours/day in the rotogravure workshop (Pelclová et al. 1990). Additionally, increased sister chromatid exchange frequencies were not observed in peripheral lymphocytes from former printers, 4 months to 2 years after cessation of exposure to toluene, compared with 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).

Some studies indicate that exposure to rotogravure ink mist may contribute to genotoxic findings in some rotogravure printers, as they are potential sources of polycyclic aromatic hydrocarbons from carbon black (Hammer et al. 1998; Pelclova et al. 1990, 2000). However, printing inks were not mutagenic in *Salmonella typhimurium* bacterial assays (Pelclova et al. 2000).

Evidence for genotoxicity in occupational studies of industries with exposure to toluene plus other solvents is also inconsistent. Solvent exposure-related changes in chromosome aberrations or sister chromatid exchanges in peripheral lymphocytes were not induced in Swedish paint industry workers exposed to toluene air concentrations ranging from 1 to 1,257 mg/m^3 (1–334 mg/m^3), compared with matched controls (Haglund et al. 1980). Sister chromatid exchanges were also not increased in Bulgarian shoe workers exposed to mean current TWA toluene concentrations of 76 or 236 mg/m^3 toluene (20 or 63 ppm), compared with unexposed referents (Pitarque et al. 2002). DNA damage, assessed by the Comet assay, was increased in whole blood or leukocytes in Brazilian shoe workers exposed to solvent-based adhesive (mainly toluene, air concentration not reported) (Heuser et al. 2005, 2007) and Mexican painters exposed to unreported concentrations of toluene (Moro et al. 2012), compared with unexposed controls. However, no exposure-related differences in DNA damage in leukocytes were found between Bulgarian shoe workers exposed to 96.0–412.3 mg/m^3 toluene (28–121 ppm) and unexposed controls, as assessed by the Comet Assay (Pitarque et al. 1999). Increased micronuclei induction was observed in peripheral lymphocytes or buccal cells in Bulgarian shoe workers exposed to mean current TWA toluene concentrations of 76 or 236 mg/m^3 toluene (20 or 63 ppm) (Pitarque et al. 2002) and Mexican shoe workers exposed to median toluene air concentrations of 31.6 mg/m^3 (8 ppm) (Gonzalez-Yebra et al. 2009), compared with controls. Using multivariate analysis of age, BMI, smoking, alcohol consumption, exposure duration, and exposure levels of acetone, ethyl acetate, methyl ethyl ketone, and toluene in Mexican shoe workers, the only variable statistically significantly associated with micronuclei induction was toluene concentration (Gonzalez-Yebra et al. 2009). However, micronuclei induction was not

associated with solvent exposure in Brazilian shoe makers exposed to solvent-based adhesive (Heuser et al. 2005, 2007) or Mexican painters exposed to unreported concentrations of toluene (Moro et al. 2012).

Human Solvent Abuse Study. DNA damage, determined by the Comet assay, was significantly increased in peripheral lymphocytes of chronic glue sniffers, compared with age-matched controls (Cok et al. 2004). However, when only nonsmokers were assessed, there was no difference in the mean total comet score between glue sniffers and controls. Exposure levels are unknown; however, the mean value of the hippuric acid and *ortho*-cresol excretion rates for glue sniffers were 73- and 1,582-fold higher, respectively, than in controls.

Controlled Exposure Study. 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 three occasions at 2-week intervals (Richer et al. 1993).

Animal Studies. Most *in vivo* tests in laboratory animals have not reported toluene-induced genotoxicity. 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 8 weeks, as assessed by Comet assay (Plappert et al. 1994). Similarly, DNA damage and repair were not induced in lymphocytes of rats exposed to paint thinner composed of toluene, acetone, ethanol, isobutyl acetate, isobutanol, butyl glycol, ethyl-benzene, and trimethylbenzene at toluene levels of 3,000 ppm twice daily for 15 minutes for 6 weeks, as assessed by Comet assay and H_2O_2 damage and repair (Martinez-Alfaro et al. 2010). Toluene did not induce micronuclei formation in bone marrow cells or erythrocytes of mice following exposure to 100 ppm 6 hours/day for 8 days (Bird et al. 2010; Wetmore et al. 2008). Toluene did not induce dominant lethal mutations in sperm cells 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). In a Russian study, toluene was reported to induce chromosomal aberrations in the bone marrow cells of rats following chronic inhalation exposure (duration and concentrations not available) (Dobrokhotov and Enikeev 1977). Additionally, when grasshopper (*Melanoplus sanguinipes*) embryos (chorion intact) were suspended in sealed containers and exposed to toluene vapors of 0, 40,000, 200,000, 400,000, or 800,000 ppm for 16 hours, mitotic arrest was induced at 200,000 ppm (higher concentrations were lethal) (Liang et al. 1983).

In vitro Studies. Toluene did not induce gene mutations in bacterial cells with or without metabolic activation (Bos et al. 1981; Fluck et al. 1976; Connor et al. 1985; Nakamura et al. 1987; Nestmann et al. 1980; NTP 1990), sister chromatid exchange or chromosomal aberrations in human lymphocytes without

metabolic activation (Gerrner-Smidt and Friedrich 1978; NTP 1990), or micronuclei in human lymphocytes with or without metabolic activation (Zarani et al. 1999). DNA damage was increased in human promyelocytic leukemia HL-60 cells treated with 1.14 and 2.74 nM toluene without metabolic activation, compared with untreated controls, as evidenced by significant, dose-related increases in tail moment in the Comet assay (Sarma et al. 2011).

3.4 TOXICOKINETICS

Overview. Studies with volunteers and laboratory animals indicate that toluene is rapidly absorbed from the respiratory tract with less rapid absorption occurring in the gastrointestinal tract and skin (Bushnell et al. 2007; Nadeau et al. 2006; Pyykko et al. 1977; Sullivan and Conolly 1988; Thrall and Woodstock 2002; Thrall et al. 2002a). Studies with animals exposed by inhalation or oral routes showed that absorbed toluene is distributed widely to tissues throughout the body with preferential distribution to adipose tissue, brain, bone marrow, liver, and kidney. Absorbed toluene is distributed in pregnant animals, as well as to the developing fetus. The primary initial steps in toluene metabolism in humans and laboratory animals are side-chain hydroxylation (to form benzyl alcohol) catalyzed predominantly 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). Remaining absorbed toluene is excreted unchanged in exhaled air and urine and as conjugates of minor metabolites in urine (Ducos et al. 2008; Janasik et al. 2008, 2010; Lof et al. 1993; Ogata 1984; Pierce et al. 2002; Tardif et al. 1998). Analyses of kinetic data for toluene concentrations in blood, exhaled breath, adipose tissue, or urine following inhalation exposure of humans 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 (Janisik et al. 2008; Leung and Paustenbach 1988; Lof et al. 1993; Nise et al. 1989; Pellizzari et al. 1992; Pierce et al. 1996, 1999, 2002). For example, elimination kinetics for toluene-exposed workers have been described by a three-phase elimination model with half-times of 9 minutes, 2 hours, and 90 hours for toluene in blood and a single median elimination half-time of 79 hours for toluene in fat (Nise et al. 1989). In a study of

volunteers exposed by inhalation to about 50 ppm for 4 hours, a two-phase decline of urinary toluene concentration was observed with half-lives of 0.88 and 12.9 hours (Janisik et al. 2008).

3.4.1 Absorption

3.4.1.1 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). In volunteers exposed to 50 ppm toluene for 7 hours while exercising or at rest, concentrations of toluene in expired air and *ortho*-cresol (a toluene metabolite), in urine collected at the end of exposure were higher during exercise than during periods at rest (~140–200% increase for blood concentrations and ~120% increase for urinary *ortho*-cresol) (Nadeau et al. 2006). The results are indicative of higher rates of uptake (and excretion) of inhaled toluene during exercise than at rest.

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). Toluene concentrations in blood and brain increased rapidly during 60-minute inhalation exposures of physically active and sedentary rats to 2,000 ppm (Bushnell et al. 2007). Physical activity during inhalation increased the uptake of toluene into the blood and brain. After 60 minutes of exposure, physically active rats showed toluene concentrations in blood and brain that were ~26 and ~40% higher, respectively, than concentrations in sedentary rats (Bushnell et al. 2007).

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

3.4.1.2 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). 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; Sullivan and Conolly 1988). In these rat studies, maximum blood concentrations were observed 1.5–3 hours after gavage administration, whereas maximum blood levels following inhalation were reached in 15–30 minutes.

No statistically significant differences between physically active and sedentary rats were found in the time course of the increase of concentrations of toluene in blood and brain following administration of single gavage doses of ~800 mg/kg (Bushnell et al. 2007). Comparison with results from inhalation exposure suggests that physical activity may not influence the uptake of orally administered toluene to the same degree that it influences uptake of inhaled toluene.

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.

3.4.1.3 Dermal Exposure

Results from early studies indicated that toluene is absorbed slowly through human skin (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. EPA (1992b) estimated a human dermal permeability coefficient, Kp, of 1 cm/hour, based on these data. 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. Transdermal uptake of toluene directly from the air is expected to be low, accounting for 1–2% of the total body burden received following exposure to toluene vapors (Brooke et al. 1998; Weschler and Nazaroff, 2014).

Soaking the skin of two 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.

Dermal permeability coefficients were estimated for human subjects wearing swimsuits who were submerged to neck level for up to 25–30 minutes in warm tap water initially containing 500 µg/L toluene (Thrall et al. 2002a). Subjects were provided purified breathing air, and exhaled breath was continuously analyzed for toluene before, during, and after exposure to track the absorption and exhalation of toluene. A human PBPK model, modified from a rat model for dermal absorption (Thrall and Woodstock 2002), was fit to each exhalation time course dataset to estimate a dermal permeability coefficient, Kp. Estimated Kp values (n = 6) ranged from 0.03 to 0.020 cm/hour, with an average value of 0.012 (standard deviation [SD]=0.007) cm/hour. In a second phase involving dermal and inhalation exposure, subjects breathed room air while they were submerged, and exhaled breath was collected and analyzed. Transient peak concentrations of toluene in exhaled breath occurred earlier and were about 50% greater than levels

during dermal-only exposure; these observations are consistent with a more rapid absorption through the respiratory tract than through skin. Thrall et al. (2002a) concluded, based on room air monitoring data and comparison of exhaled breath profiles for dermal-only and dermal+inhalation exposures, that inhalation exposure to volatilized toluene under these conditions was transient and contributed little to the overall total body from bathing in toluene-contaminated water.

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.

A dermal permeability coefficient, Kp, of 0.74 cm/hour (SD=0.005) was measured for male F344 rats exposed under occluded conditions to aqueous toluene solutions of 0.5 or 0.2 mg/mL applied to a 2.5-cm diameter patch of clipper-shaved skin on the back (Thrall and Woodstock 2002). Immediately following application, individual rats were placed in an off-gassing chamber, and exhaled breath of toluene was monitored continuously as chamber concentrations. Rapid absorption was indicated with peak chamber concentrations noted within 1–1.5 hours after exposure started; chamber concentrations showed steady declines thereafter through 4–5 hours. Based on amounts of toluene remaining on the skin at the end of exposure, average percentage absorptions of the applied doses were 45 and 42%, respectively, for the low- and high-dose levels (1.75 and 4.14 mg/kg). Using a method similar to Thrall et al. (2002a), a PBPK model was used to estimate a Kp for each exhaled breath dataset; the average Kp value across datasets was 0.074 cm/hour (SD=0.005).

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.

3.4.2 Distribution

3.4.2.1 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, toluene appears to be associated with 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 for distribution to the brain 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 1983). 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 1983). These regions have a high concentration of white matter.

Toluene was detected in blood, brain, auditory nerves, and the organ of Corti, but not in cerebrospinal fluid or inner ear fluids sampled from rats immediately following inhalation exposure to 1750 ppm toluene for 10 hours (Campo et al. 1999).

In mice exposed nose-only to 0, 0.9, 9, 50, or 90 ppm toluene for 30 minutes, toluene concentrations in the hippocampus showed statistically significant ($p<0.05$) concentration-dependent increases after exposure to air concentrations ≥9 ppm, compared with control values (Nakajima et al. 2006). This study used a solid-phase microextraction fiber inserted into the hippocampus and gas chromatography and mass

spectrometry to determine brain concentrations in mice. Examination of mice exposed to 50 ppm toluene showed that toluene concentrations in the brain returned to control levels 60 minutes after exposure ceased.

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.

Following repeated inhalation exposure of pregnant rats to 8,000 or 12,000 ppm for 15, 30, or 45 minutes twice daily from GD 8 through GD 20, measurable concentrations of toluene were found in the placenta, amniotic fluid, and fetal brain collected from exposed dams on GD 20 (Bowen et al. 2007). Regardless of daily exposure regimen or exposure level, amniotic fluid concentrations were ~<10% of concentrations in GD 20 maternal saphenous blood samples. Concentrations in fetal brain were similar to maternal saphenous blood concentrations at GD 20, whereas concentrations in placenta were higher. Concentrations of toluene in maternal cerebellum, liver, kidney, and heart tissue on GD 20 (but not lung) were higher than concentrations in GD 20 maternal saphenous blood. The results, consistent with those of Ghantous and Danielsson (1986), indicate that toluene was transported to the fetal brain, and that fetal

brain concentrations were similar to maternal blood levels, but were lower than maternal brain concentrations.

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.

3.4.2.2 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).

3.4.2.3 Dermal Exposure

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

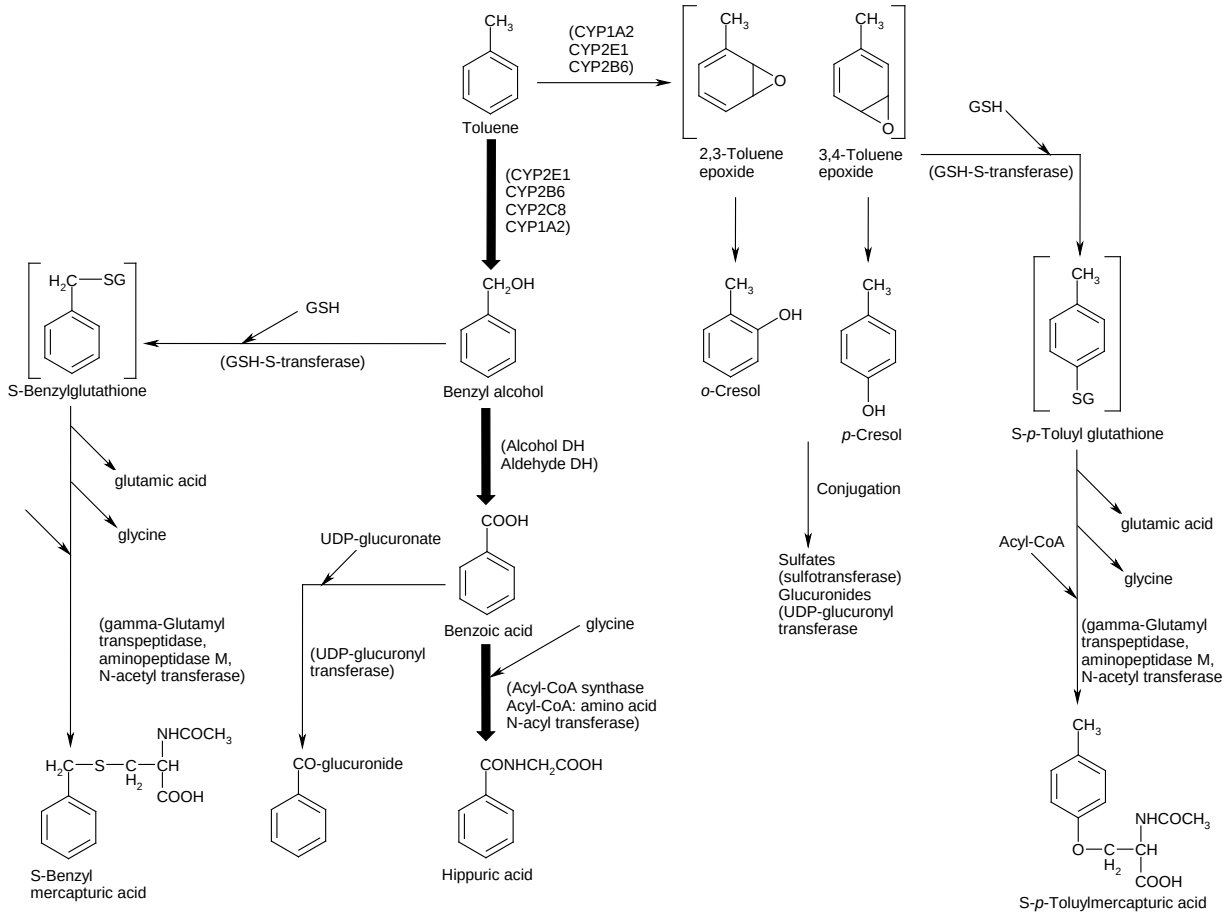
3.4.2.4 Other Routes of Exposure

In baboons intravenously injected with ^{11}C-labeled toluene, maximal concentrations of radioactivity in different brain regions were attained within 1–4 minutes of injection (Gerasimov et al. 2002a, 2002b). Concentrations of radioactivity steadily declined thereafter through about 45 minutes of monitoring. The results are indicative of rapid distribution from the blood to the brain and rapid clearance. Half-times for clearance from the brain ranged from about 10 to 20 minutes in three experiments. Similar experiments with mice showed similarly rapid distribution to the brain and rapid clearance from the brain following intravenous injection (Gerasimov et al. 2002a, 2002b).

3.4.3 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*-toluyl-mercapturic 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 3-3.

Figure 3-3. 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

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 hydroxylation 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 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). Comparison of the *in vitro* catalytic activities of human recombinant CYP2E1, CYP2A6, and CYP2A13 (a form that is highly expressed in human respiratory tract tissues) to form benzyl alcohol from toluene indicated the following order of catalytic activities: CYP2A13 > CYP2A6 > CYP2E1 (Fukami et al. 2008). The latter observations suggest that there may be tissue specificities in the principal forms of CYPs catalyzing the initial step in toluene metabolism. Kinetic constants (e.g., Km and Vmax) were comparable for recombinant wild-type CYP2E1 (CYP2E1.1) and allelic variants of CYP2E1 (CYP2E1.2, CYP2E1.3, and CYP2E1.4) to convert toluene to benzyl alcohol (Hanioka et al. 2010). The variants were made by site-directed mutagenesis involving amino acid

substitutions to reflect CYP2E1 polymorphisms identified in human populations at frequencies ranging from 1.3 to 2.6%.

Benzyl alcohol is thought to be converted to benzoic acid in two steps by alcohol dehydrogenase and aldehyde dehydrogenase (see Figure 3-3). 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). In plasma samples collected in baboons 30 minutes following intravenous injection of ^{11}C-toluene, relative amounts of radioactivity were 34% in unchanged toluene, 40% in benzoyl glucuronide, 18% in hippuric acid, 1% in benzyl alcohol and benzaldehyde, and 3% in benzoic acid (Gerasimov et al. 2002a).

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 3-3). 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 exposure at 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 1983). 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.

3.4.4 Elimination and Excretion

3.4.4.1 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 3.4.3) and, to a lesser extent, as non-metabolized toluene in exhaled air and urine (Ducos et al. 2008; Janasik et al. 2008, 2010; 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). Although unchanged toluene in urine is expected to be a minor elimination route based on mass balance, elimination kinetics data for toluene in urine following acute exposure of volunteers are consistent with the recommended use of this end point as a biomarker of exposure for toluene-exposed workers (ACGIH 2010; Ducos et al. 2008; Janisik et al. 2008, 2010). For example, following 4-hour exposure to 200 mg/m^3 (53 ppm), a two-phase decline of urinary toluene concentration was observed with half-lives of 0.88 and 12.9 hours. The correlation coefficient between toluene air concentrations (20, 60, or 100 mg/m^3; ~5, 16, or 27 ppm) and urinary toluene concentrations in the last 2 hours of exposure was 0.998 (Janisik et al. 2008).

Analyses of kinetic data for toluene concentrations in blood, exhaled breath, or adipose tissue or urine 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 (Leung and Paustenbach 1988; Lof et al. 1993; Nise et al. 1989; Pellizzari et al. 1992; Pierce et al. 1996, 1999, 2002). 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). The time courses of toluene concentrations in blood and subcutaneous fat of rotogravure printers exposed to 35–246 mg/m^3 (9–65 ppm) toluene during and after a 5-day workweek were described by a three-phase elimination model with half-times of 9 minutes, 2 hours, and 90 hours for toluene in blood and a single median elimination half-time of 79 hours for toluene in fat (Nise et al. 1989). 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 $^1H_8$-toluene and 50 ppm $^2H_8$-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). Analysis of rates of exhalation of nonmetabolized toluene and urinary excretion of metabolites from 2-hour exposures of male subjects to 50 ppm $^2H_8$-toluene indicated the following distribution of the total dose: 13% $^2H_8$-toluene in exhaled breath, and 75% $^2H_5$-hippuric acid, 0.31% $^2H_7$-*ortho*-cresol, 0.53% $^2H_7$-*meta*-cresol, and 11% $^2H_7$-*para*-cresol in urine (Pierce et al. 2002). 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).

Correlations between work place air concentrations of toluene and urinary excretion of unchanged toluene, hippuric acid, or *ortho*-cresol have been noted in a number of field studies (see ACGIH 2001 and 2010 for reviews and citations of these studies). Currently, ACGIH (2010, 2013) recommends using a

combination of three biological exposure indices (BEIs®) to assess exposure of workers to toluene in the workplace: *ortho*-cresol and unchanged toluene levels in blood immediately prior to the last shift of a workweek. The recommendation was made based on analyses of numerous field studies examining toluene blood concentrations and urinary concentrations of *ortho*-cresol and toluene in workers exposed to varying workplace air concentrations of toluene (see ACGIH 2010 for review). The specific values for these BEIs® correspond to concentrations likely to be observed in individuals exposed by inhalation to 20 ppm, the current ACGIH 8- hour TWA Threshold Limit Value (TWA-TLV®) for occupational exposure to toluene (ACGIH 2010). Previously, the level of hippuric acid in urine at the end of a workshift was recommended as a biomarker of exposure, but this recommendation was withdrawn because background urinary hippuric acid (from consumption of benzoate in foods and beverages) is expected to mask contributions from workplace exposure to toluene, especially at concentrations <50 ppm (ACGIH 2001, 2010). Other urinary biomarkers of exposure that have been proposed include benzylmercapturic acid (Inoue et al. 2002, 2004; Maestri et al. 1997) and S-*p*-toluylmercapturic acid (Angerer et al. 1998a).

3.4.4.2 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.

3.4.4.3 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. As discussed in Section 3.4.1., studies in humans and rats have monitored concentrations of unchanged toluene in exhaled breath during and following dermal exposure to aqueous solutions of toluene to estimate dermal permeability coefficients (Thrall et al. 2002a; Thrall and Woodstock 2002). Like the study by Monster et al. (1993), they demonstrate that dermally absorbed toluene can be exhaled unchanged, but they do not provide information about the relative importance of urinary excretion of unchanged toluene or metabolites following dermal exposure.

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

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

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

PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

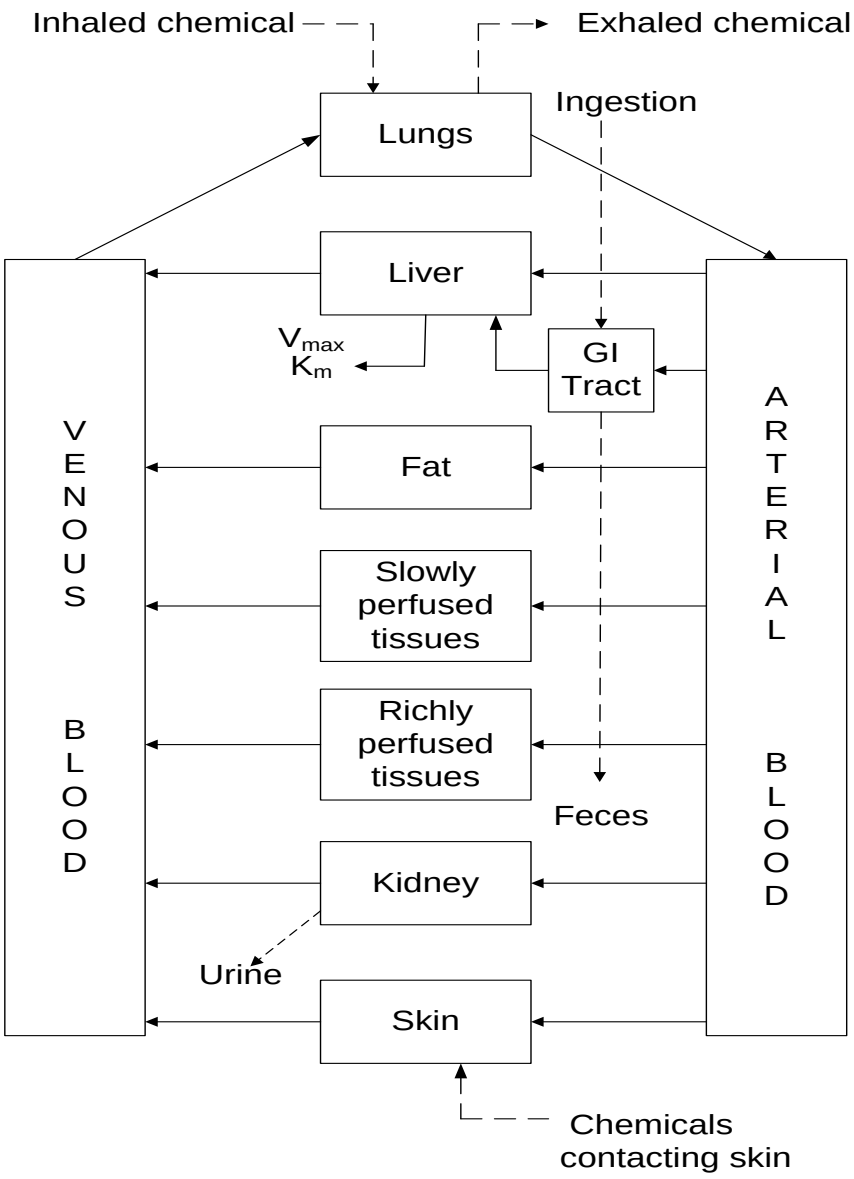
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically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

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. However, if the uptake and disposition of the chemical substance(s) are adequately described, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

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

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

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



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.

Source: Krishnan and Andersen 1994

PBPK models are available that describe the kinetics of toluene after inhalation exposure for humans (Benignus et al. 2006; Fisher et al. 1997; Jonsson and Johanson 2001; Sari-Minodier et al. 2009; Nong et al. 2006; Pierce et al. 1996, 1999; Tardif et al. 1995, 2002) and rats (DeJongh and Blaauboer 1996, 1997; Kenyon et al. 2008; Oshiro et al. 2011; Tardif et al. 1993; van Asperen et al. 2003). PBPK models to describe the kinetics of dermally applied aqueous solutions of toluene are also available for humans (Thrall et al. 2002a) and rats (Thrall and Woodstock 2002), but models to describe kinetics following oral exposure to toluene have not been developed.

Available 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,

(3) changes in the amount of toxicant in three nonmetabolizing tissue compartments (adipose tissue, slowly perfused tissues, and richly 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.

Human Models. 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 coefficients 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 richly 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 richly 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 $^1H_8$-toluene and 50 ppm $^2H_8$-toluene) for a 2-hour period (Pierce et al. 1996). Venous blood concentrations of $^1H_8$- and $^2H_8$-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 ACGIH 8-hour TWA TLV for toluene in 1998). Calculated values were $\leq$10 μmol/m^3 for samples taken just before the final shift of a workweek and $\leq$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.

Jonsson and Johansen (2001) modified the Pierce et al. (1996) model to incorporate, and estimate inter- and intra-individual variability in, exercise-induced changes in perfusion of fat tissue. The modified model split the muscle compartment into working and resting muscle compartments, and the fat compartment into perirenal and subcutaneous fat. Model parameters were expressed as prior distributions, rather than point estimates, based on literature values. The model was fit by Markov chain Monte Carlo simulation to time-course data for toluene concentrations in arterial blood, exhaled breath, and subcutaneous fat for six individuals exposed by inhalation to 80 ppm for 2 hours during rest and moderate to heavy exercise (50–150 W). The fitting exercise indicated that the kinetic data was best described when no increase in perfusion of subcutaneous fat with exercise was allowed and increased perfusion of perirenal fat induced by physical work was set to a constant level, rather than scaled proportionally to the increase in oxygen uptake with increasing workload.

The model by Jonsson and Johansen (2001) was further modified to a population-based model by replacing point-estimates for physiological model parameters with literature-based log-normal distributions (Mörk et al. 2014). Using Monte-Carlo techniques with the population-based model, estimates of the distributions for an internal dose (Cmax in blood) for various subpopulations under various exercise scenarios were calculated, and the ratio between the 50th percentile values and higher percentiles (90, 95, or 99th percentiles) were used to indicate human variability in toxicokinetic disposition of toluene. Based on this modeling exercise, toluene-specific adjustment factors for human toxicokinetic variability (AF_{HK}) were 1.2–1.8 for the general population, 1.4–2.1 for chronically-exposed workers (under various exposure scenarios), and 1.4–3.9 for acutely-exposed workers (under various exposure scenarios).

Another human PBPK model has been developed for volatile organic compounds that simulates 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 richly 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) TLV (50 ppm) throughout a workday. The model predicted that such an infant would have a daily intake of 0.46 mg toluene, 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.

Tardif et al. (1995) developed a four-compartment PBPK model for toluene in humans by modification of a four-compartment rat model developed by Tardif et al. (1993), in which all metabolism occurred in the liver. Physiological parameters for the human model were taken from the literature (e.g., alveolar ventilation rate and cardiac outputs had values of 18.0 L/hour/kg body weight for subjects at rest). Coefficients for human tissue:blood partitioning were calculated by dividing rat tissue:air values by a human blood:air coefficient (15.6) taken from the literature. The metabolic constant, Km (0.55 mg/L), was assumed to be the same in rats and humans, and the human Vmax (4.8 mg/hour/kg) was converted from the rat value by using body weight$^{0.75}$ allometric scaling. The model was linked to a similar model for xylene; linking occurred by competitive metabolic inhibition in the liver. The linked models were evaluated by comparing predicted and observed values for toluene (and xylene) concentrations in alveolar air and blood. The predicted values were judged to be “in accord” with observed values from a study of volunteers exposed to 50 ppm toluene, 40 ppm xylene, or 50 ppm toluene+40 ppm xylene for 7 hours (Tardif et al. 1991). Simulations with the model indicated that blood concentrations and extent of metabolism for the two solvents during combined exposure at 50 ppm toluene and 100 ppm xylene would not be more than 10% changed, compared with exposure to the individual solvents alone (Tardif et al. 1995).

Tardif et al. (2002) modified the unlinked human PBPK model developed by Tardif et al. (1995) to include the description of urinary excretion of *ortho*-cresol. The fraction of total metabolites excreted as *ortho*-cresol (0.00078) was estimated by fitting the model to data for urinary *ortho*-cresol levels in toluene-exposed workers (Truchon et al. 1999). Values for urinary excretion rate of creatinine

(12.06 µmol/hour/kg) and urine output (1.848 mL/hour/kg) were taken from the literature. The model was used with Monte Carlo simulation to explore how published estimates of human population variance for parameters for excretion, physiology, and metabolism would influence concentrations of toluene in blood and *ortho*-cresol in urine. The resultant distribution for *ortho*-cresol excretion at the end of the first 8-hour workday in the week with exposure to 50 ppm toluene had a geometric mean of 0.635 mmol/mol creatinine with lower and upper 95% confidence limits of 0.23 and 1.75 mmol/mol creatinine, respectively. For blood toluene concentration prior to the last workday in the week, a somewhat smaller distribution of values was indicated with a geometric mean of 120.6 µg/L and lower and upper 95% confidence limits of 64.5 and 225.7, respectively.

Nong et al. (2006) modified the adult PBPK model developed by Tardif et al. (1995) to incorporate data on age, body weight, liver volume, and hepatic CYP2E1 content from a study by Johnsrud et al. (2003) of 116 children (41 males, 75 females) ranging from newborn to 17 years of age. The model calculated hepatic clearance normalized on hepatic CYP2E1 content. The modified model, using child-specific data, was used to simulate time courses of toluene concentrations in blood of individual children of varying ages exposed by inhalation to 1 ppm toluene for 24 hours. The results indicated that the AUCs for simulated toluene blood concentrations in these children varied by a factor of about 6, compared with a factor of about 20 for the range of hepatic CYP2E1 contents. In neonates (<1 month old) with low and high hepatic CYP2E1 content (<3.69 and >3.69 pmol/mg protein), 95th percentile simulated AUC values were 3.9-fold and 2.5 higher than the 50th percentile AUC value for adults. The 95th percentile AUC values in children in older age groups (1 month–1 year; 1–11 years; 12–17 years) were less elevated; ratios of 95th percentile AUCs for children: the 50th percentile adult AUC value for these age groups were 1.57, 1.49, and 1.35, respectively.

Sari-Minodier et al. (2009) made further refinements of the Tardif et al. (2002) model to include a kidney compartment, and different physiological parameters (alveolar air ventilation, cardiac output, and fractions of total cardiac output to each compartment) for different levels of physical activity (measured in watts of work, W). Also included were modified values for the fraction of toluene metabolized to *ortho*-cresol (0.0012) and the *ortho*-cresol urinary excretion rate based on the best fit of simulations to observed time course data for volunteers exposed to 50 ppm for 7 hours (Tardif et al. 1998). The revised model was evaluated by comparing simulated values to observed data for concentrations of unchanged toluene in blood and urine in six volunteers exposed to 5.3, 16.0, or 26.6 ppm toluene for 8 hours at rest (Janasik et al. 2008), and for alveolar toluene concentrations and urinary *ortho*-cresol concentrations in four volunteers exposed to 50 ppm for 7 hours with different scenarios of physical activity (Nadeau et

al.2006). Sari-Minodier et al. (2009) considered the comparisons between simulated and empirical values to be "satisfactory." The modified model was used to simulate values for blood toluene concentration at the end of an 8-hour work shift and urinary concentrations of toluene and *ortho*-cresol at the end of a workweek under different levels of activity. The model predicted that these biological exposure indices for toluene were increased by ~2–3-fold by 50 W activities versus 12.5 W resting activities at an air concentration of 20 ppm toluene.

Thrall et al. (2002a) developed a human PBPK model for toluene with six compartments (lung, richly perfused tissues, slowly perfused tissues, skin, fat, and liver) to simulate dermal exposure scenarios. The model was modified from a model for rats of similar structure as described by Thrall and Woodstock (2002). In these models, absorption to blood occurs though the lung and the skin, metabolism only occurs in the liver, and the skin compartment represents exposed skin; unexposed skin is lumped in the slowly perfused tissue compartment. Metabolic constants were the same as those used by Tardif et al. (1995). Physiological parameters included 348 L/hour for cardiac output and alveolar ventilation. Blood flow to compartments was distributed as percentages of cardiac output: liver 25%; fat 6%, richly perfused 49%; slowly perfused 15%; and total skin 5%. Tissue volume as percentages of body weight were: 4% liver; 20% fat; 5% richly perfused; 52% slowly perfused; and 4% total skin. Partition coefficients were: 1.75 saline:air; 13.9 blood:air; 83.5 liver:air; 1021 fat:air; 27.7 muscle:air; and 43.0 skin:air. Dermal exposures were simulated by an equation relating the rate of change in toluene concentration in the skin to dermal flux and dermal perfusion. Body weight was an input so that that the model could be used to estimate dermal permeability coefficients (Kp, a parameter for the dermal flux equation) for individual subjects of varying body weights, by optimization of fit to exhaled breath data collected before, during, and after subjects were dermally exposed to aqueous solutions of toluene. Estimated Kp values for six subjects ranged from 0.004 to 0.020 cm/hour, with an average of 0.012 cm/hour (SD=0.007).

Benignus et al. (2006) combined a commercially available general whole-body human physiological model (named QCP2004) with a human PBPK model modified from the Tardif et al. (1995) model. The combined model (named GPAT for general physiology and toxicokinetic model) was developed to reduce the calculational complexity associated with time-dependent changes in exposure concentrations and human physical activity. The QCP2004 model provided estimates of physiological parameters required by the PBPK model under varying levels of human physical activity. The Tardif et al. (1995) human PBPK model was modified to include a brain compartment and new partition coefficients estimated by Thrall et al. (2002b). QCP2004 model simulations for minute ventilation, cardiac output, and hepatic blood flow were compared with observed data for these end points in humans with varying levels of

physical activity (0–~200 W). The simulated values for these physiological parameters were mostly within 95% confidence limits for regression lines relating observed values and levels of activity from a number of published studies for each physiological end point. Values for blood toluene concentrations were simulated with the GPAT model and compared with published sets of observed values measured in human subjects exposed to toluene for short periods (30–120 minutes) under varying levels of physical activity. Simulated values and observed values for blood toluene concentrations in the published studies, regardless of exposure concentration or activity level, were plotted and fitted to a linear regression model. Comparison of an "identity line" (on which all points would lie if the model predicted perfectly) and the regression line indicated that the identity line was within the 95% confidence limits of the regression line.

Animal Models. The four-compartment rat PBPK model for toluene developed by Tardif et al. (1993) restricted metabolism to the liver compartment. 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 richly 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 was 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.

van Asperen et al. (2003) modified the five-compartment DeJongh and Blaauboer (1996) rat model with slightly different metabolic constants (Vmax of 4 mg/kg/hour and Km of 0.2851 mg/L) and a modified blood:air coefficient (13 versus 18 in the previous model). Other physiological parameters and partition coefficients were identical to those in the Dejongh and Blaauboer (1996) model. The model with the revised blood:air coefficient provided better fit of time-course data for blood and brain toluene concentration in Wistar-derived rats exposed for 7.5 hours to a constant concentration of about 2,667 ppm or five fluctuating peak concentrations of about 8,000 ppm for 0.5 hours separated by 1-hour intervals without toluene. Blood and brain concentrations were determined several times during and after exposure up to 24 hours after the start of exposure. The modified model was used to examine predicted brain dose-effect relationships in a visual discrimination task in rats exposed to toluene via constant and fluctuating exposure scenarios. Effects on end points measuring “disinhibition of responding” were most pronounced in groups with the highest estimated brain concentrations at the time of testing, but a monotonic relationship for increasing magnitude of effect and increasing brain concentration was not evident.

Thrall and Woodstock (2002) developed a rat PBPK model for toluene with six compartments (lung, richly perfused tissues, slowly perfused tissues, skin, fat, and liver) to simulate dermal exposure

scenarios. The rat model was adapted from the rat model developed by Tardif et al. (1993). In the modified model, absorption to blood occurs though the lung and the skin, metabolism only occurs in the liver, and the skin compartment represents exposed skin; unexposed skin is lumped in the slowly perfused tissue compartment. Dermal exposures were simulated by an equation relating the rate of change in toluene concentration in the skin to dermal flux and dermal perfusion. Body weight was an input so that that the model could be used to estimate dermal permeability coefficients (Kp, a parameter for the dermal flux equation) for individual rats of varying body weights. This was done by optimization of fit of the model to exhaled breath data collected when rats were dermally exposed to aqueous solutions of toluene. Metabolic constants were the same as those used by Tardif et al. (1993). Physiological parameters included 5.4 L/hour for cardiac output and alveolar ventilation. Blood flow to compartments was distributed as percentages of cardiac output: liver 25%; fat 5%, richly perfused 51%; slowly perfused 15%; and total skin 5%. Tissue volume as percentages of body weight were: 4% liver; 8% fat; 5% richly perfused; 64% slowly perfused; and 10% total skin. Partition coefficients were: 1.2 saline:air; 18.0 blood:air; 83.5 liver:air; 1021 fat:air; 27.7 muscle:air; and 43.0 skin:air. The rat PBPK model was used to estimate a Kp for each exhaled breath dataset; the average Kp value across datasets was 0.074 cm/hour (SD=0.005).

Kenyon et al. (2008) developed a seven-compartment PBPK model for inhalation exposure of rats to toluene that included compartments for the lung, brain, richly perfused tissues, slowly perfused tissues, fat, gastrointestinal tract, and liver. Metabolism was restricted to the liver, and metabolic constants were those determined by Tardif et al. (1993). Partition coefficients were those determined by Thrall et al. (2002b). Values for cardiac output and alveolar ventilation were calibrated/optimized by fitting of the model to time-course data for toluene blood concentrations in Long-Evans rats under three different experimental conditions: “normal” rats with a diurnal cycle of being active and fed at night; “sedentary, day-acclimated” rats acclimated to be fed and active during the light diurnal cycle, but not performing a task; and “active, day-acclimated” rats acclimated to be fed and active during light hours and performing a lever pressing task. Blood flow fractions to tissues and volumes of tissue compartments were based on literature values for Long-Evans rats. The calibrated/optimized model was evaluated for its ability to predict time-course data for blood and brain concentrations of toluene collected from rats in several other pharmacokinetic studies under various physical activity and exposure conditions. Statistical analyses of plots of predicted versus observed blood concentration data from five other studies indicated no significant difference from a fitted regression line and a “unity” line. For predicted versus observed brain concentration data from four studies, the fitted regression had a slope of 0.850 that was statistically significantly less than the unity line slope of 1.0. Most of this discrepancy was attributed to brain

concentration data from a single study of Wistar rats for which the model consistently overpredicted brain concentration; when these data were excluded, the slope of the regression line was 1.01.

Oshira et al. (2011) modified the Kenyon et al. (2008) rat model for inhalation exposure to include hepatic enzyme induction as an empirical step function. When the Kenyon et al. (2008) model was used to simulate toluene brain concentrations during exposures of Long-Evans rats to 775 or 1,125 ppm for 24 hours, the model adequately described brain concentrations at 1 and 6 hours of exposure, but overpredicted brain concentrations at 24 hours and at 6 hours after exposure ceased. The modified model (which included graded fold increases of the maximum rate of toluene metabolism in the liver [VmaxC] used in the original model) provided better predictions of brain concentrations at 24 and 30 hours after exposure started. The modified model was used to predict brain concentrations of toluene during 1- and 24-hour exposures to several air concentrations and examined relationships between estimated brain concentrations and effects on a trained behavior task (visual signal detection). The dose-effect relationship for response latency in this task after 1 hour of exposure was shifted to the left on the dose axis, compared with the 24-hour dose-effect relationship. The results are consistent with the hypotheses that the rats developed a behavioral tolerance to toluene within 24 hours of exposure and that behavioral effects from 24-hour exposures cannot be accurately extrapolated from 1-hour exposure data.

3.5 MECHANISMS OF ACTION

3.5.1 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 1983; Sullivan and Conolly 1988), and dermal route (Dutkiewicz and Tyras 1968; Thrall and Woodstock 2002; Thrall et al. 2002a). 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 (Bergman 1983; Bowen et al. 2007; Campo

1999; Ghantous and Danielsson 1986; Ikeda 1990; Nakajima et al. 2006; Kenyon et al. 2008; van Asperen et al. 2003).

Metabolism. Studies of urinary metabolites in toluene-exposed humans 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. CYP2E1 is thought to be the principal enzyme responsible for catalyzing the formation of benzyl alcohol from toluene, but other CYP forms capable of catalyzing this reaction include CYP1A1, CYP1A1, CYP2A6, CYP2A13, CYP2B6, and CYP2C8 (Fukami et al. 2008; Nakajima et al. 1997). Benzyl alcohol is thought to be converted to benzoic acid by alcohol dehydrogenase and aldehyde dehydrogenase. Hippuric acid formation from benzoic acid is catalyzed by acyl-CoA synthetase and acyl-CoA:amino acid N-acyltransferase, whereas benzoyl glucuronide formation from benzoic acid is catalyzed by UDP-glucuronyl transferase (Nakajima and Wang 1994). The formation of *ortho*- and *para*-cresol is thought to be catalyzed from benzoic acid by CYP1A2, CYP2E1, and CYP2B6 through epoxide intermediates (Nakajima et al. 1997). The liver appears to be the principal site of metabolism of toluene, but evidence of metabolism in other tissues like the lung is available (Fukami et al. 2008; Wheeler et al. 1992).

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 unchanged toluene and minor metabolites including S-benzyl mercapturic acid, S-*p*-toluoyl mercapturic acid, and conjugates of *ortho*- and *para*-cresol account for less than 5% of absorbed toluene, but assays for urinary levels of unchanged toluene and *ortho*-cresol can provide reliable biomarkers of toluene exposure (ACGIH 2010; Ducos et al. 2008; Janisik et al. 2008, 2010). Excretion of nonmetabolized toluene in exhaled air can represent from 7 to 20% of absorbed toluene, depending on exposure conditions (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).

3.5.2 Mechanisms of Toxicity

3.5.2.1 Mechanisms of Neurotoxicity

Dysfunction of the central nervous system is a critical human health concern following acute, intermediate, or chronic inhalation exposure to toluene. Therefore, the mechanisms of toluene toxicity have been investigated predominately in the nervous system. Proposed mechanisms of toxicity include altered membrane and membrane channel properties; direct damage to brain structures via lipid damage, oxidative stress, and/or apoptosis; altered neurotransmitter synthesis, release, degradation, and receptor binding; disruption of the hypothalamic-pituitary-adrenal axis; and neuroinflammation.

Mechanisms of Central Nervous System Depression and Narcosis. 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). Similarly, increased Na+-K+-ATPase activities were observed in brain homogenates of rats sacrificed 15 minutes after an intraperitoneal injection of 35 mg/kg toluene or *ortho*-cresol (Calderon-Guzman et al. 2005). In culture systems, 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); near-significant increases in calcium leakage were observed in synaptosomes of PND 25–43 offspring exposed to 1,800 ppm for 6 hours/day from GD 7 to 20, compared with unexposed offspring (Edelfors et al. 2002); and increased LDH and calcium leakage were observed in a neuroblastoma cell line (SH-SY5Y) following *in vitro* toluene exposure (McDermott et al. 2007). 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).

More recently, acute effects of toluene and other volatile organic chemicals on neuronal functions have been proposed to involve interactions with voltage- and ligand-gated ion channels in nerve tissue. In various cell preparations, toluene has been shown to disrupt currents mediated by GABA receptors (Beckstead et al. 2000; Gmaz and McKay 2014), N-methyl-D-aspartate (Cruz et al. 1998, 2000, 2003), nicotinic acetylcholine receptors (Bale et al. 2002, 2005), muscarinic acetyl choline receptors (Tsuga et al. 1999; Wu et al. 2002), voltage-sensitive Ca^{+2} channels (Shafer et al. 2005; Tillar et al. 2002), calcium-activated and G-coupled potassium channels (Del Re et al. 2006), and gap junctions (Del Re and Woodward 2005).

Mechanisms of Structural Brain Damage. 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) suggests 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), degenerative changes and ultrastructural damage in the hippocampus, frontal cortex, and brain stem (Kanter 2008a, 2008b, 2001, 2013), decreased or shrunken cells in the hippocampus (Gelazonia et al. 2006a; Gotohda et al. 2002; Korbo et al. 1996; Zhvania et al. 2012), impaired dendritic outgrowth in the frontal cortex (Pascual and Bustamante 2010), impaired neurite growth in neonatal rat cerebellar granular neurons stimulated with L1 cell adhesion molecule (suggesting impaired L1-lipid raft interactions) (White et al. 2016), and white matter damage in the anterior commissure (Duncan et al. 2012). It is uncertain if toluene-induced changes in membrane phospholipid content may be caused by increased breakdown of phospholipids or inhibition of synthesis.

Brain damage may be mediated through apoptotic pathways. Increased apoptosis has been observed in the rat brain following acute or intermediate-duration oral exposure to 650 mg/kg/day (Kamel and

Shehata 2008). Following exposure to 1,500–1,800 ppm, 6 hours/day from GD 7 to 20, increased apoptosis was observed in the cerebellum of rat pups at PNDs 21 and 27 (but not PNDs 11, 22, 24, or 90) (Dalgaard et al. 2001; Ladefoged et al. 2004). No changes in hippocampal apoptosis were observed. Additionally, a significant increase in caspase-3 activity was observed in the cerebellum on PND 6, but not PNDs 22, 24, or 27, indicating increased caspase-3 mediated apoptosis (Ladefoged et al. 2004). A study in rat astrocyte cultures also reported that observed apoptosis following *in vitro* toluene exposure was caspase-dependent, and that stimulation of p42/44 mitogen activated protein kinase (MAPK) by toluene functions to promote cell survival (Lin et al. 2002).

Damage to brain structures may also result from oxidative stress, as increased markers of oxidative stress have been observed in the rat brain following acute- or intermediate-duration inhalation exposure to concentrations as low as 10 ppm (Baydas et al. 2003, 2005; Burmistrov et al. 2001; Coskun et al. 2005; Kodavanti et al. 2011, 2015; Royland et al. 2012). Increased markers of oxidative stress have also been observed in rats following acute or intermediate-duration oral exposure to 650 mg/kg/day (Kamel and Shehata 2008). Following gestational exposure to 1,800 ppm for 6 hours/day from GD 7 to 20, increased reactive oxygen species were produced in response to *in vitro* exposure to toluene in cultured synaptosomes from PND 25 to 43 offspring (Edelfors et al. 2002). Lipid peroxidation in rat brain was also increased following intraperitoneal injections of toluene or its metabolites (*ortho*-, *para*-, and *meta*-cresol) (Calderon-Guzman et al. 2005). This mechanism is plausible in humans, as increased markers of oxidative stress have been observed in workers exposed to solvent mixtures (Halifeoglu et al. 2000; Kim et al. 2011; Won et al. 2011).

Mechanisms of Mild Neurological Impairment. Mechanistic understanding is poor of effects that have been associated with intermediate and chronic exposure to toluene in workplace air such as increased incidence of self-reported neurological symptoms (Guzelian et al. 1988; Matsushita et al. 1975; Orbaek and Nise 1989; Ukai et al. 1993; Yin et al. 1987), performance deficits in neurobehavioral tests (Boey et al. 1997; Eller et al. 1999; Foo et al. 1990; Kang et al. 2005; Matsushita et al. 1975; Orbaek and Nise 1989); hearing loss (Morata et al. 1997), changes in auditory and/or visual-evoked potentials (Abbate et al. 1993; Vrca et al. 1995, 1996, 1997a, 1997b), and color vision loss (Campagna et al. 2001; Cavalleri et al. 2000; 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 1,000–4,000 ppm for 30 minutes (Kim et al. 1998), 1,000 ppm for 8 hours (Rea et al. 1984), or up to 105 mg/kg/day in drinking water for 28 days (Hsieh et al. 1990b, 1991); 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. 1983b); 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); significantly increased dopamine levels, decreased levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and dihydroxyphenylalanine (DOPA), and altered monoamine neurotransmitter synthesis (tyrosine and tryptophan hydroxylase enzyme activities) in rats exposed to 40 ppm toluene 104 hours/week for 16 weeks (Berenguer et al. 2003, 2004; Soulage et al. 2004); and significant localized changes in dopamine and noradrenaline neurotransmitter levels and utilization in 8-week-old adult male rats exposed to 80 ppm for 6 hours/day from PND 1 to 7, compared with controls (von Euler et al. 1989). Altered neurotransmitter levels have also been reported in several animal studies at concentrations modeling solvent abuse (>1,000 ppm) (Apawu et al. 2014; Alfaro-Rodriguez et al. 2011; Gerasimov et al. 2002c; Koga et al. 2007; O'Leary-Moore et al. 2007, 2009; Ono et al. 1999; Paez-Martinez et al. 2008; Tsuga and Honma 2000; Williams et al. 2005) and following toluene injection (Calderon-Guzman et al. 2005; Riegel et al. 2004; Win-Shwe et al. 2007b). Based on *in vitro* and *in vivo* microdialysis studies, Riegel et al. (2007) suggested that observed increases in dopamine levels may result from direct stimulation of dopamine neurons in the ventral tegmental area, resulting in increased dopamine release into the nucleus accumbens of the mesolimbic system. Another *in vivo* microdialysis study reported dose-related decreases in extracellular acetylcholine levels in the striatum and hippocampus following intraperitoneal injections of 200–2,000 mg/kg toluene, which peaked 2–3 hours post-injection (Honma and Suda 2004). However, acetylcholine levels in striatal and hippocampal homogenates were significantly increased, suggesting that decreased extracellular levels were due to decreased acetylcholine release from nerve terminals.

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 was 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. Additionally, in another animal study, no significant exposure-related changes in serum prolactin levels in rats were reported with 4-week exposures to concentrations up to 320 ppm (Hillefors-Berglund et al. 1995). There were also no changes in dopamine D3 receptor binding 4 weeks after cessation of a 4-week exposure to 80 ppm toluene (von Euler et al. 2000). However, there is some evidence of changes in glutamate and GABA binding in male rats exposed by inhalation to toluene at concentrations of 50–1,000 ppm for 4 weeks or 500 ppm for 12 weeks (Bjornaes and Naalsund 1988) and binding to muscarinic acetylcholine receptors in male rats following exposure to ≥500 ppm for 6 hours (Tsuga and Honma 2000). Various alterations in receptor subunit levels were observed in the mesolimbic system of the rat brain following exposure to 8,000 ppm 30 minutes/day for 10 days, including increases in $GABA_A\alpha1$, NR1, NR2, and GluR2/3 subunits in the medial prefrontal cortex and decreased $GABA_A\alpha1$ and NR1 subunits in the substantia nigra (Williams et al. 2005). Additionally, agonist binding to the GluN2B receptor subunit was significantly decreased in mesocorticolimbic regions (nucleus accumbens, dorsal striatum, and caudal anterior cingulate cortex) (Dick et al. 2015).

Further evidence of alterations in neurotransmitter systems comes from injection studies. Following intraperitoneal injections of 250–750 mg/kg, rats showed dose-related increases in locomotor activity, motor incoordination, and memory impairment during neurobehavioral testing (Lo et al. 2009). Pretreatment with dopamine D1, D2, and D3 receptor antagonists (SCH23390, raclopride, nafadotride, respectively) prevented locomotor effects, and all behavioral alterations were prevented with pretreatment with d-serine, a co-agonist at the glycine binding site of NMDA receptors (Lo et al. 2009). Similarly, the NMDA co-agonist, sarcosine, was able to attenuate neurobehavioral alterations in rats following intraperitoneal injections of 250–750 mg/kg toluene (Chan et al. 2012) and the D2 antagonist remoxipride was able to attenuate locomotor effects in rats following intraperitoneal injections of 600–1,200 mg/kg toluene (Riegel and French 1999). Additionally, locomotor effects following an intraperitoneal injection of 600 mg/kg were significantly attenuated when neurotransmission in the mesolimbic nucleus accumbens was altered via 6-hydroxydopamine lesions (6-OHDA) or the mGlu2/3 receptor agonist

LY379268 (Riegel et al. 2003). Observed increases in depressive-like behavior in the forced swim and tail suspension tests, 1 and 4 days following intraperitoneal injection of 500 mg/kg toluene, were prevented with administration of serotonin-reuptake inhibitors (fluoxetine and imipramine) (Yang et al. 2010). Decreased NMDA-mediated calcium signaling and NR2B subunits have been reported in cerebellar granule cells cultured from rat pups following daily intraperitoneal injections of 200–1,000 mg/kg/day from PND 4 to 10 (Chen et al. 2004, 2005). Following daily intraperitoneal injections of 500 mg/kg/day toluene on PNDs 4–9 or 21–26, NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) were enhanced following electrical stimulation, but decreased in response to exogenous NMDA in PND 30–38 hippocampal slices (Chen et al. 2011). However, paired-pulse facilitation of NMDA currents was only observed in rats exposed to 500 mg/kg/day toluene from PND 4 to 9 (Chen et al. 2011).

Significant decreases (28 or 47%) in rat brain 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 having 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. Decreased GFAP was also observed in neonatal rat brains following daily intraperitoneal injections of 750 mg/kg from PND 4 to 10 (Burry et al. 2003) and in a dose-related manner in cultured astrocyte precursor cells following *in vitro* exposure to toluene, suggesting that toluene exposure could disrupt early brain development (Yamaguchi et al. 2002). Additionally, *in vitro* toluene exposure inhibited proliferation of primary rat cortical astrocyte cultures in a dose-related manner (Burry et al. 2003). The possible mechanistic connections of these observations to toluene-induced changes in neurobehavior are uncertain. In contrast, GFAP levels were significantly increased in the hippocampus, cerebellum, and spinal cord of male rats exposed to 1,500 ppm, 4 hours/day for 4–10 days (Gotohda et al. 2000a, 2000b) and in the hippocampus, cortex, and cerebellum of male rats exposed to 3,000 ppm paint thinner (66% toluene) 1 hour/day for 45 days (Baydas et al. 2003).

Alterations in adult neurogenesis in the subventricular zone of the lateral ventricle (SVZ) of the hippocampus may contribute to cognitive impairments associated with toluene abuse. Exposure to toluene in paradigms modeling abuse (500–6,000 ppm) led to an initial burst of neurogenesis, followed by a decrease in both neurogenesis and cell survival in the SVZ in mice (Franco et al. 2014; Paez-Martinez et

al. 2013). Seo et al. (2010) also reported a reduced rate of adult hippocampal neurogenesis in mice following a single intraperitoneal injections of 500 mg/kg toluene. As reviewed by these study reports, cognitive impairments and drug seeking behaviors have been associated with abnormalities in adult hippocampal neurogenesis in animal models.

Numerous gene expression changes have been observed in brain tissues of rats and mice following acute or intermediate inhalation exposure or injection; however, current understanding is inadequate to determine the mechanistic significance of these findings. Genes demonstrating significantly altered expression levels are involved in synaptic transmission and plasticity (Ahmed et al. 2007; Hester et al. 2011, 2012), memory (Win-Shwe and Fujimaki 2012; Win-Shwe et al. 2007a, 2010b, 2010c), neurotrophic factors (Win-Shwe et al. 2010a; Gotohda et al. 2000b), and oxidative stress (Win-Shwe et al. 2010a). Results of these studies vary greatly, and indicate that gene expression changes are species-, strain-, dose-, duration-, and lifestage-dependent. A series of studies suggest that neurobehavior may be altered through neuroimmune effects, such as neuroinflammation, and that immune status may affect gene expression changes in brain neuroinflammation (Win-Shwe et al. 2010a, 2011, 2012a, 2012b). Epigenetic alterations (histone acetylation) were seen in the dentate gyrus of rats exposed to 1,000–6,000 ppm for 30 minutes or 10 days (30 minutes, 2 times/day) (Huerta-Rivas et al. 2012), and in the nucleus accumbens and ventral tegmental area of rats exposed to 6,000 ppm for 10, 30-minute sessions over 8 days (Sanchez-Serrano et al. 2011).

Mechanisms of Hearing Loss. There is evidence that hearing loss 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 exposed to 1,700–2,000 ppm toluene, 6 hours/day, 5 days/week for 6 weeks (Campo et al. 2008), and rats exposed to a single gavage dose of 1,500 mg/kg (Campo et al. 2008). Inhibition of P450 metabolism by SKF525-A in guinea pigs resulted in altered auditory thresholds following exposure to 1,750 ppm toluene 6 hours/day, 5 days/week for 4 weeks; toluene administration alone did not change auditory thresholds (Waniusiow et al. 2009). Acivicin pretreatment, which inhibits γ-glutamyl transferase and the production of cysteine *S*-conjugates during toluene metabolism, did not alter hearing loss in rats exposed to 1,750 ppm toluene 6 hours/day, 5 days/week for 4 weeks, suggesting that toluene-induced hearing loss is not mediated by the cysteine *S*-conjugate metabolic pathway (Waniusiow et al. 2008).

Multiple studies in animals indicate that hearing loss observed with occupational toluene exposure may be due, in part, to direct damage to the OHCs of the cochlea that are responsible for amplifying incoming sound waves prior to signal transduction (Campo et al. 1997, 1998; Johnson and Canlon 1994; Lataye and Campo 1997; Lataye et al. 1999; Waniusiow et al. 2008). Phenobarbital pretreatment also reduced OHC loss in rats exposed to 1,700–2,000 ppm toluene 6 hours/day, 5 days/week for 6 weeks, compared with toluene-treatment alone (Campo et al. 2008). Additionally, 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 BAEP) and OHC 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 OHC 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 limited regarding how toluene exposure leads to a loss of OHCs in the ear and the degree to which toluene effects on neural cell membranes may be involved. However, a series of recent papers propose that toluene modifies the response of protective acoustic reflexes to loud noises via anticholinergic effects, which could potentially result in increased noise-induced hearing loss (Campo et al. 2007; Lataye et al. 2007; Maguin et al. 2009; Venet et al. 2011). Following an injection of 116.2 nM toluene directly into the carotid artery supplying the tested cochlea, electrophysiological responses of OHCs (cochlear microphonic potentials; CMPs) to noise (65–95-dB SPL) were significantly increased in an intensity-dependent manner in anesthetized rats (Campo et al. 2007; Lataye et al. 2007). This suggests that toluene affects: (1) endocochlear potential or OHC membrane conductance; (2) how OHC stereocilia move; and/or (3) the inner- or middle-ear muscle reflex via alterations in the efferent pathway (preventing protective reflexive actions). The intensity-dependent increase suggests that the increased CMP amplitudes were due to altered inner-ear and/or middle ear acoustic reflexes (Lataye et al. 2007). Since acoustic reflexes can be stimulated in both ears following loud noises in one ear, further support for toluene-mediated alterations in acoustic reflexes comes from increased CMP amplitudes following both ipsilateral (same side) and contralateral (opposite side) noise stimulation (Campo et al. 2007). Additionally, toluene exposure did not alter CMPs in rats with severed middle ear muscles (Campo et al. 2007); however, CMPs were not altered from baseline following noise exposure prior to toluene injections, limiting the interpretation of these results. Since the auditory efferent system is a cholinergic descending pathway, and toluene has been shown to alter currents in

nicotinic acetylcholine receptors (Bale et al. 2002, 2005) and muscarinic acetylcholine receptors (Tsuga et al. 1999; Wu et al. 2002), Campo et al. (2007) hypothesized that observed alterations in acoustic reflexes in middle ear muscles result from anticholinergic actions of toluene. In support, CMP responses following injections of antagonists of cholinergic receptors and antagonists of calcium-gated acetylcholine channels were of similar magnitude to responses to toluene (Lataye et al. 2007; Maguin et al. 2009). Another study examined noise-induced distortion product otoacoustic emissions (DPOAE) to evaluate the effect of toluene exposure on noise-induced suppression of cochlear responses (Venet et al. 2011). Similar to CMP responses, DPOAE amplitude suppression to ipsilateral and contralateral stimuli was decreased in rats injected with 116 nM toluene; however, no cochlear damage was observed (Venet et al. 2011). Additionally, a clear dose-response effect was not observed, as contralateral noise-induced suppression is increased in rats injected with 58 nM toluene, and no clear effects were observed at 58 nM toluene with ipsilateral stimuli or 87 nM toluene with either ipsilateral and contralateral stimuli (Venet et al. 2011).

Mechanisms of Vision Impairment. The molecular mechanism and pathogenesis of color vision impairment (dyschromatopsia) associated with occupational and intentional abuse 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 demyelination of optic nerve fibers may be involved (Muttray et al. 1997, 1999; Zavalic et al. 1998a, 1998b, 1998c). Alterations observed in VEPs may be mediated through alterations in glutamatergic receptors, as treatment with MK801 (a non-competitive antagonist of the NMDA receptor) prevented the reduction of VEP amplitude observed following acute exposure to 2,000 ppm toluene (Bale et al. 2007).

3.5.2.2 Mechanisms of Toxicity to Other Systems

Mechanistic studies in other systems are limited, but suggested mechanisms are similar to those observed for neurotoxicity, including altered membrane properties, apoptosis, oxidative stress, and gene expression alterations.

Altered Membrane Properties. As observed in nervous system cultures, altered membrane properties were reported following toluene exposure in Jurkat T cells, as evidenced by increased LDH and calcium leakage (McDermott et al. 2007).

Apoptosis and Oxidative Stress. As observed in nervous tissue, increased markers of oxidative stress have been observed in liver, kidney, and testes in rats following acute or intermediate-duration oral exposure to 650 mg/kg/day (Kamel and Shehata 2008). Additionally, increased apoptosis has been observed in the liver, but not kidney or testes, in rats following intermediate-duration oral exposure to 650 mg/kg/day (Kamel and Shehata 2008). Additional evidence for oxidative stress in reproductive organs include increased activities for glutathione peroxidase in ovaries of rats exposed to 500 ppm toluene for 4 hours/day, 5 days/week for 1 month (Burmistrov et al. 2001), attenuation of sperm parameter changes and testicular damage in rats exposed to 6,000 ppm toluene for 2 hours/day for 5 weeks following pre-treatment with antioxidant thymoquinone (Kanter 2011b), and increased 8-oxodG formation in testes along with decreased sperm counts and serum testosterone in male rats given daily intraperitoneal injections of 50 or 500 mg/kg for 10 days (Nakai et al. 2003). In another study, no significant changes in testicular or epididymal levels of several markers of oxidative stress were observed in rats exposed to 1,500 ppm 4 hours/day for 7 days (Tokunaga et al. 2003).

An *in vitro* study in human leukemia cells reports significant increases in apoptosis and dose-related (but nonsignificant) increases in reactive oxygen species production (Sarma et al. 2011). In porcine proximal tubular cells (LLC-PK1), both apoptosis and lipid peroxidation were significantly increased following *in vitro* exposure to toluene (Al-Ghamdi et al. 2003, 2004).

Gene Expression Changes in Tissues Other than the Brain. Changes in mRNA levels for genes involved in oxidative stress thrombosis, vasoconstriction and inflammation were noted in rat cardiac tissue following single gavage doses of 1,000 mg/kg toluene (Gordon et al. 2010). In a series of studies from a single laboratory, intermediate-duration inhalation exposures of normal or allergy-challenged (ovalbumin [OVA]-immunized and challenged) mice to toluene concentrations ranging from 5 to 90 ppm have been reported to produce non-monotonic dose-related changes in the mRNA levels for inflammatory cytokines, neurotrophins, neurotrophin receptors or other immune regulatory transcription factors in lung, BAL fluid, thymus, or spleen (Fujimaki et al. 2009b, 2010, 2011; Liu et al. 2010; Yamamoto et al. 2009; Win-Shwe et al. 2007a). As with the neurological gene expression studies, current understanding is inadequate to determine the mechanistic significance of these findings.

3.5.2.3 Potential Mechanisms of Metabolite-mediated Toxicity

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 3.4.3),
(3) results from animal studies and studies of toluene-exposed workers do not identify the liver as the most sensitive target organ (see Section 3.2.1), 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 repeated high-level exposures such as those experienced by toluene abusers.

3.5.3 Animal-to-Human Extrapolations

Many laboratory animal species have been used to describe toluene toxicity, but the most commonly used species is the rat. As described in Section 3.4, 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-duration MRLs for oral exposure to toluene are based on extrapolating effects in rats to humans (see Section 2.3 and Appendix A).

As discussed in Section 3.4.5, a number of PBPK models are available that describe the kinetics of toluene after inhalation exposure for humans (Benignus et al. 2006; Fisher et al. 1997; Jonsson and Johanson 2001; Sari-Minodier et al. 2009; Nong et al. 2006; Pierce et al. 1996, 1999; Tardif et al. 1995, 2002) and rats (DeJongh and Blaauboer 1996, 1997; Kenyon et al. 2008; Oshiro et al. 2011; Tardif et al. 1993; van Asperen et al. 2003). PBPK models to describe the kinetics of dermally applied aqueous solutions of toluene are also available for humans (Thrall et al. 2002a) and rats (Thrall and Woodstock 2002), but models to describe kinetics following oral exposure to toluene have not been developed.

Benignus et al. (2007) used available PBPK models for inhaled toluene in humans (Benignus et al. 2006) and rats (Kenyon et al. 2008) to examine the relative sensitivity of neurobehavioral end points in humans and rats acutely exposed to inhaled toluene. End-point-specific dose-response relationships were constructed relating brain concentrations (estimated with pertinent PBPK models) with the magnitude of effect (expressed as a logistic function where a value of 1 means that the end point has been maximally affected) from studies of toluene effects on neurobehavioral end points in humans and rats. A single human dose-response relationship was constructed from six studies of choice reaction times in toluene-exposed human subjects. This relationship was compared with relationships constructed from studies of four neurobehavioral end points in toluene-exposed rats: VEPs, signal detection accuracy, signal detection reaction time, and escape-avoidance correct response rate. The comparison showed that the relationships for human choice reaction time and rat VEP were close to overlapping, consistent with human and rat equivalence in sensitivity to these two types of neurobehavioral end points. The dose-response relationships for the other rat neurobehavioral end points were shifted to the right on the brain concentration x-axis and showed lower slopes in the following progression: rat signal detection reaction time, rat signal detection accuracy, and rat escape-avoidance correct response rate.

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 in deriving oral MRLs for toluene.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*,

initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Current data do not provide consistent evidence of endocrine disruption in toluene-exposed humans. 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 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), 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), and increased risk of preterm birth with increased environmental levels of toluene (Poirier et al. 2015). However, small numbers and lack of adjustment for possible confounding factors in some of these studies precludes drawing definite conclusions. Additionally, studies of blood levels of reproductive hormones in repeatedly exposed workers or acutely

exposed human subjects have not provided strong and consistent evidence of exposure-related effects (Luderer et al. 1999; Svensson et al. 1992a, 1992b).

Similarly, evidence for endocrine effects in animals following acute- or intermediate-duration inhalation exposure to toluene is not consistent across studies and does not clearly identify toluene as an endocrine-disrupting chemical. Female rats exposed to 30 or 300 ppm toluene 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). Additionally, increased adrenal weight and adrenocortical cell size, along with serum ACTH and corticosterone levels, were observed in in male rats exposed to 1,500 ppm 4 hours/day for 7 days (Gotohda et al. 2005). This exposure scenario was shown to cause, in companion studies, neuronal damage and an increase in glucocorticoid receptors in the hippocampus, suggesting a possible disruption in the neuroendocrine axis (Gotohda et al. 2000a, 2000b, 2002). However, 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; Roberts et al. 2003; NTP 1990; Von Oettingen et al. 1942), or dose-related changes in endocrine hormones (Andersson et al. 1980, 1983b).

There is limited evidence that exposure to toluene may damage the reproductive organs in animals, but available data do not support that toluene effects reproductive performance. Effects on male reproductive tissues have been observed in a few studies of animals exposed by inhalation to concentrations ≥2,000 ppm (e.g., reduced sperm count, motility, and quality; and altered reproductive organ weight and histology) (Kanter 2011b; Ono et al. 1996, 1999), but changes in sperm count and epididymis weight were not accompanied by any change in indices of reproductive performance (e.g., fertility) in male rats exposed to 2,000 ppm for 60 days before mating (Ono 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 (NTP 1990). However, no effects on the 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). 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). However, no histopathological effects on the prostate, testes, uterus, or ovaries were observed in rats and female mice gavaged with 312–2,500 mg/kg/day or exposed 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). Studies in rats exposed repeatedly by inhalation to toluene, including a 2-generation reproductive toxicity study, have shown no evidence of adverse effects on mating or fertility at tested concentrations as high as 1,200–2,000 ppm (API 1981, 1985; Ono

et al. 1996; Roberts et al. 2003; Thiel and Chaboud 1997). In addition, the majority of numerous gestational exposure studies reported no exposure-related changes in reproductive indices (API 1978, 1991; Bowen and Hannigan 2013; Bowen et al. 2005, 2007, 2009a, 2009b; Courtney et al. 1986; Dalgaard et al. 2001; Gospe and Zhou 2000; Hass et al. 1999; Hougaard et al. 2003; Jones and Balster 1997; Klimisch et al. 1992; Ladefoged et al. 2004; NIOSH 1983; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Seidenberg et al. 1986; Thiel and Chahoud 1997; Warner et al. 2008).

There is evidence that toluene exposure can perturb the hypothalamic-pituitary axis in rats leading to persistent increases in serum levels of prolactin. Elevated prolactin levels were reported in rats after exposure to 80 ppm toluene 6 hours/day, 5 days/week for 4 weeks (Von Euler et al. 1994) or 80–1,000 ppm 6 hours/day for 3 days (Andersson et al. 1983b). 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. However, in other studies, no changes in prolactin levels were found in rats after exposure to 40–320 ppm 6 hours/day, 5 days/week for 4 weeks (Hillefors-Berglund et al. 1995), 500 ppm toluene 6 hours/day for 3 days, or 1,000 ppm 6 hours/day for 5 days (Andersson et al. 1980). In addition, a study of toluene-exposed workers found no evidence for changed prolactin levels, compared with control subjects (Svensson et al. 1992a, 1992b).

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

3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to adverse health effects from exposure to hazardous chemicals, but whether there is a difference depends on the chemical(s) (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to exposure-related 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 that are most sensitive to disruption from exposure to hazardous substances. Damage from exposure in one stage 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). Past literature has often described the fetus/infant as having an immature (developing) blood-brain barrier that is leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the brain, there are differences between fetuses/infants and adults that are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; whether these mechanisms provide protection for the developing brain or render it more vulnerable to

toxic injury is an important toxicological question. Chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

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 given their low glomerular filtration rate and not having developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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

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 a critical target of toluene toxicity (see Chapter 2 and Sections 3.2 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 a 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 reported following toluene inhalation exposure in young animals (neonatal-young adult) are similar to those reported in adults, including changed levels of brain neurotransmitters (O'Leary-Moore et al. 2009; von Euler et al. 1989); high frequency hearing loss (Pryor and Rebert 1992; Pryor et al. 1984a); increased locomotor activity (Bowen et al. 2007; Samuel-Herter et al. 2013); impaired motor coordination (Samuel-Herter et al. 2013); and altered pain perception (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 clear toluene from the body 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 nonprimate animals.

Recent studies investigating age-related susceptibility to “binge” toluene exposure in rats do not clearly support increased susceptibility in young versus adult rats. Following 15- or 30-minute exposures to ~5,000 ppm toluene, increased locomotor activity, altered exploration, and impaired motor coordination and gait were observed in adolescent (1 month), young adult (2–3 months), adult (5–6 months) and older adult (10–12 months) rats, with no apparent age-related effects (Samuel-Herter et al. 2013). However, the

duration to recover from toluene-induced motor impairments was significantly longer in adolescent and young adults (Samuel-Herter et al. 2013). Following exposure to 0, 2,000, 4,000, or 8,000 ppm toluene for 20 minutes/day for 10 days, age- and sex-dependent increases in locomotion were observed in adolescent (PND 28), young adult (PND 44), and adult (PND 70) rats; however, adolescent rats were not identified as a susceptible group (Bowen et al. 2007). In the adolescent rats, increases were significant in females from all exposure groups and males from the 4,000 and 8,000 ppm groups during week 2 of exposure. In the young adult and adult rats, increases were significant in females from the 4,000 and 8,000 ppm groups and in males from the 8,000 ppm group during week 2 of exposure; these increases were significantly greater in magnitude than observed changes in adolescents from the same dose groups. Additionally, adult females and males from the 8,000 ppm group showed significantly increased locomotor behavior during week 1, from day 1 and 3, respectively. These results suggest that while effects may be observed at lower doses in adolescents, the effects may take longer to manifest and may be smaller in magnitude, compared with adults (Bowen et al. 2007). Another study evaluated potential differences in toluene-induced locomotor effects in adolescent (PND 28) and adult (PND 90) male rats exposed to 0, 8,000, or 16,000 ppm for 12 days using three different exposure patterns: standard (two 15-minute exposures separated by a 105-minute break), rapid (two 15-minute exposures separated by a 15-minute break), and paced (six 5-minute exposures separated by 25-minute breaks) (Batis et al. 2010). Locomotor activity was assessed during each exposure period and during a 30-minute "recovery" period immediately following the final toluene exposure each day. While locomotor activity was significantly altered in both exposure groups at both ages, subtle differences were noted. Adolescents displayed greater toluene-induced locomotor activity on the first day and generally greater increases in activity over all days than adults during toluene exposure; however, adults displayed greater toluene-induced locomotor activity than adolescent in the "recovery" period following exposure on the first and subsequent days. Age group differences were most pronounced in the paced "binge-like" exposure protocol. The subtle age-related effects described in these studies may be mediated by age-dependent neurochemical changes observed (O'Leary-Moore et al. 2009). Following acute, high-dose exposure to 8,000–12,000 ppm toluene, no significant changes were found in neurotransmitter, n-acetyl-aspartate (NAA), choline-containing compounds, creatine, glutamate, glutamine, GABA, or lactate levels in adolescent brains; however, decreased levels of choline and GABA in the frontal cortex and striatum, decreased glutamine and NAA levels in the frontal cortex, and a wide-ranging increase in lactate were observed in young adult brains (O'Leary-Moore et al. 2009).

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). Following repeated inhalation exposure of pregnant rats to higher concentrations (8,000 or 12,000 ppm) for 15, 30, or 45 minutes twice daily from GD 8 to 20, amniotic fluid concentrations were ~<10% of concentrations in maternal blood samples, concentrations in fetal brain were similar to maternal blood concentrations, and concentrations in placenta were higher (Bowen et al. 2007). Concentrations of toluene in fetal brain were less than concentrations in maternal brain.

The results from animal studies indicate that toluene did not cause maternal or developmental toxic effects in animals at inhalation exposure levels <1,000 ppm administered for 6–7 hours/day during gestation (API 1978, 1991, 1992; Jones and Balster 1997; Klimisch et al. 1992; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997; Tsukahara et al. 2009; Win-Shwe et al. 2012a, 2012b; Yamamoto et al. 2009) or at oral exposure levels of 1,800–2,350 mg/kg/day during the period of organogenesis in two developmental screening studies (NIOSH 1983; Seidenberg et al. 1986). With higher inhalation exposure levels (≥1,000 ppm), predominant effects include retarded fetal growth and skeletal development and altered development of behavior in offspring, and were almost always accompanied by signs of maternal toxicity (API 1991, 1992; Bowen and Hannigan 2013; Bowen et al. 2005, 2009a; Callan et al. 2015; Dalgaard et al. 2001; Hass et al. 1999; Hougaard et al. 2003; Roberts et al. 2007; Jones and Balster 1997; Ono et al. 1995; Saillenfait et al. 2007; Thiel and Chahoud 1997). Other animal studies reported that continuous, 24-hour/day exposure during gestation caused maternal body weight depression and effects on fetuses including depressed body weight and delayed skeletal ossification at toluene concentrations as low as 133–399 ppm in rats, mice, and rabbits (Hudak and Ungvary 1978; Ungvary and Tatrai 1985). In a comprehensive developmental toxicity study in rats, a statistically significant increase in the incidence of a dilated renal pelvis in the left kidney was observed in fetuses from dams exposed to 1,250 mg/kg/day on GDs 16–19 via gavage, compared with controls (Warner et al. 2008). No changes were observed in any other developmental end point. However, exposure of pregnant rats to gavage doses of 650 mg/kg/day toluene in corn oil on GDs 6–19 produced offspring with decreased body weights, delayed ossification, smaller brain volumes, decreased forebrain myelination per cell, and decreased cortical cell proliferation and migration (Gospe and Zhou 1998, 2000; Gospe et al. 1996).

Performance deficits in a few neurobehavioral tests were observed in one study in offspring of pregnant mouse dams exposed by inhalation to 2,000 ppm, but not 200 or 400 ppm, for 60 minutes 3 times/day on GDs 12–17 (Jones and Balster 1997). Performance deficits were not observed in offspring of pregnant rat dams exposed by inhalation to up to 2,000 ppm for 6 hours/day during gestation (Hougaard et al. 2003; Ono et al. 1995; Thiel and Chahoud 1997). Drinking water exposure during gestation and lactation at doses of 106 mg/kg/day resulted in changes in postweaning open-field locomotor activity in rat offspring (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 (Janisik et al. 2008; Leung and Paustenbach 1988; Lof et al. 1993; Nise et al. 1989; Pellizzari et al. 1992; Pierce et al. 1996, 1999, 2002; see Section 3.4.4). Thus, mobilization during pregnancy or lactation of stored toluene from preconception 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) 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, 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), and a daily oral intake for a 10-kg child (2.0 mg/day) associated with the intermediate oral MRL for toluene (0.2 mg/kg/day). 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.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

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, 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 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by toluene are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the

biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Toluene

In a number of field studies, correlations have been noted between workplace air toluene concentrations and toluene concentrations in blood or urine, or hippuric acid or *ortho*-cresol concentrations in urine of workers (see ACGIH 2001 and 2010 for reviews and citations of these studies). Other urinary biomarkers of exposure that have been examined include benzyl alcohol (Ikeda et al. 2008; Kawai et al. 2007), benzylmercapturic acid (Ikeda et al. 2008; Inoue et al. 2002, 2004, 2008; Maestri et al. 1997), and S-*p*-toluylmercapturic acid (Angerer et al. 1998a; Cosnier et al. 2013, 2014; Ikeda et al. 2008; Inoue et al. 2008). A preliminary study also examined toluene concentration in saliva as a possible biomarker of exposure, which showed a correlation coefficient with air concentrations of 0.77, compared with 0.93 for urinary toluene concentration (Ferrari et al. 2008).

Currently, ACGIH (2010, 2013) recommends using a combination of three biomarkers to assess exposure of workers to toluene: *ortho*-cresol and unchanged toluene levels in urine at the end of a workshift and toluene levels in blood immediately prior to the last shift of a workweek. The recommendation was made based on analyses of numerous field studies examining toluene blood concentrations and urinary concentrations of *ortho*-cresol and toluene in workers exposed to varying workplace air concentrations of toluene. Previously, the level of hippuric acid in urine at the end of a workshift was recommended as a biomarker of exposure, but this recommendation was withdrawn because background urinary hippuric acid from consumption of benzoate in foods and beverages is expected to mask contributions from workplace exposure to toluene, especially at concentrations below 50 ppm (ACGIH 2001, 2010). Results from studies comparing the effectiveness of various biomarkers at high (>50 ppm) and low (<10 ppm) air concentrations indicate that toluene concentrations in blood or urine are more accurate at low air concentration than urinary concentrations of examined metabolites (Ikeda et al. 2008; Inoue et al. 2008; Kawai et al. 2008; Lovreglio et al. 2010; Takeuchi et al. 2002; Ukai et al. 2007).

3.8.2 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 MRI or 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, low-level exposures as are likely to occur with environmental releases. Micronuclei induction in buccal cells and/or peripheral lymphocytes have been suggested as biomarkers of effect for solvent exposure (Gonzalez-Yebra et al. 2009; Heuser et al. 2007; Pitarque et al. 2002); however, evidence for micronuclei induction following exposure to toluene or mixed solvents is inconsistent (Gonzalez-Yebra et al. 2009; Heuser et al. 2005, 2007; Moro et al. 2012; Nise et al. 1991; Pitarque et al. 2002) and induction of micronuclei in these cells is not expected to be only produced by solvents. Similarly, altered gene expression profiles in blood cells could potentially serve as biomarkers of effect, but human (Hong et al. 2016; Kim et al. 2011; Song and Ryu 2015) and animal studies (Ahmed et al. 2007; Fujimaki et al. 2009a, 2009b, 2011; Hester et al. 2011, 2012; Kodavanti et al. 2011; Liu et al. 2010; Royland et al. 2012; Takeda et al. 2013; Win-Shwe et al. 2007a, 2010a, 2010b, 2010c, 2011; Yamamoto et al. 2009) have not identified concentration-related gene expression changes in blood, nervous, or immune tissue following toluene exposure. A detailed discussion of the effects of toluene exposure is included in Section 3.2.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Alteration of toluene metabolism may influence toluene's toxic effects because toluene metabolism predominately represents a detoxification process (see Section 3.5.2). 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), rats exposed to 1,700–2,000 ppm toluene, 6 hours/day, 5 days/week for 6 weeks (Campo et al. 2008), and rats exposed to a single gavage dose of 1,500 mg/kg (Campo et al. 2008). 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 OHC 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 nondrinkers.

Concurrent chronic ethanol ingestion and acute toluene inhalation in rats was associated with a modest elevation in plasma AST 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, *ortho*-cresol, and *para*-cresol. The excretion of metabolites was investigated in four groups of workers who were exposed in the workplace to benzene or 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). Exposure to 50 ppm benzene and 50 or 100 ppm toluene, 6 hours/day on 8 separate days (over a period of 15 days) caused a significant induction of hepatic CYP 2E1, which did not occur with individual exposure to benzene or toluene (Wetmore et al. 2008). Additionally, rats exposed to 50 ppm benzene plus 100 ppm toluene (but not 50 ppm toluene) showed a significant increase in the number of micronucleated bone marrow cells and erythrocytes (~50–70%), compared with exposure to 50 ppm benzene alone (Bird et al. 2010; Wetmore et al. 2008). Micronuclei induction in erythrocytes following exposure to 50 ppm benzene plus 100 ppm toluene, 6 hours/day for 8 consecutive days was also

significantly greater than exposure to 50 ppm benzene alone (~200%); however, it was ~70% less than micronuclei induction by 150 ppm benzene alone (Bird et al. 2010). Exposure to toluene alone at 50 or 100 ppm did not induce micronuclei (Bird et al. 2010; Wetmore et al. 2008). Therefore, while these findings are suggestive that toluene enhances benzene toxicity, there is not clear evidence for the mechanism of joint toxic action.

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 a better visual fit to empirical data than non- or uncompetitive inhibition metabolic terms, using 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. Using an interactions-based biological hazard index, PBPK-based model predictions indicated that interactions between toluene, *m*-xylene, and ethylbenzene are not strictly additive (especially at higher concentrations levels); however, competitive inhibition is expected to be negligible at low individual solvent concentrations (e.g., 20 ppm) (Haddad et al. 1999). Toxicokinetic data from volunteers acutely exposed to toluene, *m*-xylene, and ethylbenzene provided a good fit to predictions of the Haddad et al. (1999) PBPK model (Marchand et al. 2015).

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. However, in eight healthy male volunteers who were exposed to combinations of toluene and *n*-hexane via feeding tubes at rates of 1.5 or 4 mg/minutes and 0.3 or 1.0 mg/minutes, respectively, for 60 minutes, the high-dose of *n*-hexane significantly decreased urinary hippuric acid excretion, but toluene exposure did not alter the urinary excretion of 2,5-hexanedione (Baelum et al. 1998). 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). In support, *n*-hexane metabolism (as measured by end-of-shift free-2,5-hexanedione levels in urine) was not modified by co-exposure to toluene in workers exposed to various solvent mixtures in an adhesive tape factory at concentrations below occupational exposure limits, as determined by multiple regression analysis (Kawai et al. 2000).

McDermott et al. (2008) examined potential interactions of *in vitro* solvent exposure on LDH leakage, calcium levels, and glutathione redox status of human Jurkat T-cells. Nine binary mixtures of toluene, *n*-hexane, and methyl ethyl ketone (MEK) were evaluated, using three exposure levels per solvent based on concentration-response data for the individual solvents. The resulting data were analyzed using both isobolographic and Berenbaum's combination index analysis to test for interaction. The findings indicated greater-than-additive interactions between toluene+*n*-hexane and toluene+MEK for both LDH leakage and glutathione redox status (GSH and GSSG levels, GSH/GSSG ratio). Greater-than-additive interactions were also observed between toluene+*n*-hexane for perturbations in calcium levels; however, less-than-additive interactions were observed between toluene+MEK at higher MEK concentrations (McDermott et al. 2008).

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 GD 12 and exposed to toluene at concentrations of 1,000, 2,000, or 3,600 mg/m^3 (265, 531, or 956 ppm) on GDs 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 GDs 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.

3.10 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. Factors involved with increased susceptibility 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 6.7, Populations with Potentially High Exposures.

One of the primary target organs 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 3.5.2). 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 3.4.3).

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 via CYP 2E1 respectively enhanced or inhibited toluene-induced hearing loss in rats (Campo et al. 1998, 2008; 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. Additionally, smokers may have an increased risk of toxicity due to a possible repression of CYP 2E1 via epigenetic modifications (Jimenez-Garza et al. 2015). Differences in the relative efficiency of enzymes found in ethnic populations may lead to differences in toluene susceptibility, as 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, 2015). For example, DNA damage in leukocytes from Brazilian shoe workers exposed to solvent-based adhesive (mainly toluene, air concentration not reported)

was significantly increased in workers with polymorphisms in the glutathione *S*-transferase P1 gene (*GSTP1; Ile/Val or Val/Val*) compared with *GSTP1 Ile/Ile* workers (Heuser et al. 2007). In Indian paint workers exposed to toluene as the major solvent used in paint thinner, sister chromatid exchanges were significantly increased in workers heterozygous or homozygous for the mutant allele of *CYP1A1m2* or *CYP2E1*, compared with unexposed controls of the same genotype; however, sister chromatid exchanges were not significantly increased in workers homozygous for the wild-type alleles, compared with unexposed controls of the same genotype (Priya et al. 2015). Similarly, increased micronuclei in Bulgarian shoe workers were only observed in workers with a null glutathione *S*-transferase M1 (*GSTM1*) genotype, although sister chromatid exchanges were not associated with *GSTM1*genotype (or solvent-exposure) (Pitarque et al. 2002). Additionally, in healthy South Korean males living in Ulsan City, which houses several industrial complexes, urinary 8-OHdG and hippuric acid levels were only significantly correlated in individuals with *GSTM1*-null, glutathione *S*-transferase T1(*GSTT1)*-null, and aldehyde dehydrogenase 2 (*ALDH2*) *2/*2 genotypes (Kim et al. 2011). Genetic diversity of *GSTM1* (null/present), glutathione *S*-transferase T1 gene (*GSTT1*, null/present), and *CYP2E5* (C1/C1, C1/C2, C2/C2 variants) was also evaluated in a study of Korean and foreign-born workers exposed to toluene at a Korean printing company (Kim et al. 2015). The allelic frequency of the *GSTM1* present genotype ranged from 0.71 in Chinese workers to 0.15 in Vietnamese workers, with a frequency of 0.4–0.6 in Korean, Sri Lankan, and Cambodian workers, and there was a slight, near-significant (p=0.081) positive correlation between the presence of the *GSTM1* gene allele and hippuric acid levels in the blood of workers. For *GSTT1* present genotype, the allelic frequency ranged from 0.75 in Indonesian workers to 0.18 in Chinese workers, with a frequency of 0.4–0.6 in Korean, Vietnamese, and Cambodian workers, and there was a significant correlation between the presence of the *GSTT1* gene allele and urinary toluene/blood toluene ratio. For *CYP2E5* C1 genotype, the allelic frequency ranged from 1.00 in Sri Lankan workers to 0.55 in Vietnamese workers, with a frequency of 0.6–0.7 in Korean, Indonesian, and Chinese workers; however, no correlation was found between toluene metabolism and the *CYP2E5* gene allele in this study.

Studies in various inbred and outbred mouse strains indicate that genetic differences can lead to differential susceptibility to various chemicals, particularly drugs of abuse (Crabbe et al. 1994, 2005). Bowen et al. (2010) investigated the effects of acute toluene exposure on locomotor behavior in four genetically divergent strains of mice, Balb/CBYJ, C57BL/6J, DBA/2J, and Swiss Webster. During a 30-minute exposure to 100, 2,000, 8,000, or 10,000 ppm toluene, all strains showed a qualitatively biphasic increase in locomotor activity, compared with pre-exposure activity levels, which is consistent with reports of initial increases in locomotion at lower concentrations followed by a decrease in activity at

concentrations of 3,000–10,000 ppm (Bowen and Balster 1998; Conti et al. 2012; Kim et al. 1998; Lopez-Rubalcava and Cruz 2000). However, while increased activity was observed in all exposure groups for the inbred Balb/CBYJ, C57BL/6J, and DBA/2J mice, activity was not significantly increased in outbred Swiss Webster mice until ≥2,000 ppm. Furthermore, when the mice were exposed to a 2,000-ppm toluene challenge, following exposure to 8,000 ppm for 30 minutes/day for 14 days, DBA/2J mice showed the greatest sensitization to toluene-induced locomotor effects among the four strains. Bowen et al. (2010) concluded that genetic differences must account for the differential sensitivity to the effects of toluene exposure, and suggested that inter-strain variations in the dopaminergic system may underlie the observed differences.

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).

Both children and aging adults could potentially have increased susceptibility to toluene exposure. Children’s susceptibility is discussed in Section 3.7. No studies investigating toluene exposure in aging humans were located. A limited number of oral exposure studies in rats did not identify a clear pattern for age-related susceptibility for toluene-induced changes in locomotor activity, oxidative stress markers, cardiac biomarkers, or gene expression changes (Gordon et al. 2010; Kodavanti et al. 2011; MacPhail et al. 2012; Royland et al. 2012).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to toluene. 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, board certified medical toxicologists, board-certified occupational medicine physicians and/or other medical specialists with expertise and experience treating patients overexposed to toluene can be consulted for medical advice. The following texts provide specific information about treatment following exposures to toluene:

Gummin DD. 2014. Hydrocarbons. In: Goldfrank LR, Flomenbaum NE, Lewin NA, eds. Goldfrank's toxicologic emergencies. 10th ed. New York, NY: McGraw-Hill, 1303-1322.

Leikin JB, Pauloucek FP. 2008. In: Leikin JB, Pauloucek FP, eds. 4th ed. Boca Raton, FL: CRC Press, Taylor & Francis Group, 1195-1196.

Shannon MW, Borron SW, Burns MJ. 2007. In: Shannon MW, Borron SW, Burns MJ, eds. Haddad and Winchester's clinical management of poisoning and drug overdose. 4th ed. Philadelphia, PA: WB Saunders, 1370-1374.

Additional relevant information can be found in the front section of this profile under QUICK REFERENCE FOR HEALTH CARE PROVIDERS.

3.11.1 Reducing Peak Absorption Following Exposure

The absorption of toluene is rapid and virtually complete following acute 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; therefore a reduction of physical activity during exposure is likely to reduce absorption (Bushnell et al. 2007; Nadeau et al. 2006; Rahill et al. 1996). However, there is no effective way to reduce peak absorption following inhalation exposure. Emesis is contraindicated in cases of toluene ingestion due to the risk of aspiration. Since gastric lavage is often associated with spontaneous emesis, and current data do not support that it is

effective in reducing risk of pulmonary complications following hydrocarbon exposure, lavage is generally not indicated following hydrocarbon exposure (Gummin 2014). Similarly, the use of activated charcoal is generally not indicated for hydrocarbon exposure due to limited ability to reduce absorption coupled with risk of stomach distension and predisposition to vomiting and aspiration. Activated charcoal may be justified in cases of mixed chemical overdoses (Gummin 2014).

Following skin exposure, immediate washing of the skin with soap (if available) and water will reduce the opportunity for dermal absorption; rinsing the skin with water alone may not be sufficient (Gummin 2014). If toluene is spilled on clothing, clothes should be removed promptly and safely discarded to prevent further skin and/or inhalation exposure. If the eyes are affected, proper rinsing procedures should be followed (Gummin 2014).

3.11.2 Reducing Body Burden

The total body burden of toluene is reduced by measures that increase the rate of metabolism and excretion. Oxygen therapy, positive-pressure ventilation, high-frequency jet ventilation, and extracorporeal membrane oxygenation have been used as emergency treatments in cases of severe hydrocarbon toxicity, often associated with episodes of toluene abuse (Graham 1990; Gummin 2014). These procedures promote the loss of unmetabolized toluene from the lungs.

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).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

In cases where toluene has caused cardiac arrhythmias, management of dysrhythmias is critical. Interventions may include evaluation and treatment of potential electrolyte and acid-base abnormalities as well as administration of antiarrhythmic medications to control the heart beat (Graham 1990; Gummin 2014). 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.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 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 adverse health effects (and techniques for developing methods to determine such health effects) of toluene.

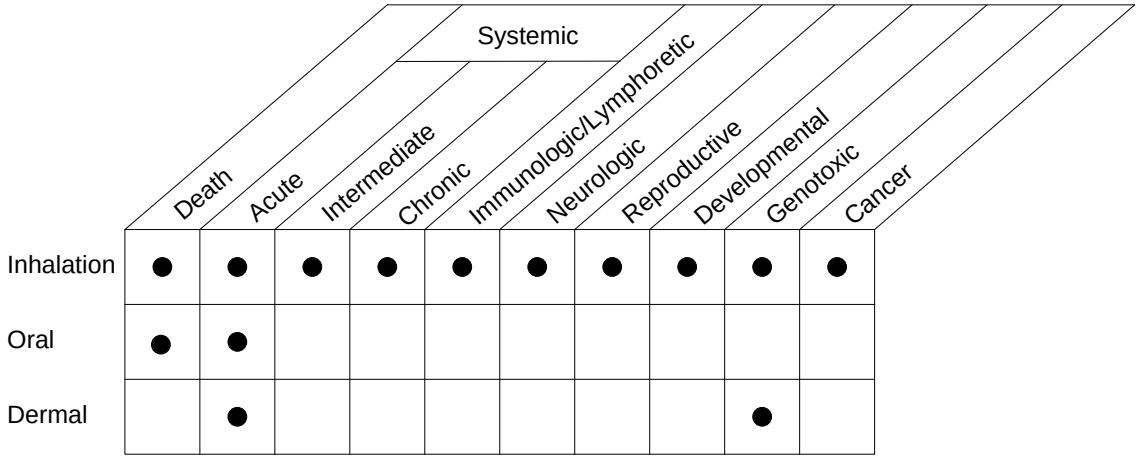
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health risk assessment. This definition should not be interpreted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

3.12.1 Existing Information on Health Effects of Toluene

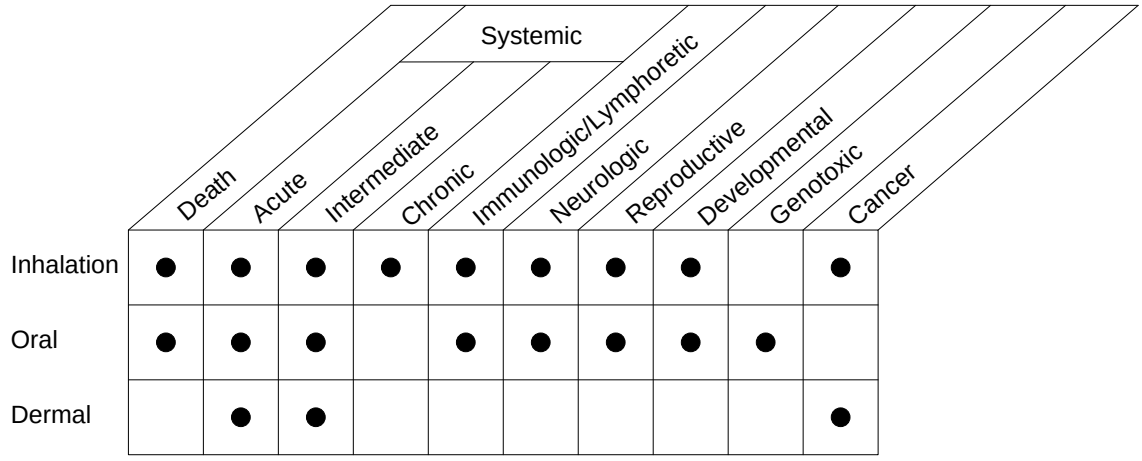
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to toluene are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of toluene. Each dot in the figure indicates that one or more 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* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-5, 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 expected to be the main routes by which

Figure 3-5. Existing Information on Health Effects of Toluene



Human



Animal

● Existing Studies

humans are exposed to toluene. Figure 3-5 also shows that considerable animal toxicity data for inhalation exposure are available. However, there are limited oral and dermal data from animal studies.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The acute effects of toluene exposure in humans have been well-studied, and identify the nervous system as a critical acute toxicity target of toluene, with subtle neurological effects at exposures >40 ppm in healthy individuals and as low as 15 ppm in clinically sensitive individuals (Andersen et al. 1983; Baelum et al. 1985; Dick et al. 1984; Echeverria et al. 1991; Gamberale and Hultengren 1972; Kobald et al. 2015; Little et al. 1999; Orbaek et al. 1998; Osterberg et al. 2000, 2003; Rahill et al. 1996; von Oettingen et al. 1942). Supporting data for neurological effects as a critical acute effect are provided by studies of animals after inhalation exposure that consistently report altered locomotor activity at exposure levels ≥500 ppm and cognitive deficits at concentrations as low as 125 ppm (Arito et al. 1988; Boyes et al. 2007; Bowen and Balster 1998; Bowen et al. 2010; Bruckner and Peterson 1981a, 1981b; Bushnell et al. 1985; Conti et al. 2012; Cruz et al. 2001; Ghosh et al. 1989, 1990; Hinman 1987; Hogie et al. 2009; Huerta-Rivas et al. 2012; Johnson 1992; Johnson et al. 1988; Kishi et al. 1988; Li et al. 1992; Little et al. 1998; Lopez-Rubalcava and Cruz 2000; McWilliams et al. 2000; Mullin and Krivanek 1982; Paez-Martinez et al. 2003; Rebert et al. 1989b; Takeuchi and Hisanaga 1977; Taylor and Evans 1985; Tomaszycki et al. 2013; Wood and Colotla 1990; Wood et al. 1983), as well as numerous additional studies in animals following exposure to “binge-like” levels (1,000–12,000 ppm) modeling human solvent abuse (Apawu et al. 2014; Bale et al. 2007; Batis et al. 2010; Beckley et al. 2013; Beyer et al. 2001; Bowen et al. 2007; Bushnell et al. 2007; Gerasimov et al. 2002c; Gmaz et al. 2012; Gotohda et al. 2000a, 2000b, 2002, 2007; Lammers et al. 2005b; O’Leary-Moore et al. 2007; Oshiro et al. 2007; Paez-Marinez et al. 2008, 2013; Pascual and Bustamante 2010; Perit et al. 2012; Riegel and French 2002; Samuel-Herter et al. 2013; Schiffer et al. 2006; Williams et al. 2005). Further support is provided by acute oral exposure studies examining neurological effects in animals (Burns et al. 1994; Dyer et al. 1988; Gordon et al. 2007, 2010; Mehta et al. 1998). It is unlikely that additional standard acute-duration exposure studies in animals would provide new key information on the toxicity of toluene, but special studies that involve a range of exposure levels (including low levels) and employ sensitive, behavioral, ultra structural, and biochemical measurements may be useful—especially if findings are correlated with observed neurobehavioral alterations. Data for the dermal exposure route are limited; however, this is not expected to be the main route of human exposure. Sufficient data for the oral and inhalation routes were available to derive an acute inhalation MRL based on minimally adverse neurological effects in volunteers with multiple chemical sensitivity exposed to 15 ppm for 20 minutes

(Little et al. 1999) and an acute oral MRL based on changes in FEPs observed in mice exposed to 250 mg/kg toluene (Dyer et al. 1988).

Intermediate-Duration Exposure. The database is lacking studies on intermediate-duration exposure in humans. Several studies are available on repeated-dose exposure of animals to toluene after inhalation exposure. Similar to acute exposure studies, animal studies report neurological effects following intermediate-duration inhalation exposure at concentrations ranging from 100 to 2,500 ppm (API 1997; Arito et al. 1988; Boyes et al. 2015; Campo et al. 1997; Kyrklund et al. 1987; McWilliams et al. 2000; NTP 1990; Pryor et al. 1984b; von Oettingen et al. 1942; Wiaderna and Tomas 2002; Wood and Cox 1995). However, NOAELs 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, and could potentially be used to derive an intermediate-duration MRL for inhalation exposures. Adequate oral intermediate-duration exposure studies have been conducted in rats and mice (Hsieh et al. 1989, 1990a, 1991; NTP 1990), and sufficient data were available to derive an intermediate-duration oral MRL based on toluene-induced immune depression observed at 84–105 mg/kg/day (Hsieh et al. 1989, 1990a, 1991). Neurobehavioral alterations following intermediate-duration oral toluene exposure were limited to increased open-field activity in young mice following pre- and postnatal exposure to 106 mg/kg/day toluene (Kostas and Otchin 1981). Intermediate-duration studies designed to assess neurobehavioral alterations and/or mechanisms of neurotoxicity following oral exposure to toluene may be useful in evaluating human health risk, and could potentially serve as a basis for the deriving the intermediate-duration oral MRL. Studies following dermal exposure are lacking; however, this is not expected to be the main route by which humans are exposed to toluene.

Chronic-Duration Exposure and Cancer. Numerous case reports have associated chronic toluene abuse in humans at levels inducing narcosis and euphoria (4,000–12,000 ppm, as estimated by Gospe et al. 1994) with persistent neurological damage (Aydin et al. 2002, 2003; Byrne et al. 1991; Caldemeyer et al. 1996; Camarra-Lemarroy et al. 2015; Capron and Logan 2009; Deleu and Hanssens 2000; Devathasan et al. 1984; Filley et al. 1990; Fyu et al. 1998; Gupta et al. 2011; Hormes et al. 1986; Hunnewell and Miller 1998; Ikeda and Tsukagoshi 1990; Kamran and Bakshi 1998; King et al. 1981; Kiyokawa et al. 1999; Kucuk et al. 2000; Maas et al. 1991; Maruff et al. 1998; Meulenbelt et al. 1990; Miyagi et al. 1999; Nomura et al. 2016; Papageorgiou et al. 2009; Poblano et al. 1996; Rosenberg et al. 1988a, 1988b, 2002; Ryu et al. 1998; Suzuki et al. 1983; Uchino et al. 2002; Yamanouchi et al. 1995).

Neurological alterations are also critical effects reported following occupational exposure. Self-reported neurological symptoms and reduced ability in tests of cognitive and neuromuscular function have been reported in humans occupationally exposed to average concentrations as low as 40–150 ppm (Boey et al. 1997; Eller et al. 1999; Foo et al. 1990; Kang et al. 2005; Matsushita et al. 1975; Murata et al. 1993; Nordling Nilson et al. 2010; Orbaek and Nise 1989; Ukai et al. 1993; Yin et al. 1987). Studies of occupationally exposed workers also indicate that chronic exposure to average concentrations as low as 50–130 ppm can damage 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; Vrca et al. 1995, 1996, 1997a, 1997b; Zavalic et al. 1998a, 1988b, 1988c). Sufficient data for the inhalation route were available to derive a chronic MRL based on a lack of adverse effects in subjective neurological symptoms, performance on psychomotor tasks, color vision, and hearing in groups of German photogravure printers occupationally exposed to toluene (Schäper et al. 2003, 2004, 2008; Seeber et al. 2004, 2005; Zupanic et al. 2002).

The chronic effects of toluene have not been investigated in humans or animals 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. Available studies do not clearly identify toluene as a genotoxic agent. Findings from human occupational exposure studies to predominantly toluene are inconsistent, and studies are limited by lack of reporting of historical exposure levels and small cohort sizes (Bauchinger et al. 1982; Forni et al. 1971; Hammer 2002; Hammer et al. 1998; Maki-Paakkenen et al. 1980; Nise et al. 1991; Pelclova et al. 1990; Schmid et al. 1985). *In vivo* animal studies are limited, and findings are also inconsistent (API 1981; Dobrokhotov and Enikeev 1997; Liang et al. 1983; Martinez-Alfaro et al. 2010; Plappert et al. 1994; Wetmore et al. 2008). *In vitro* studies in bacteria and animal cells were almost exclusively negative (Bos et al. 1981; Connor et al. 1985; Fluck et al. 1976; Gerner-Smidt and Friedrich 1978; Nakumura et al. 1987; Nestmann et al. 1980; NTP 1990; Zarani et al. 1999) with the exception of toluene-induced DNA damage in human HL-60 cells (Sarma et al. 2011). To evaluate the potential of toluene to cause chromosomal damage, additional 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 concurrent/previous exposure to other chemicals (human studies) (Bauchinger et al. 1982; Dobrokhotov and Enikeev 1997; Hammer 2002; Hammer et al. 1998; Liang et al. 1983; 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 may 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. 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) or decreased fecundity (Plenge-Böenig and Karmaus 1999), 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. One population-based cohort study reported increased risk of preterm birth with increasing environmental toluene exposure (Poirier et al. 2015); concurrent exposure to multiple pollutants (which were not controlled for in statistical analyses) limits the conclusions that can be drawn from this study. In addition, one study reported that toluene-exposed male workers showed decreasing plasma levels of LH, FSH, and testosterone with increasing concentrations of toluene (8–<111 ppm) (Svensson et al. 1992a, 1992b). Effects on male reproductive tissues have been observed in a few studies of animals exposed by inhalation to concentrations ≥2,000 ppm (e.g., reduced sperm count, motility, and quality, and altered reproductive organ weight and histology) (Kanter 2011b; Ono et al. 1996, 1999), but changes in sperm count and epididymis weight were not accompanied by any change in indices of reproductive performance (e.g., fertility) in male rats exposed to 2,000 ppm for 60 days before mating (Ono et al. 1996). A single study reported abundant vacuoles, lytic areas, and mitochondrial degeneration in the antral follicles in the ovaries of female rats following a 7-day exposure to 3,000 ppm (Tap et al. 1996), but no histological evidence of structural damage to the reproductive organs was noted 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 or alterations in pregnancy outcomes was found in the majority of animal assays at exposure levels as high as 12,000 ppm (inhalation) or 2,350 mg/kg/day (oral) (API 1978, 1991; Bowen and Hannigan 2013; Bowen et al. 2005, 2007, 2009a, 2009b; Courtney et al. 1986; Dalgaard et al. 2001; Gospe and Zhou 2000; Hass et al. 1999; Hougaard et al. 2003; Jones and Balster 1997; Klimisch et al. 1992; Ladefoged et al. 2004; NIOSH 1983; Ono et al. 1995, 1996; Roberts et al. 2007; Saillenfait et al. 2007; Seidenberg et al. 1986; Thiel and Chahoud 1997) (including a 2-generation study of rats exposed to up to 2,000 ppm, 6 hours/day [API 1985; Roberts et al. 2003]). However, 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 (Ungvary and Tatrai 1985)

and exposure to 5,000 ppm 6 hour/day during days 6–15 of pregnancy resulted in increased post-implantation loss and complete fetal resorption in 6/9 rats (API 1992). 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 exposure.

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 and Wilkins-Haug 1990; Arnold et al. 1994; Erramouspe et al. 1996; Goodwin 1988; Hersh 1988; Hersh et al. 1985; Lindemann 1991; Pearson et al. 1994; Wilkins-Haug and Gabow 1991a). 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 (API 1991, 1992; Dalgaard et al. 2001; Hass et al. 1999; Hougaard et al. 2003; Hudak and Ungvary 1978; Jones and Balster 1997; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; 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 133–2,000 ppm with 3–6-hour/day protocols (API 1978, 1991, 1992; Jones and Balster 1997; Klimisch et al. 1992; Ono et al. 1995; Roberts et al. 2007; Saillenfait et al. 2007; Thiel and Chahoud 1997). 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 in two developmental screening studies (NIOSH 1983; Seidenberg et al. 1986), but a statistically significant increase in the incidence of a dilated renal pelvis in the left kidney was observed in fetuses

from dams exposed to 1,250 mg/kg on GDs 16–19 via gavage in a comprehensive developmental toxicity study in rats (Warner et al. 2008). 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, 2000; 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 modeling solvent abuse (8,000–16,000 ppm, 15–30 minutes/day) can impair behavioral development of rat offspring (Bowen and Hannigan 2013; Bowen et al. 2005, 2009a; Callan et al. 2015). At lower exposure levels (≤2,000 ppm), maternal exposure for 6 hours/day did not result in altered offspring behavior in rats (Hougaard et al. 2003; Ono et al. 1995; Thiel and Chahoud 1997); however, maternal exposure to 2,000 ppm for 60 minutes 3 times/day can lead to impaired behavioral development of mouse offspring (Jones and Balster 1997). 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 expected to be the main route of human exposure.

Immunotoxicity. Human studies of immunological end points in toluene-exposed subjects do not identify consistent or strong evidence for toluene effects on immune system end points such as counts of blood lymphocytes or levels of blood immunoglobulins (Little et al. 1999; Pelclova et al. 1990; Stengel et al. 1998; Yin et al. 1987) or development of autoimmune disorders (Chaigne et al. 2015; Diot et al. 2002; Marie et al. 2014). In animal studies, evidence for toluene effects on the immune system following inhalation exposure are limited to the finding of decreased resistance to mortality from respiratory infection by *S. zooepidemicus* in a study of mice exposed for 3 hours to toluene concentrations as low as 2.5 ppm (Aranyi et al. 1985). However, animal data using the oral route of exposure provide some evidence of impaired immune function following intermediate-duration toluene exposure (Hsieh et al. 1989, 1990a, 1991), and these effects were used to derive an MRL of 0.2 mg/kg/day for intermediate-duration oral exposure toluene. 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 (Abbate et al. 1993; Andersen et al. 1983; Baelum et al. 1985; Boey et al. 1997; Dick et al. 1984; Echeverria et al. 1991; Eller et al. 1999; Foo et al. 1990; Gamberale and Hultengren 1972; Kang et al. 2005; Little et al. 1999; Matsushita et al. 1975; Morata et al. 1997; Murata et al. 1993; Nordling Nilson et al. 2010; Orbaek and Nise 1989; Orbaek et al. 1998; Osterberg et al. 2000, 2003; Rahill et al. 1996; Ukai et al. 1993; von Oettingen et al. 1942; Vrca et al. 1995, 1996, 1997a, 1997b; Yin et al. 1987; Zavalic et al. 1998a, 1988b, 1988c) and are the basis for the acute and chronic 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; Boyes et al. 2007, 2016; Bowen and Balster 1998; Bruckner and Peterson 1981a, 1981b; Bushnell et al. 1985; Conti et al. 2012; Cruz et al. 2001; Dashniani et al. 2014; Ghosh et al. 1989, 1990; Hinman 1987; Hogie et al. 2009; Huerta-Rivas et al. 2012; Johnson 1992; Johnson et al. 1988; Kishi et al. 1988; Li et al. 1992; Little et al. 1998; Lopez-Rubalcava and Cruz 2000; McWilliams et al. 2000; Mullin and Krivanek 1982; Paez-Martinez et al. 2003, 2013; Rebert et al. 1989b; Takeuchi and Hisanaga 1977; Taylor and Evans 1985; Tomaszycki et al. 2013; Wood and Colotla 1990; Wood et al. 1983). 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 2 ppm for acute inhalation exposure (based on a study by Little et al. 1999) and a chronic-duration MRL of 1 ppm (based on a series of studies by Schäper et al. 2003, 2004, 2008; Seeber et al. 2004, 2005; Zupanic et al. 2002).

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 a critical target of toluene toxicity following acute exposure. An acute MRL of 0.8 mg/kg/day was developed based on a change in FEP waveforms in rats exposed to a single dose of toluene (Dyer et al. 1988). Neurobehavioral changes following intermediate-duration oral toluene exposure were limited to increased open-field activity in young mice following pre- and postnatal exposure to 106 mg/kg/day toluene (Kostas and Otchin 1981). Intermediate-duration studies designed to assess neurobehavioral alterations and/or mechanisms of neurotoxicity following oral exposure to toluene may be useful in evaluating human health risk, and could potentially serve as a basis for the deriving the intermediate-duration oral MRL (which is currently based

on immune depression). No data on dermal exposure are available, but this is not expected to be the main 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. Results from studies comparing the effectiveness of various biomarkers at high (>50 ppm) and low (<10 ppm) air concentrations indicate that toluene concentrations in blood or urine are more accurate at low air concentration than urinary concentrations of examined metabolites (Ikeda et al. 2008; Inoue et al. 2008; Kawai et al. 2008; Lovreglio et al. 2010; Takeuchi et al. 2002; Ukai et al. 2007).

Currently, ACGIH (2013, 2010) recommends using a combination of three biomarkers to assess exposure of workers to toluene: *ortho*-cresol and unchanged toluene levels in urine at the end of a workshift and toluene levels in blood immediately prior to the last shift of a workweek.

Other urinary biomarkers of exposure that have been examined include benzyl alcohol (Ikeda et al. 2008; Kawai et al. 2007), benzylmercapturic acid (Ikeda et al. 2008; Inoue et al. 2002, 2004, 2008; Maestri et al. 1997), and S-*p*-toluylmercapturic acid (Angerer et al. 1998a; Ikeda et al. 2008; Inoue et al. 2008). A preliminary study also examined toluene concentration in saliva as a possible biomarker of exposure, which showed a correlation coefficient with air concentrations of 0.77, compared with 0.93 for urinary toluene concentration (Ferrari et al. 2008). 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 1983; Bray et al. 1949; Bushnell et al. 2007; Campo et al. 1999; Carlsson 1982; Carlsson and Ljungquist 1982; Chand and Clausen 1982; Dossing et al. 1983c; Ducos et al. 2008; Furman et al. 1998; Ghantous and Danielsson 1986; Hjelm et al. 1988; Ikeda et al. 1990; Janasik et al. 2008, 2010; Kawai et al. 1992a, 1992b, 1993, 1996; Leung and Paustenbach 1988; Lof et al. 1990, 1993; Maestri et al. 1997; Nadeau et al. 2006; Nakajima and Wang 1994; Nakajima et al. 1991, 1992a, 1992b, 1993, 1997, 2006; Ng et al. 1990; Nise et al. 1989; Ogata 1984; Pellizzari et al. 1992; Pierce et al. 1996, 1999, 2002; Paterson and Sarvesvaran 1983; Takeichi et al. 1986; Tardif et al. 1998; Tassaneeyakul et al. 1996; van Doorn et al. 1980; Wang and Nakajima 1992; Zahlsen et al. 1992).

Limited data are available on the quantitative absorption and excretion of toluene by the oral and dermal routes. 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; Sullivan and Conolly 1988). Studies of humans and animals indicate that dermal absorption of toluene is slow (Aitio et al. 1984; Dutkiewicz and Tyras 1968; Thrall and Woodstock 2002; Thrall et al. 2002a), 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.

PBPK models are available that describe the kinetics of toluene after inhalation exposure for humans (Benignus et al. 2006; Fisher et al. 1997; Jonsson and Johanson 2001; Mörk et al. 2014; Nong et al. 2006; Pierce et al. 1996, 1999; Sari-Minodier et al. 2009; Tardif et al. 1995, 2002) and rats (DeJongh and Blaauboer 1996, 1997; Kenyon et al. 2008; Oshiro et al. 2011; Tardif et al. 1993; van Asperen et al.

2003). PBPK models to describe the kinetics of dermally applied aqueous solutions of toluene are also available for humans (Thrall et al. 2002a) and rats (Thrall and Woodstock 2002), but models to describe kinetics following oral exposure to toluene have not been developed. 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 model was developed.

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. In cases where toluene has caused cardiac arrhythmias, interventions may include evaluation and treatment of potential electrolyte and acid-base abnormalities as well as administration of antiarrhythmic medications to control the heart beat (Graham 1990; Gummin 2014). 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. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

The 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 (Bowen et al. 2007; Castilla-Serna et al. 1991; O'Leary-Moore et al. 2009; Pryor and Rebert 1992; Pryor et al. 1984a; Samuel-Herter et al. 2013; von Euler et al. 1989) 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. Age-series inhalation studies do not provide consistent evidence of increased neurobehavioral or neurochemical alterations in juvenile or young adult rats, compared with adult rats (Batis et al. 2010; Bowen et al. 2007; O'Leary-Moore et al. 2009; Samuel-Herter et al. 2013). Additional research on the development of metabolic capabilities in newborn and very young children 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 3.7). 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.

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

3.12.3 Ongoing Studies

Nine ongoing research efforts have been identified that may provide data related to the toxic actions of toluene (RePORTER 2014, 2016). These projects are summarized in Table 3-7.

Human studies: Dr. Anneclaire De Roos of Drexel University is conducting metanalysis of 13 case-control studies to determine if exposure to solvents, including trichloroethylene, perchloroethylene, benzene, toluene, and xylene, is a risk factor for multiple myeloma. Dr. Brad Racette of Washington University is conducting a retrospective, population-based study evaluating the potential association between occupational solvent exposure (including toluene) and Parkinson Disease in Finland. Dr. Wynne Schiffer of the University of Minnesota is conducting a study on functional and structural changes in the adolescent brain following solvent abuse.

Animal studies: Dr. Jacob Thomas Beckley of the Medical University of South Carolina is evaluating short- and long-term effects of acute toluene exposure on neuroplasticity, and Dr. Matthew Tracy of Virginia Commonwealth University is evaluating neurobehavioral changes associated with toluene exposure in mice.

Toxicokinetic studies: Dr. Wayne L. Backes of Louisiana State University Medical Center is continuing his efforts to better characterize the P450 monooxygenase system.

Mechanistic studies: Dr. Keith Shelton of Virginia Commonwealth University is investigating the neuropharmacological mechanisms of action of toluene (and other intentionally solvents) in mice, focusing on toluene's modulatory effects on the GABA receptor. Dr. John Woodward of the Medical University of South Carolina is also investigating the neuropharmacological mechanisms of action of toluene, specifically in the glutamatergic system.

Table 3-7. Ongoing Research for Toluene

Investigator	Affiliation	Research description	Sponsor
Backes, W	Louisiana State University Medical Center, New Orleans, Louisiana	Toxicological significance of alkylbenzene metabolism	National Institute of Environmental Health Sciences
Beckley, JT	Medical University of South Carolina, Charleston, South Carolina	Neuroplasticity associated with acute toluene inhalation	National Institute on Drug Abuse
De Roos, AJ	Drexel University, Philadelphia, Pennsylvania	Multiple myeloma consortium study of occupational exposures and family history	National Institute of Environmental Health Sciences
Racette, BA	Washington University St. Louis, Missouri	Risk of Parkinson Disease associated with solvent exposures in Finland	National Institute of Environmental Health Sciences
Schiffer, WK	University of Minnesota, Minneapolis, Minnesota	Imaging the causes and consequences of adolescent inhalant abuse	National Institute on Drug Abuse
Shelton, KL	Virginia Commonwealth University, Richmond, Virginia	Discriminative stimulus effects of abused inhalants	National Institute on Drug Abuse
Tracy, M	Virginia Commonwealth University, Richmond, Virginia	Acute and chronic effects of inhalants in intracranial-self-stimulation (ICSS)	National Institute on Drug Abuse
Woodward, JJ	Medical University of South Carolina, Charleston, South Carolina	Neural actions of toluene	National Institute on Drug Abuse

Source: RePORTER 2014, 2016